Sunitinib for advanced renal cell cancer

Clinical and pharmacodynamic aspects

Astrid A.M. van der Veldt
The studies presented in this thesis were performed at the Department of Medical Oncology, VU University Medical Center, Amsterdam, in collaboration with the Department of Medical Oncology, The Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, the Netherlands.

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VRIJE UNIVERSITEIT

Sunitinib for advanced renal cell cancer

Clinical and pharmacodynamic aspects

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Chapter 1

Targeted therapy for advanced and metastatic renal cell cancer

Astrid A.M. van der Veldt, John B.A.G. Haanen, Alfons J.M. van den Eertwegh, Epie Boven

Parts of this chapter have been published in Discov Med 2010;10:394-405.
1.0 Introduction
In the past 5 years, the introduction of targeted therapy has dramatically changed the treatment armamentarium of advanced and metastatic renal cell cancer (mRCC) and has significantly improved the perspectives of patients with this disease. In December 2005, the Food and Drug Administration approved the first targeted agent, sorafenib, for the treatment of patients with cytokine-refractory disease. Thereafter, five other targeted agents have been registered for treatment of mRCC, including sunitinib, temsirolimus, everolimus, bevacizumab in combination with interferon-α, and more recently pazopanib. Currently, sunitinib is the most widely prescribed targeted drug in patients with mRCC. Although sunitinib has gained remarkable success in mRCC, a number of issues still need to be addressed to better understand the pharmacodynamics of this effective drug. Then, the management of patients with mRCC can be further improved. In this chapter, the literature on renal cell cancer (RCC) is summarized and the current treatment strategies for patients with mRCC in the era of targeted therapy are reviewed. Thereafter, sunitinib is discussed in more detail and the other chapters of this thesis are introduced.

2.0 Renal cell cancer
2.1 Epidemiology
Kidney tumors comprise 2% of all adult malignancies and account for 208,000 new diagnoses and 102,000 deaths worldwide per year (1). RCC represents the vast majority of all kidney tumors. Over the last years, the incidence of all stages of RCC has increased (2;3), leading to a rising mortality rate. Men have a two times higher risk of developing RCC than women (1). The disease is most frequently diagnosed in the sixth and seventh decades of life (4). Most patients with RCC do not have an identifiable risk factor. Few risk factors for RCC have been established such as cigarette smoking, obesity, and hypertension (5-9), whereas fruit and vegetable consumption may have a protective effect (10;11). Approximately 2-3% of cases are familial and several hereditary RCC syndromes have been described including von Hippel-Lindau syndrome, hereditary papillary RCC, hereditary leiomyomatosis RCC, Birt-Hogg-Dubé, and tuberous sclerosis (12). Among these, the von Hippel-Lindau syndrome is most notable. A defect in one allele of the von Hippel-Lindau (VHL) gene, a tumor suppressor gene on the short arm of chromosome 3 (3p25–26), is responsible for this autosomal dominant syndrome, which is characterized by several vascular tumors including RCC, central nervous system hemangioblastoma and pheochromocytoma (13). Most people with the von Hippel-Lindau syndrome inherit a germline mutation of the VHL gene from the affected parent and a normal wild type gene from the unaffected parent. People who have already inherited
one mutated copy of the gene have a high probability of developing a second mutation in at least one other cell in their organs. According to the two-hit hypothesis, tumorigenesis occurs when both VHL alleles are inactivated (13).

2.2 Clinical presentation
Patients with RCC can present with local or systemic symptoms. However, most patients with localized disease are incidentally diagnosed with RCC, as a result of the extensive use of abdominal imaging. Local symptoms of RCC comprise hematuria, flank pain or a palpable abdominal mass, whereas systemic symptoms can be caused by metastases or paraneoplastic symptoms, such as weight loss, fatigue, fever, hypertension, hypercalcemia and erythrocytosis (14). Computed tomography (CT) scans are mainly applied for staging of RCC and to allow determination of local invasion, involvement of lymph nodes and distant metastases (15). Approximately 30% of all patients with RCC have metastatic disease at presentation (16). After complete resection of the primary tumor, recurrence develops in another 30% of patients (16). Metastatic disease is most frequently found in lungs (50-60%), followed by bone (30-40%), liver (30-40%) and brain (5%) (16). In addition, metastases from RCC can be detected at unusual sites such as thyroid gland, skin and underlying soft tissue.

2.3 Pathology
Histopathological examination of tumor tissue is necessary to confirm the diagnosis of RCC. Then, RCC is classified histologically into different subtypes (Figure 1). Clear cell carcinoma is the most common subtype and accounts for 75% of the cases (17). Clear cell refers to the high lipid content in cytoplasm that is dissolved during histological preparation, resulting in a lucent or clear cytoplasm. In the remaining cases, papillary carcinoma, chromophobe carcinoma and collecting duct carcinoma are described, which represent 10-15%, 5-10% and 1% of the cases, respectively (17).

Figure 1. Representative examples of (A) clear cell, (B) papillary, and (C) chromophobe renal cell carcinoma. Haematoxylin and eosin staining, 20 × [Reprinted with permission; (18)].
2.4 Staging

Table 1A displays the tumor-node-metastasis (TNM) classification applied for staging RCC (19). Based on this classification RCC is further categorized into four stages of disease (Table 1B).

**Table 1A. Definition of TNM classification for renal cell cancer**

<table>
<thead>
<tr>
<th>Tumor (T)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TX</td>
<td>Primary tumor cannot be assessed</td>
</tr>
<tr>
<td>T0</td>
<td>No evidence of primary tumor</td>
</tr>
<tr>
<td>T1</td>
<td>Tumor ≤ 7 cm in greatest dimension, limited to the kidney</td>
</tr>
<tr>
<td>T1a</td>
<td>Tumor ≤ 4 cm in greatest dimension, limited to the kidney</td>
</tr>
<tr>
<td>T1b</td>
<td>Tumor &gt; 4 cm but not &gt; 7 cm in greatest dimension, limited to the kidney</td>
</tr>
<tr>
<td>T2</td>
<td>Tumor &gt; 7 cm in greatest dimension, limited to the kidney</td>
</tr>
<tr>
<td>T2a</td>
<td>Tumor &gt; 7 cm but ≤ 10 cm in greatest dimension, limited to the kidney</td>
</tr>
<tr>
<td>T2b</td>
<td>Tumor &gt; 10 cm, limited to the kidney</td>
</tr>
<tr>
<td>T3</td>
<td>Tumor extends into major veins or perinephric tissues but not into the ipsilateral adrenal gland and not beyond Gerota’s fascia</td>
</tr>
<tr>
<td>T3a</td>
<td>Tumor grossly extends into the renal vein or its segmental (muscle containing) branches, or tumor invades perirenal and/or renal sinus fat but not beyond Gerota’s fascia</td>
</tr>
<tr>
<td>T3b</td>
<td>Tumor grossly extends into the vena cava below the diaphragm</td>
</tr>
<tr>
<td>T3c</td>
<td>Tumor grossly extends into the vena cava above the diaphragm or invades the wall of the vena cava</td>
</tr>
<tr>
<td>T4</td>
<td>Tumor invades beyond Gerota’s fascia (including contiguous extension into the ipsilateral adrenal gland)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Regional lymph node (N)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NX</td>
<td>Regional lymph nodes cannot be assessed</td>
</tr>
<tr>
<td>N0</td>
<td>No regional lymph node metastasis</td>
</tr>
<tr>
<td>N1</td>
<td>Metastases in regional lymph node(s)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Distant metastasis (M)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MX</td>
<td>Presence of distant metastasis cannot be assessed</td>
</tr>
<tr>
<td>M0</td>
<td>No distant metastasis</td>
</tr>
<tr>
<td>M1</td>
<td>Distant metastasis</td>
</tr>
</tbody>
</table>

**Table 1B. Stage grouping for renal cell cancer**

<table>
<thead>
<tr>
<th>Stage</th>
<th>T</th>
<th>N</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>T1</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>II</td>
<td>T2</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>III</td>
<td>T1 or T2</td>
<td>N1</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>N0 or N1</td>
<td>M0</td>
</tr>
<tr>
<td>IV</td>
<td>T4</td>
<td>Any N</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>Any T</td>
<td>Any N</td>
<td>M1</td>
</tr>
</tbody>
</table>
2.5 **Prognosis**

The prognosis of RCC is related to the stage of the disease. When applying the TNM classification to predict the prognosis, the 5-year cancer specific survival is 78%, 73%, 55% and 17% for TNM stages I, II, III, and IV, respectively (20). In case of metastatic disease (stage IV), the prognosis is poor. Nevertheless, the prognosis among patients with distant metastases is highly variable. In 2002, Motzer et al (21) have introduced the Memorial Sloan-Kettering Cancer Center (MSKCC) criteria to predict the prognosis in patients with distant metastases. To date, these criteria (21) are still applied for risk stratification of mRCC patients. The MSKCC criteria are based on five risk factors including low Karnofsky performance status (< 80%), high lactate dehydrogenase (> 1.5 times the upper limit of normal), low serum hemoglobin, high corrected serum calcium (> 10 mg/dL), and time from initial diagnosis to systemic treatment < 1 year. When patients have none of these risk factors they are categorized into the favorable risk group, whereas patients with 1-2 and ≥ 3 risk factors are categorized into the intermediate and poor risk groups, respectively. Accordingly, the MSKCC criteria are highly prognostic for survival in patients with mRCC when treated with first-line interferon-α. In such patients, the median overall survival was 30, 14 and 5 months when belonging to the favorable, intermediate and poor risk groups, respectively (21).

3.0 **Surgical treatment**

3.1 **Localized disease**

The primary treatment of RCC consists of surgical excision of the primary tumor. The surgical procedure is mainly determined by the size and location of the primary tumor as well as the TNM stage. Therefore, careful physical examination, routine laboratory tests and adequate tumor staging are required before surgery. Radical nephrectomy is the gold standard curative operation for patients with localized RCC. For primary tumors with a size less than 4 cm, a nephron-sparing partial nephrectomy is generally recommended (15), as the recurrence-free and long-term survival are similar to those of patients treated with radical nephrectomy (22). In addition, partial nephrectomy may be considered in case of bilateral RCC, a solitary kidney, renal cell insufficiency or other situations in which radical nephrectomy is contraindicated. To decrease surgery-associated morbidity, laparoscopic nephrectomy and minimally invasive percutaneous ablative approaches (e.g., radiofrequent heat ablation and cryoablation) for small tumors can be considered (15).
3.2 Primary metastatic disease

When mRCC patients present with potentially resectable primary tumors and/or a solitary metastasis, nephrectomy and/or metastasectomy should still be considered, as long-term survival has been reported in some patients (23). In the cytokine era, responses of the primary tumor were very rare (24) and patients presenting with primary metastatic disease and a resectable primary tumor usually underwent cytoreductive nephrectomy. This strategy was based on the results of two randomized phase III trials, which had shown that nephrectomy followed by interferon-α improved overall survival (OS) as compared with interferon-α alone (25;26). A combined analysis of these two trials demonstrated a median OS benefit of 5.8 months in patients who underwent nephrectomy (27). In the era of targeted therapy however, the current role of cytoreductive nephrectomy has not yet been defined.

4.0 Systemic therapy for metastatic disease

4.1 Cytokine-based therapy

Until the last decade, the treatment options for patients with metastatic RCC have been disappointing, since mRCC is resistant to standard cytotoxic chemotherapy (28). As some RCC tumors are able to evoke an immune response, immunotherapy was usually applied to treat patients with mRCC. The most consistent antitumor effects have been observed with cytokine-based therapy consisting of interferon-α and/or interleukin-2. Although few mRCC patients, especially those with lung metastases and a previous nephrectomy, may achieve a long-lasting complete remission, cytokine-based therapy results in modest response rates and provides modest survival benefit (29). Interferon-α, the most frequently administered cytokine, leads to an objective response of 7.5% and a median OS of 13 months (30). Currently, the targeted agents (Table 2) have replaced immunotherapy in the majority of mRCC patients.

4.2 Biological pathways for targeted therapy in renal cell cancer

An increased understanding of the biology of RCC has identified relevant targets for molecular therapy in RCC tumor cells and their microenvironment (Figure 2). Discovery of the VHL gene in families affected with the von Hippel-Lindau syndrome has clarified the pathogenesis of hereditary RCC (43). The VHL gene is a tumor suppressor gene of which biallelic inactivation promotes a phenotype at risk to develop malignancies. Of interest, in more than 90% of sporadic RCC, in particular the clear cell subtype, one VHL allele is inactivated through a deletion (loss of heterozygosity) (44). The other VHL allele is inactivated through either gene mutation (in approximately 80% of clear cell tumors) or through gene silencing by methylation [in approximately 19% of cases; (45;46)].
Table 2. Major phase III trials for drugs approved for treatment of mRCC

<table>
<thead>
<tr>
<th>Drug</th>
<th>Targets</th>
<th>Administration</th>
<th>Dose</th>
<th>Eligibility</th>
<th>Study design</th>
<th>N</th>
<th>Experimental arm</th>
<th>Control arm</th>
<th>Median PFS (months)</th>
<th>Median OS (months)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bevacizumab</td>
<td>VEGF</td>
<td>IV</td>
<td>10 mg/kg, every 2 weeks</td>
<td>First-line</td>
<td>Double-blind RCT</td>
<td>649</td>
<td>IFN-α (9 MU, s.c., 3 times a week) + bevacizumab</td>
<td>IFN-α (9 MU, s.c., 3 times a week) + placebo</td>
<td>10.2 vs. 5.4*</td>
<td>23.3 vs. 21.3</td>
<td>(31,32)</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>VEGF</td>
<td>IV</td>
<td>10 mg/kg, every 2 weeks</td>
<td>First-line</td>
<td>RCT</td>
<td>732</td>
<td>IFN-α (9 MU, s.c., 3 times a week) + bevacizumab</td>
<td>IFN-α (9 MU, s.c., 3 times a week)</td>
<td>8.5 vs. 5.2*</td>
<td>18.3 vs. 17.4</td>
<td>(33,34)</td>
</tr>
<tr>
<td>TKI</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorafenib</td>
<td>VEGFR-1, -2, and -3; PDGFR-α and β</td>
<td>Oral</td>
<td>50 mg/day, 4 weeks-on/4 weeks-off</td>
<td>First-line</td>
<td>RCT</td>
<td>750</td>
<td>Sorafenib</td>
<td>IFN-α (9 MU, s.c., 3 times a week)</td>
<td>11 vs. 5*</td>
<td>26.4 vs. 21.8</td>
<td>(35,36)</td>
</tr>
<tr>
<td>Pazopanib</td>
<td>VEGFR-1, -2, and -3; PDGFR-α and β</td>
<td>Oral</td>
<td>800 mg/day</td>
<td>Second-line, first-line</td>
<td>Double-blind RCT</td>
<td>435</td>
<td>Pazopanib</td>
<td>Placebo</td>
<td>9.2 vs. 4.2*</td>
<td>n.d.</td>
<td>(39)</td>
</tr>
<tr>
<td>mTOR inhibitor</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temsirolimus</td>
<td>mTOR</td>
<td>IV</td>
<td>25 or 15 mg/week</td>
<td>First-line</td>
<td>RCT</td>
<td>626</td>
<td>Temsirolimus (25 mg) or temsirolimus (15 mg) + IFN-α (3–6 MU, s.c., 3 times a week)</td>
<td>IFN-α (3–6 MU, s.c., 3 times a week)</td>
<td>5.5* and 4.7 vs. 3.1</td>
<td>10.9* vs. 7.3</td>
<td>(40)</td>
</tr>
<tr>
<td>Everolimus</td>
<td>mTOR</td>
<td>Oral</td>
<td>10 mg/day</td>
<td>Second-line</td>
<td>Double-blind RCT</td>
<td>416</td>
<td>Everolimus</td>
<td>Placebo</td>
<td>4.9 vs. 1.9*</td>
<td>14.8 vs. 14.4</td>
<td>(41,42)</td>
</tr>
</tbody>
</table>

TKI, tyrosine kinase inhibitor; mTOR, mammalian target of rapamycin; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; PDGFR, platelet-derived growth factor receptor; FLT-3, FMS-like tyrosine kinase-3; c-KIT, c-KIT protein; IV, intravenous; RCT, randomized controlled trial; IFN-α, interferon-α; MU, million U; s.c., subcutaneously; PFS, progression-free survival; OS, overall survival; vs., versus; n.d., not determined; * indicates significant difference in outcome; †, starting dose of 3 MU 3 times a week for the first week, raised to 9 MU 3 times a week for the second week, and raised to 18 MU 3 times a week for the third week. Patients unable to tolerate these doses received the highest tolerable dose; ‡, starting dose of 3 MU 3 times a week for the first week, raised to 6 MU 3 times a week thereafter.
Defective VHL gene function leads to overexpression of a series of proteins that can be used as targets for treatment. The VHL gene encodes the von Hippel-Lindau protein that is required for degradation of the crucial transcription factor hypoxia-inducible factor (HIF) (47). Under hypoxic conditions in tumors, the HIF protein is usually upregulated. When VHL is inactivated, the HIF protein cannot be degraded and the amount of HIF is increased even under normoxic conditions. Activated HIF translocates to the nucleus of tumor cells and results in transcription of a large repertoire of genes including vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) (48). VEGF is a potent proangiogenic protein which plays a key role in tumor angiogenesis [Figure 3; (49)] and exerts its effects by binding to the VEGF receptor (VEGFR) on endothelial cells (50). The high angiogenic potential of RCC, especially in tumors in which VHL is inactivated, results in highly vascularized tumors. Several drugs affecting VEGF signaling have been approved for treatment of mRCC. The activity of VEGF can be inhibited by bevacizumab (51), which is a monoclonal antibody that binds circulating VEGF, thereby preventing binding of VEGF to its receptor.
The tyrosine kinase inhibitors (TKIs) sunitinib (35), sorafenib (37), and pazopanib (39) inhibit VEGF signaling by targeting the intracellular domain of its receptor on tumor-associated endothelium, leading to reduced tumor angiogenesis. In addition, these TKIs are able to inhibit PDGFR in the microenvironment of tumors, thereby enhancing the anti-angiogenic effects of these drugs (52).

Next to the VHL gene, HIF expression is regulated by signaling through the mammalian target of rapamycin (mTOR) pathway (53). This name is based on the precedent that the pathway was first discovered through genetic and molecular studies using the immunosuppressant drug rapamycin, also known as sirolimus (54). Activation of mTOR, which is a serine-threonine protein kinase, is regulated through a series of complex signaling interactions that link growth factor receptor signaling and other cell stimuli to activation of the phosphoinositide-3 kinase and Akt/protein kinase pathway (55). The mTOR pathway regulates cell proliferation, cell survival, cell motility, transcription, and protein synthesis. In RCC, mTOR is frequently activated (53;56). Presently, temsirolimus and everolimus, which are rapamycin derivatives or rapalogues, have been approved for treatment of mRCC. Temsirolimus and everolimus bind to an intracellular protein, FKBP-12, forming a complex that inhibits the mTOR serine-threonine kinase (57). Consequently, these drugs induce cell cycle arrest and inhibition of tumor angiogenesis by reducing synthesis of VEGF (58).
4.3 Inhibitors of VEGF signaling

4.3.1 Bevacizumab
Bevacizumab is a recombinant humanized monoclonal antibody that neutralizes the circulating VEGF protein without affecting VEGF bound to its receptor (51). The drug is administered intravenously (IV). In RCC, bevacizumab is usually administered at 10 mg/kg with an interval of 2 weeks. Initially, bevacizumab has shown clinical benefit as compared to placebo in patients with cytokine-refractory mRCC (59). In this setting, the median progression-free survival (PFS) in patients receiving bevacizumab (10 mg/kg IV every 2 weeks) was significantly longer than that in the placebo group (4.8 versus 2.5 months). The response rate of monotherapy bevacizumab was low (10%). As a next step, bevacizumab was added to interferon-α (9 million U subcutaneously 3 times a week) and was compared with interferon-α alone in the first-line setting (31;33). In two phase III randomized trials, the combination resulted in an improved objective response rate as compared to that in patients receiving interferon-α alone (26-31% versus 13%). Furthermore, adding bevacizumab significantly improved median PFS (8.5-10.2 versus 5.2-5.4 months). The combination did not significantly improve OS, the primary endpoint of these studies (32;34), which may be the result of second-line treatment with TKIs confounding the OS analysis.

Combination of bevacizumab with interferon-α is more toxic than either drug alone. Most frequently reported toxicities (> 20% of all grades according to Common Terminology Criteria for Adverse Events (CTCAE)) include fatigue (33-93%), proteinuria (18-71%), anorexia (36-71%), nausea (58%), pyrexia (45%), neutropenia (43%), bleeding (33%), asthenia (32%), hypertension (26-28%), influenza-like illness (24%), and headache (23%) (31;34). Of these toxicities, fatigue is a frequently reported grade 3 side-effect (> 20%). Hypertension and proteinuria are predominantly associated with the administration of bevacizumab. In particular, the development of hypertension is a commonly reported side-effect after administration of drugs that target VEGF signaling. The underlying mechanism of the rise in blood pressure has not yet been fully clarified yet, but is sought in a reduction in microvascular density of the peripheral microcirculation (60).

4.3.2 Sunitinib
Sunitinib targets several receptors including VEGFR-1, -2, and -3, PDGFR-α and -β, c-Kit protein (c-Kit), and FMS-like tyrosine kinase-3 (FLT-3). Sunitinib is an oral TKI that is routinely administered at 50 mg once a day in a treatment cycle of 6 weeks consisting of a 4 weeks-on/2 weeks-off schedule. In a randomized phase III trial, sunitinib showed significant benefit as compared to interferon-α in first-line treatment of 750 patients with
mRCC (35). In the final analysis, the response rate was 47% in mRCC patients treated with sunitinib, whereas the objective response rate was 12% in patients treated with interferon-α (36). In addition, sunitinib improved PFS (11 versus 5 months) and OS (26.4 versus 21.8 months) (36). This improvement in OS did not achieve statistical significance (36), which is most likely due to cross-over of interferon-α treated patients to sunitinib and/or other active targeted therapy at the time of disease progression. In this phase III trial, the median OS was extended as compared with historical controls, implying that targeted therapy has significantly improved the survival of patients with mRCC. In addition, sunitinib has shown efficacy as second-line drug in mRCC patients progressive on previous cytokine-based therapy (61;62). In 168 cytokine-refractory mRCC patients, sunitinib treatment was associated with a response rate of 42% and a median PFS of 8.1 months (61).

In some patients, intermittent dosing of sunitinib may cause flare-up symptoms in the 2-week rest period. Therefore, Escudier et al (63) have evaluated the efficacy and tolerability of sunitinib when administered continuously at 37.5 mg daily in cycles of 6 weeks. In this phase II study, mRCC patients who had failed previous cytokine therapy were enrolled. When administered continuously, the efficacy and tolerability of sunitinib were similar to the findings with the intermittent schedule (61;62); the median PFS and OS were 8.2 and 19.8 months, respectively. A meta-analysis of pharmacokinetic and efficacy data from all clinical studies with single-agent sunitinib in mRCC, however, has shown that the probability of greater response and survival increased with higher drug exposure (64). Therefore, dosing of 50 mg in a 4 weeks-on/2 weeks-off schedule is still recommended in patients who tolerate sunitinib well and do not experience clinical deterioration during the rest-period.

Non-hematological toxicities that are most frequently (> 20% of all grades) associated with sunitinib treatment are diarrhea (53%), fatigue (51%), nausea (44%), stomatitis (25%), vomiting (24%), and hypertension [24%; (35)]. Clinically relevant hematological toxicities include leucopenia (78%), neutropenia (72%), and thrombocytopenia (65%). Although sunitinib-induced toxicities can be severe and may significantly impede patients in their daily activities, the incidence of grade 3 and 4 toxicities is low.

### 4.3.3 Sorafenib

Sorafenib was originally developed as a RAF kinase inhibitor, targeting the RAS-RAF-MEK-ERK pathway, but was subsequently discovered to target other tyrosine kinases including VEGFR-2 and -3, PDGFR-α and -β, c-Kit, FLT-3, and RET (65;66). Sorafenib is an oral TKI and is administered at 400 mg twice a day. The efficacy of sorafenib has been demonstrated in a phase III randomized trial (37). In this study, 903 patients with cytokine-refractory mRCC were assigned to sorafenib or placebo. At a preplanned interim
analysis, the PFS of sorafenib-treated patients was superior to that of patients assigned to receive placebo. On the basis of these data, the protocol was amended to offer sorafenib to patients assigned to placebo. Although a partial response was reported in only 10% of patients, sorafenib significantly improved PFS as compared with that of placebo (5.5 versus 2.8 months) (37). However, sorafenib-treated patients did not have a significantly better OS than placebo-assigned patients (17.8 versus 15.2 months) (38). This lack of substantial improvement in OS was probably due to the cross-over design of the study (38).

Sorafenib can induce rather severe toxicities, especially of the skin and gastrointestinal tract. Often (> 20% of all grades), sorafenib treatment is associated with diarrhea (48%), rash and desquamation (41%), alopecia (31%), hand-foot syndrome (33%), and fatigue (29%) (38). For specific toxicities, the incidence of grade 3 toxicities is low (< 10%). Nevertheless, grade 3 and 4 toxicities have been reported in 29% of patients.

4.3.4 Pazopanib

Pazopanib is a novel TKI and has more recently been approved for the treatment of mRCC. This drug inhibits VEGFR-1, -2, and -3, PDGFR-α and -β, and c-Kit (67). In general, pazopanib is orally administered at 800 mg once a day. A randomized, double-blind, placebo-controlled phase III trial was the basis for drug approval (39). Initially, the study inclusion was restricted to patients progressive on previous cytokine-based therapy. The protocol was amended to include treatment-naïve patients, as the use of cytokines had decreased. In the overall study population, the independently assessed objective response rate was 30%. In the treatment-naïve and cytokine-pretreated population, response rates were 32% and 29%, respectively. Pazopanib showed an overall PFS benefit as compared with that of placebo (9.2 versus 4.2 months). In the treatment-naïve subpopulation, the PFS benefit was 8.3 months for pazopanib as compared to placebo (11.1 versus 2.8 months), whereas the PFS benefit was 3.2 months over placebo in the cytokine-pretreated subpopulation (7.4 versus 4.2 months). These favorable objective response rates and PFS benefits of pazopanib are comparable with those of sunitinib (35;36). Currently, the results for OS have not yet been published.

The toxicity of pazopanib is similar to, if not less severe than, that of similar molecules such as sunitinib (35) and sorafenib (37). Diarrhea (52%), hypertension (40%), hair color changes (38%), nausea (26%), anorexia (22%), and vomiting (21%) account for the most frequently (> 20% of all grades) reported non-hematological side effects (39). Among the hematological toxicities, leucopenia (37%), neutropenia (34%), and thrombocytopenia (32%) are commonly reported. Grade 3-4 diarrhea and hypertension are reported in 3% and 4% of patients, respectively.
4.4 Inhibitors of mTOR signaling

4.4.1 Temsirolimus
Temsirolimus is an inhibitor of mTOR. The drug is administered IV at 25 mg per week. The efficacy of temsirolimus was first demonstrated in a phase II trial in patients with treatment-refractory mRCC. In a retrospective analysis of this study, the benefit of temsirolimus seemed pronounced in a subset of patients with poor prognosis (68). Hence, a randomized phase III trial (40) was conducted in 626 treatment-naïve mRCC patients having at least three out of six poor prognostic factors, which were defined as more than two metastatic sites of involvement plus five criteria according to the MSKCC prognostic criteria (21). Patients were randomized to either temsirolimus (25 mg weekly) or temsirolimus (15 mg weekly) plus interferon-α subcutaneously 3 times a week (3 million U with escalation to 6 million U) versus interferon-α alone subcutaneously 3 times a week (3 million U with escalation to 18 million U) (40). The objective response rates among patients receiving interferon-α (4.8%), temsirolimus (8.6%), and combination therapy (8.1%) did not differ significantly. Temsirolimus treatment improved median PFS as compared with that of interferon-α alone (5.5 versus 3.1 months). In addition, temsirolimus-treated patients had a better OS than patients treated with interferon-α alone (10.9 versus 7.3 months). However, when patients on the combination regimen were compared with the interferon-α group, PFS and OS were similar, being 4.7 and 8.4 months, respectively. Temsirolimus is well tolerated as compared with interferon-α. After administration of temsirolimus, asthenia (51%), rash (47%), nausea (37%), anorexia (32%), dyspnea (28%), hyperlipidemia (27%), infection (27%), diarrhea (27%), peripheral edema (27%), hyperglycemia (26%), hypercholesterolemia (24%), and fever (24%) are most frequently (> 20% of all grades) observed (40). Among these toxicities, most common grade 3 and 4 side effects are asthenia and hyperglycemia (both in 11% of patients).

4.4.2 Everolimus
The mTOR-targeting agent everolimus can be given orally; it is administered at 10 mg per day. In a double-blind randomized trial, the efficacy of everolimus has been investigated in 416 mRCC patients who had progressed on sunitinib, sorafenib, or both (41;42). Patients were assigned in a 2:1 ratio to receive everolimus (N = 277) or placebo (N = 139). At disease progression, patients were unblinded and those assigned to placebo were offered open-label everolimus. The objective response rate of everolimus was very low and almost similar to that in the placebo group [1.8 % versus 0%; (41)]. Treatment with everolimus significantly improved median PFS as compared with that of placebo (4.9 versus 1.9 months), but did not result in improved OS (14.8 versus 14.4
months). Lack of increased OS was likely the result of the cross-over design of the study. A subsequent phase II study showed efficacy of the combination of bevacizumab (10 mg/kg IV every 2 weeks) plus everolimus (10 mg per day) in treatment-naïve and previously treated mRCC patients (69); overall response rates were 30% and 23%, respectively. Additionally, median PFS was 9.1 and 7.1 months in these patients, respectively. These favorable results require further study of this promising combination and phase III studies are ongoing.

Everolimus-induced toxicities are comparable with those associated with temsirolimus treatment (40). Stomatitis (44%), infections (37%), asthenia (33%), fatigue (31%), diarrhea (30%), rash (29%), nausea (26%), anorexia (25%), peripheral edema (25%), vomiting (24%), thrombocytopenia (23%), hypercholesterolemia (77%), hypertriglyceridemia (73%), and hyperglycemia (57%) are commonly (> 20% of all grades) described after treatment with everolimus (41). Among these toxicities, grade 3 and 4 toxicities are rather rare. Grade 3 hyperglycemia is the most often reported grade 3-4 toxicity and is observed in 15% of patients. In addition, everolimus-induced pneumonitis is a remarkable side-effect, of which grade 3 pneumonitis develops in 4% of patients.

5.0 Targeted drugs in renal cell cancer: focus on sunitinib

5.1 Chemistry

Sunitinib malate (SU11248, Sutent®) is small molecule with a molecular weight of 398.5 g/mol (532.6 g/mol as malate salt). The chemical name of sunitinib is 5-[5-Fluoro-2-oxo-1,2-dihydroindol-(3Z)-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid(2-diethylamino ethyl)amide (70). The chemical structure of sunitinib is shown in Figure 4.

Figure 4. Chemical structure of sunitinib.
5.2 Mechanism of action
As mentioned earlier, sunitinib inhibits several receptor tyrosine kinases including VEGFR-1, -2, and -3, PDGFR-α and –β, c-Kit, and FLT-3 (71). In biochemical tyrosine kinase and cellular proliferation assays, inhibition of these receptor tyrosine kinases was shown in the low nano-molar range (72-75). In addition, sunitinib targets RET (76) and colony-stimulating factor 1 receptor (CSF-1R) (77). Receptor tyrosine kinases are transmembrane proteins at the cell surface and transduce extracellular signals to the cytoplasm (78) (Figure 5).

Figure 5. Sunitinib interacts with the adenosine triphosphate (ATP) binding site of the tyrosine kinase receptors and prevents autophosphorylation, thereby inhibiting the downstream signaling of proteins. L, extracellular ligand; R, receptor; TK, tyrosine kinase; P, phosphorylation site.

These proteins have extracellular domains, which enable binding of ligands, and intracellular catalytic domains. Some receptors act as monomers, whereas others (e.g., PDGFR-β) dimerize on ligand binding. When a ligand binds to the extracellular domain of the receptor, it results in autophosphorylation of the cytoplasmatic domain and stimulation of the tyrosine kinase activity. Then, tyrosine kinase activation stimulates
multiple downstream signaling pathways that are involved in DNA synthesis, proliferation, growth, migration and cell death (78). Sunitinib acts through competitive inhibition of adenosine triphosphate at its binding site, preventing autophosphorylation and kinase activity in the catalytic domain. As a result, transmembrane signaling is blocked, thereby affecting multiple downstream processes involved in tumor growth, metastasis formation and angiogenesis.

5.3 Targets in renal cell cancer
In RCC, the main targets for sunitinib are thought to be located in the microenvironment of tumor cells (Figure 2). The VEGF and PDGF pathways are supposed to be the most important pathways for sunitinib in RCC. VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1) are expressed on vascular endothelial cells, whereas VEGFR-3 (Flt-4) is present on lymphatic endothelium. In addition, PDGFR-β is expressed on pericytes and smooth muscle cells covering blood vessels. Inhibition of VEGFR on tumor-associated endothelium is assumed to be the major therapeutic effect of sunitinib (79;80). As a result, sunitinib impedes tumor angiogenesis which is stimulated by VEGF that is produced by tumor cells. Furthermore, inhibition of PDGFR signaling might enhance the anti-angiogenic effects of sunitinib by targeting pericytes, which are able to protect endothelial cells from apoptosis (81). Although indirect actions of sunitinib on cells in the microenvironment seem to prevail, there is increasing evidence that sunitinib also modulates tumor growth of RCC by direct actions on tumor cells (82). Apart from VEGF and PDGF signaling, sunitinib targets other pathways that may contribute to its efficacy in RCC. Although FLT-3, c-KIT and RET are assumed to be effective targets in other specific malignancies including acute myelogenous leukemia (83), gastrointestinal stromal tumors (84), and thyroid cancer (85), the additional value of targeting these receptors in RCC has not been clarified yet. In normal tissue, FLT-3 is usually expressed on immature hematopoietic progenitor cells, mature myeloid and lymphoid cells and controls the proliferation and survival of hematopoietic progenitor cells (86). Inhibition of FLT-3 may be associated with sunitinib-induced bone marrow toxicity (87). In addition, c-KIT is expressed on hematopoietic progenitor cells, mast cells, germ cells and the interstitial cell of Cajal in the gastrointestinal tract (88). At the level of melanocyte function, inhibition of c-KIT is associated with hair depigmentation (89), which is frequently reported after sunitinib treatment (90). Furthermore, CSF-1R is activated by the osteoclastogenic factor CSF, which is produced by tumor cells, thereby stimulating osteoclasts and increasing osteoclastic activity of osteoclasts (77). As a result, targeting CSF-1R may inhibit bone metastases. The clinical value of inhibiting CSF-1R has not yet been elucidated.
5.4 Preclinical activity

In preclinical studies, sunitinib has shown growth inhibition and tumor regression in various human cell lines and human xenografts models including RCC (72;80). In vitro studies in cell lines of clear cell RCC have demonstrated that pharmacologically relevant concentrations of sunitinib (~ 0.1 μmol/L) inhibited the phosphorylation of VEGFR and PDGFR-β (71;80) and blocked endothelial cell proliferation and invasion, but did not affect the viability of RCC cell lines in vitro (80). Studies in RCC xenografts have shown that sunitinib could also inhibit tumor angiogenesis in vivo, but may not affect proliferation or apoptosis of RCC cells. The results of a previous study indicated that sunitinib inhibits RCC growth primarily through an anti-angiogenic mechanism (80). In contrast to these results, Xin et al (82) have reported that sunitinib induces apoptosis in clear cell RCC cells through inhibition of signal transducer and activator of transcription 3 (Stat3). In that study, however, IC50 values required to inhibit RCC cell viability and proliferation in vitro were in the range of 5 μmol/L and above, which are much higher than the clinically relevant plasma concentrations of sunitinib, which are in the range of 50 to 100 ng/mL or 0.1 to 0.2 μmol/L (72;90).

5.5 Human studies

5.5.1 Dosing and schedule

Although initial phase I studies were planned to administer sunitinib continuously, a resting period was incorporated to allow patients to recover from potential severe bone marrow and adrenal toxicity that was observed in animal studies (71). Therefore, sunitinib was administered in a 4 weeks-on/2 weeks-off schedule in phase I studies (83;90). At doses of 75 mg daily or above, dose-limiting toxicities of fatigue, hypertension and bullous skin toxicity were encountered (83;90). As a result, the recommended phase II dose was set at 50 mg daily on a 4/2 schedule. Dosing of sunitinib is not based on body surface area (BSA), since a phase I study showed that BSA-normalized dosing would not improve the variability of sunitinib exposure (90).

5.5.2 Pharmacokinetics

After oral administration, sunitinib is mainly metabolized by the liver and eliminated by biliary excretion into the feces (91). Metabolism occurs primarily by the cytochrome P450 (CYP) 3A4 isoenzyme in hepatic microsomes (92). Sunitinib undergoes two N-de-ethylation steps (93). First, CYP3A4 N-de-ethylates sunitinib to the active metabolite SU12662. This metabolite appears to be equipotent to the parent compound in biochemical and cellular assays for inhibition of VEGFR, PDGFR and c-KIT (94). The active metabolite SU12662 is further metabolized by CYP3A4 to the inactive metabolite
SU14335 (93). This N-de-ethylation step occurs at a slower rate than the first step. As pharmacokinetic studies showed that inhibitors and inducers of CYP3A4 significantly affect the metabolism of sunitinib (92), it is recommended to avoid these drugs in patients treated with sunitinib. Consumption of grapefruit juice, which is a potent intestinal cytochrome CYP3A4 inhibitor, however, resulted in a marginal increase in sunitinib exposure (95). In addition, it was demonstrated that food did not affect the bioavailability of a single dose sunitinib of 50 mg in healthy volunteers (96). In a phase I study, the peak plasma concentration of sunitinib was reached at five hours after oral administration of 50 mg sunitinib (90). The steady state concentrations of sunitinib and its active metabolite SU12662 were achieved within 10 to 14 days (97). In a pharmacokinetic meta-analysis, the terminal half-lives of sunitinib and its active metabolite SU12662 were estimated at 69 and 80 hours, respectively (98). Daily administration of sunitinib led to accumulation of the drug. On day 28, the accumulation of sunitinib and SU12662 were respectively 3.0-5.5 and 7-15 times higher than on day 1 (90). Daily dosing of sunitinib 50 mg resulted in sufficient plasma concentrations above 50 ng/mL, as required for adequate inhibition of VEGFR-2 and PDGFR-β (72).

5.5.3 Pharmacodynamics

As mentioned in paragraph 4.3.2, sunitinib has proven efficacy with an acceptable toxicity profile in mRCC patients. In the main study (35), a randomized phase III trial comparing sunitinib with interferon-α in first-line treatment, sunitinib showed a response rate of 47% as well as an improved PFS (11 versus 5 months) and OS (26.4 versus 21.8 months) as compared with interferon-α (36). Since then sunitinib is the first-line treatment of choice for mRCC patients. Although most patients will benefit from sunitinib, a number will demonstrate progressive disease (PD). In addition, PD will occur in patients at a certain stage of sunitinib treatment. Some patients have difficulties to tolerate the drug due to a series of toxicities occurring simultaneously. At present, several other targeted drugs are available and may be more suitable for specific patients. As a result, sunitinib treatment in mRCC patients can be challenging for patients as well as their treating physicians. Therefore, a better understanding of the efficacy and toxicity of sunitinib is necessary to improve the management of mRCC patients. To this end, specific tools are required to predict the efficacy and toxicity of sunitinib in individual patients with mRCC. Then, individual mRCC patients can be appropriately selected for treatment with sunitinib.
6.0 Outline of the thesis

The current chapter gives a summary of the literature on the approved targeted drugs for treatment of mRCC, thereby focusing on sunitinib. In this thesis, a number of clinical issues concerning sunitinib treatment in mRCC patients is investigated.

Part I of the thesis is related to the efficacy of sunitinib in patients with mRCC. In chapter 2, the efficacy of sunitinib is evaluated in patients with a primary RCC tumor in a compassionate use programme. In chapter 3, the surgical implications of sunitinib for patients with primary metastatic disease are studied. In chapter 4, the efficacy of sunitinib in patients with newly diagnosed brain metastases is reported. The role of genetic polymorphisms for prediction of PFS in mRCC patients treated with sunitinib is explored in chapter 5.

Part II includes chapters in which the side-effects of sunitinib treatment are described. In chapter 6, factors that may predict severe toxicity in unselected patients with advanced RCC are investigated. In chapter 7, it is discussed whether severe cognitive disorders can develop after sunitinib treatment in elderly patients and whether these mental changes may be associated with preexisting arteriosclerotic leukoencephalopathy. In chapter 8, a multicenter study, that examines whether genetic polymorphisms are associated with sunitinib-induced toxicity, is described.

Part III reports clinical studies on the pharmacodynamic effects of sunitinib treatment in mRCC patients. In chapter 9, it is evaluated whether computed tomography (CT) based criteria, previously developed by Choi et al (99), are of additional value for early prediction of clinical outcome in mRCC patients treated with sunitinib. In chapter 10, the relation between the dosing schedule of sunitinib and the changes in hemoglobin levels during sunitinib treatment is explored. In chapters 11 and 12, the effects of sunitinib on respectively endothelial progenitor cells and circulating proteins are investigated. In chapter 13, the association between the sunitinib-induced rise in blood pressure and a change in microvascular density in the skin is examined. Finally, in Chapter 14 and 15, the results described in this thesis will be summarized and put into perspective.
References


Chapter 2

Sunitinib for treatment of advanced renal cell cancer: primary tumor response

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Sunitinib for Treatment of Advanced Renal Cell Cancer: Primary Tumor Response
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Abstract
Purpose: Nephrectomy before immunotherapy in patients with metastatic renal cell cancer (mRCC) will improve patient outcomes. In addition, the primary tumor is known to be refractory to cytokines. Sunitinib is now approved for treatment of advanced RCC, but its effect on the primary tumor has yet to be reported.

Experimental Design: All patients treated with sunitinib for advanced RCC without prior nephrectomy were reviewed and sequential computed tomography scans were evaluated for response in the primary tumor as well as metastases according to Response Evaluation Criteria in Solid Tumors. Volumes of primary tumors and central necrotic areas were measured with the perimeter method.

Results: Computed tomography scans were available for evaluation of response in 17 of 22 patients with a primary tumor in situ (1 patient with two primaries). According to Response Evaluation Criteria in Solid Tumors, 4 patients had a partial response, 12 had stable disease, and 1 had progressive disease. The one-dimensional longest diameter of the primary tumor correlated with the volumetric measurements both at baseline and at the time of evaluation of response. Excluding the patient with progressive disease, the median volume reduction was 31% associated with a median increase in the volume of necrosis of 39%. Three patients underwent nephrectomy and tumors showed extensive necrotic areas next to small fields of vital tumor cells.

Conclusions: Sunitinib can induce a significant reduction in volume of primary renal cell tumors. Further trials need to address the role of nephrectomy in advanced RCC patients on sunitinib treatment.

Until recently, cytokine therapy was the only treatment for metastatic renal cell cancer (mRCC), but less than 20% of patients benefit from a response (1). Responses of the primary tumor to immunotherapy are very rare (2–4). For that reason, patients with synchronous metastases and a resectable primary tumor to undergo a nephrectomy followed by IFN-α versus patients on IFN-α alone (5–7). An additional feature in favor of nephrectomy is the rare phenomenon of spontaneous regression of metastases (8). It has also been argued that nephrectomy may improve the response to immunotherapy by reduction of immunosuppressive factors produced by the primary tumor.

There are several subtypes of renal cell cancer (RCC), of which ~75% contain clear cell carcinoma histology. At least 60% of these tumors is associated with inactivation of the von Hippel-Lindau tumor suppressor gene. This leads to elevated protein levels of hypoxia-inducible factor a and consequent overexpression of vascular endothelial growth factor and plasmin-derived growth factor (9). These growth factors promote tumor angiogenesis, which likely contributes to the hypervascularity of RCC. Inhibitors of angiongenesis have proven efficacy in RCC, and among them is sunitinib (33911248 or Sutent; Pfizer Pharmaceuticals Group). Sunitinib is an oral tyrosine kinase inhibitor of the vascular endothelial growth factor receptors, the platelet-derived growth factor receptors, Flt-3, and c-Kit, and has been approved for the treatment of advanced RCC. In a randomized phase III clinical trial, sunitinib has shown an objective response rate of 31%, which was significantly higher than the 6% in patients that received IFN-α (10). The progression-free survival of the sunitinib treatment group was 11 months, whereas that of the IFN-α group was only 5 months (10). Based on current data available, sunitinib is now the preferred drug for frontline treatment of most mRCC patients (1). Presently, there is little experience with the effects of sunitinib on the primary tumor in patients that present with mRCC. Therefore, we conducted a retrospective analysis of the
drug-induced response in the primary tumor and also addressed the radiologic changes in contrast enhancement associated with the increase in central necrosis.

Patients and Methods

Patients and treatment. Medical records were reviewed of patients with advanced RCC without prior nephrectomy. A 0.05 was considered significant. A is the number of Vf(%) is expressed in cubic centimeters. (%)

Drug-induced response in the primary tumor and also addressed the radiologic changes in contrast enhancement associated with the increase in central necrosis.

Table 1. Antitumor effects of sunitinib in patients with advanced RCC without prior nephrectomy

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<td>1021 -52</td>
<td>228</td>
<td>-18 -54</td>
<td>12+ 13+</td>
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<tr>
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<td>5 11 50 18 SD</td>
<td>57 8 16</td>
<td>183 183 183 14 1 4</td>
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</table>

Abbreviations. RECIST primary, RECIST applied for primary tumor only; RECIST metastases, RECIST applied for metastases only; RECIST overall, overall RECIST applied for both primary tumor and metastases; PR, partial response; SD, stable disease; PD, progressive disease; Volume tumor, total volume of primary tumor; Volume necrosis, volume necrosis part of the primary tumor; Volume solid, volume solid part of the primary tumor; N (%), change in percentage; PFS, progression-free survival.

The hypodense nonenhancing part of the tumor is referred to as necrosis.

Metastases were present but not measurable.

Patient no. 6 had definite primary tumors in vivo.

Image analysis. Pretreatment phase CT scans of the abdomen were acquired (70 s after i.v. injection of a low-molecular contrast agent [Omipaque or Ultravist 300]. All were reoriented in 3-mm contiguous axial slices. Because volumetric measurements are considered as the reference standard for the assessment of the objective response, volumes of the primary tumors as visualized on CT scans were calculated using the perimeter method (12). Image viewing and manipulation were controlled with Centricity RA 600 version 6.1 software (GE Healthcare, Inc.), which allows the radiologist to draw perimeters around the contours of lesions. The software that automatically calculates the area enclosed by the periosteum and the mean density of this area in Hounsfield units (HU).

An independent radiologist (M.R.M.) blinded to the patients’ outcome examined the CT scan images. On each CT scan section in which the primary tumor was visible, the radiologist drew a line along the perimeter of the tumor to assess the area. The total volume (V) of the primary tumor was then calculated by summing the separate cross-sectional areas (A) multiplied by the section increment in millimeters (l), as follows V = \( \sum \text{A}_i \cdot \text{l} \), where \( \text{A}_i \) is the number of sections containing tumor and V is expressed in cubic centimeters.

Primary tumors of RCC are known for central heterogeneity that is associated with intranodal necrosis or hemorrhage on histologic examination (13, 14). Therefore, the volumetric measurements were corrected for the central hypodense nonenhancing part of the tumor. Nonenhancing area at a density <0 HU were arbitrarily defined as nonenhancing area.

Table 2. Radiation treatment data for RCC patients.

<table>
<thead>
<tr>
<th>Radiation Treatment</th>
<th>Dose (cGy)</th>
<th>Total Dose (cGy)</th>
<th>Treatment Time (min)</th>
<th>Fraction Time (min)</th>
<th>Cumulative Time (min)</th>
<th>Cumulative Dose (cGy)</th>
<th>Cumulative Dose (cGy)</th>
<th>Cumulative Dose (cGy)</th>
<th>Cumulative Dose (cGy)</th>
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<td>100</td>
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</tbody>
</table>

Abbreviations. RECIST primary, RECIST applied for primary tumor only; RECIST metastases, RECIST applied for metastases only; RECIST overall, overall RECIST applied for both primary tumor and metastases; PR, partial response; SD, stable disease; PD, progressive disease; Volume tumor, total volume of primary tumor; Volume necrosis, volume necrosis part of the primary tumor; Volume solid, volume solid part of the primary tumor; N (%), change in percentage; PFS, progression-free survival.
Results

Patients and treatment response. Eighty-two patients with advanced RCC had been treated with sunitinib in the expanded access program, and an additional 13 patients received the drug on doctor’s prescription following registration. Of these 95 patients, 22 patients had a primary tumor in situ. There were 17 males and 5 females, and the median age was 61 years (range, 48-77 years). 21 patients had clear cell carcinoma and 1 patient had non-clear cell carcinoma. According to the Memorial Sloan-Kettering Cancer Center criteria [based on the five risk factors: low Karnofsky performance status (<60%), high lactate dehydrogenase (>1.5 times the upper limit of normal), low serum hemoglobin, high corrected serum calcium (>10 mg/dL), and time from diagnosis to treatment <1 year; ref. 14], 16 patients were categorized into the intermediate-risk group and 6 patients into the poor-risk group. According to the prognostic criteria for vascular endothelial growth factor–targeted therapy by Choueiri et al. (ref. 15), based on the five risk factors: time from diagnosis to treatment <2 years, baseline platelet count >900 x 10^9/L, neutrophil count >5 x 10^9/L, corrected calcium >9.5 or >10 mg/dL, and initial Eastern Cooperative Oncology Group performance status ≤1, 1 patient had no adverse prognostic factor, 3 patients had 2, and 18 patients had >2 adverse prognostic factors. In 16 patients sunitinib was given as first-line treatment, and in 6 patients, as second-line treatment. A primary decision not to perform a nephrectomy was based on doctor’s prescription following registration. Of these 95 patients, 22 patients had a primary tumor in situ. There were 17 males and 5 females, and the median age was 61 years (range, 48-77 years). 21 patients had clear cell carcinoma and 1 patient had non-clear cell carcinoma. According to the Memorial Sloan-Kettering Cancer Center criteria [based on the five risk factors: low Karnofsky performance status (<60%), high lactate dehydrogenase (>1.5 times the upper limit of normal), low serum hemoglobin, high corrected serum calcium (>10 mg/dL), and time from diagnosis to treatment <1 year; ref. 14], 16 patients were categorized into the intermediate-risk group and 6 patients into the poor-risk group. According to the prognostic criteria for vascular endothelial growth factor–targeted therapy by Choueiri et al. (ref. 15), based on the five risk factors: time from diagnosis to treatment <2 years, baseline platelet count >900 x 10^9/L, neutrophil count >5 x 10^9/L, corrected calcium >9.5 or >10 mg/dL, and initial Eastern Cooperative Oncology Group performance status ≤1, 1 patient had no adverse prognostic factor, 3 patients had 2, and 18 patients had >2 adverse prognostic factors. In 16 patients sunitinib was given as first-line treatment, and in 6 patients, as second-line treatment.

Excluding the patient with progressive disease, RECIST calculations were analyzed for the primary tumor only, the metastases only, and the overall response, resulting in a median change of -12%, -28%, and -21%, respectively. Progression-free survival and survival of the individual patients are also depicted in Table 1.

Imaging. The median volume of the 18 primary tumors was 208 cm^3 at baseline. There was a high correlation between the volumetric measurements and the longest diameter of the primary tumors at baseline (Spearman’s ρ = 0.908, P <0.001; Fig. 1A) and at first evaluation after the start of sunitinib (Spearman’s ρ = 0.956, P <0.001; Fig. 1B). At baseline, large tumors seemed to have large necrotic areas; the necrotic volume correlated well with the tumor volume (Spearman’s ρ = 0.805, P = 0.01e-005). The patient with progressive disease was excluded from further analysis.

During sunitinib treatment, the median density of the solid part decreased from 32 HU (range, 54-130 HU) to 66 HU (range, 49-116 HU), P = 0.028, whereas that of the hypodense nonenhancing, necrotic part was not altered. The changes in

Fig. 1. Relation between volumetric measurements and the size of the longest diameter of the primary RCC at baseline (A) and at first evaluation after the start of sunitinib (B).

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volumetric measurements of the primary tumor after 1.2 to 3.9 months of sunitinib are listed in Table 1. The volume of the primary tumor decreased in 13 of 17 patients (Fig. 2). In patients with partial response or stable disease ($n = 16$), the volume of the primary tumor ($n = 17$) significantly decreased with a median reduction of 33% ($P = 0.001$). The decrease of the volume of the solid part was also significant (median, 54%; $P = 0.001$). In contrast, a significant increase in the volume of the central necrosis was measured (median, 93%; $P = 0.005$). Changes in the volume of the solid part were significantly correlated with changes in the total volume of the primary tumor (Spearman’s $\rho = 0.850$, $P = 1.52\times10^{-5}$) and changes in the necrotic volume (Spearman’s $\rho = 0.645$, $P = 0.005$).

Follow-up CT scans were available to evaluate the long-term effects of sunitinib in seven patients with an initial decrease in the primary tumor volume. Although six of these patients had a further decrease in the primary tumor volume during continuation of sunitinib, the longest response was observed within the first 4 months (Fig. 3A). During follow-up, the volume of the necrotic part remained more or less stable in most tumors (Fig. 3B), whereas the solid part decreased slightly further in six of eight tumors (Fig. 3C). Separate follow-up measurements of the longest diameter of the primary tumor and metastases during sunitinib treatment showed parallel decreases in size.

Nephrectomy after sunitinib treatment. Three patients (nos. 9, 10, and 12) with mRCC had their kidney removed during sunitinib treatment. In patient no. 9, the primary tumor volume decreased by 46%. After 3 months on sunitinib, nephrectomy was carried out after discontinuation of the drug for 3 weeks. Patient no. 10 had 38% reduction of the primary tumor and underwent surgery after 11 months. Sunitinib was stopped 1 week before the operation. Patient no. 12 had 30% tumor volume reduction, and after 4 months surgery was done after withdrawal of sunitinib for only 2 days.

Pathology revealed necrotic areas next to vital tumor cells (Fig. 4).

In the three patients there was no regrowth of disease in the treatment-free perioperative interval. No negative effects on hemostasis or wound healing were observed and the postoperative course was uneventful. After surgery, sunitinib was withheld in patient nos. 10 and 12 based on the excellent response in metastatic sites. Both patients presented with progressive disease 4 and 6 months after surgery, respectively. Patient no. 9 continued sunitinib after surgery until progressive disease, 6 months thereafter.

Discussion

This is the first analysis on the effects of sunitinib on the primary tumor in 17 patients with advanced RCC expressed both according to RECIST and in volumetric measurements. Partial response or stable disease was measured in 16 of these
patients. Thirteen patients had a volumetric reduction of the primary tumor of 18% to 64% associated with decrease of the longest diameter of 0% to 33%. The decrease in tumor volume was accompanied by an increase of central necrosis and a decrease of the vital solid part. In three patients with low metastatic tumor load, the primary tumor became surgically resectable. Apart from vital tumor cells, pathology showed extensive areas of necrosis. Our findings are of interest because outcome of advanced RCC patients without nephrectomy would have been poor in the cytokine era. Nowadays, when primary nephrectomy does not seem to be a valid option, patients may experience marked clinical benefit from sunitinib.

Primary tumors of RCC can grow to an enormous size as compared with their metastases, with longest diameters up to 20 cm (16). On sunitinib treatment, the longest diameter of the primary tumor shows a relatively smaller drop than that of the metastases (see Table 1). For this reason, large primary tumors will have an important effect on the overall objective response when these are included in RECIST measurements. Hence, the overall response may be underestimated because a relatively large reduction in primary tumor size is necessary to achieve a partial response as exemplified in patient nos. 12, 13, and 14. Therefore, it could be considered to exclude primary tumors in calculating the overall response according to RECIST. Volumetric measurements, although cumbersome, may be preferred because tumor growth or shrinkage can be asymmetrical in one or even three dimensions. Fortunately, in our study, volumetric changes of the primary tumor of RCC showed a high correlation with the calculations according to RECIST. This indicates that the changes in size of the primary tumors can be considered symmetrical and that RECIST measurements reflect the response to sunitinib. Note that according to one-dimensional (RECIST), two-dimensional (WHO definition), and volumetric measurements, the response criteria are different and that a partial response is reached on a decrease in tumor size of 30%, 50%, and 65%, respectively (11).

Similar to findings in primary RCC, extensive necrosis can also be induced by sunitinib in hepatocellular carcinoma, reflected by a decrease in contrast enhancement on follow-up CT scans (17). Other tyrosine kinase inhibitors, such as sorafenib in RCC and imatinib in gastrointestinal stromal cell tumors, are known to cause early and extensive necrosis as well (18, 19). Treatment-induced necrosis is not part of standard criteria to assess tumor response. Because the necrotic part of primary tumors of RCC is often heterogeneous, very irregular, and asymmetrical, measuring necrosis by the longest diameter is
difficult. Therefore, other methods to quantify necrosis are required. Here, we applied volumetric measurements and revealed an increase in necrosis in primaries. Another approach to include necrosis as part of tumor response has recently been described by Choi et al. (20, 21). In patients with gastrointestinal stromal tumors treated with imatinib, Choi response criteria (≥ 15% decrease in one-dimensional tumor size or ≥ 50% decrease in tumor HI) seemed to be more accurate than RECIST and correlated significantly with tumor-to-tumor progression and disease-specific survival (20, 21). Fatemi et al. (17) have also addressed the inadequacy of RECIST and used volumetric measurements for sunitinib-induced necrosis in hepatocellular carcinoma expressed as minor (≤50%) and major (>50%) posttreatment tumor necrosis. We show that RECIST criteria are well applicable to evaluate the response of metastases as well as of the primary RCC to sunitinib treatment. The increase in necrosis and the reduction of the solid part, however, might be a more consistent indicator for the extent of response. Noteworthy, increase of necrosis as well as tumor size would likely reflect progression of disease (patient no. 11). It remains to be investigated in mRCC treated with sunitinib whether other response criteria and functional imaging techniques will add to information already acquired by contrast-enhanced CT/US for measurements according to RECIST (22).

The pros and cons of cytoreductive nephrectomy in the presence of metastatic disease have long been a subject for debate (23–25). Historically, the nonresponsiveness of the primary tumor to tyrosine kinase therapy and the palliation of symptoms were arguments for a nephrectomy (23–24, 26) Increased perioperative and postoperative morbidity and mortality (27) in patients with incurable disease and a limited life expectancy were postulated as arguments against cytoreductive nephrectomy. Furthermore, surgery could lead to a potential delay or even cancellation of systemic therapy due to progression of metastatic disease postoperative morbidity, or decreased performance status (25). The present responses of the primary tumor to sunitinib seem encouraging and put the role of a nephrectomy in a different light as compared with the guidelines in the cytokine era. The observed response in the primary tumor and the alleviation of symptoms on sunitinib treatment seem to have made the historical arguments for a nephrectomy defective. In our patients, sunitinib also improved the performance status in those with a response. Successful resection of the primary tumor directly invading adjacent organs and structures in three patients suggests that sunitinib may be used to improve surgical resectability in doubtful cases. Whether this is beneficial for patient outcome cannot be answered on the basis of these three cases. Perspective randomized phase III clinical trials in patients presenting with mRCC are warranted to define which patients treated with sunitinib will benefit from nephrectomy, to analyze whether nephrectomy has an effect on survival, and to determine the optimal timing of a nephrectomy (28). Such trials will lead to new guidelines on the role of nephrectomy in mRCC patients in the sunitinib era.

Acknowledgments

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Neoadjuvant sunitinib for surgically complex advanced renal cell cancer of doubtful resectability: initial experience with downsizing to reconsider cytoreductive surgery

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Neoadjuvant sunitinib for surgically complex advanced renal cell cancer of doubtful resectability: initial experience with downsizing to reconsider cytoreductive surgery

Axel Bex · Astrid A. M. van der Veldt · Christian Blank · Alfons J. M. van den Eertwegh · Epic Boven · Simon Horenblas · John Haanen

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Abstract

Objective To evaluate neoadjuvant sunitinib in patients with synchronous metastatic renal cell cancer (mRCC) to downsize surgically complex tumours and reconsider cytoreductive surgery.

Patients and methods Retrospective analysis of ten consecutive mRCC patients treated with sunitinib in an expanded access program who presented with surgically complex primary tumours or bulky locoregional metastases. Surgery-limiting tumour sites (SLTSs) were defined as primary or retroperitoneal lesions with direct invasion of adjacent organs or encasement of vital structures on imaging. Patients received sunitinib 50 mg/day for 4 weeks on and 2 weeks off to be tailed by cytoreductive surgery after downsizing and individual reassessment. Response was measured according to Response Evaluation Criteria in Solid Tumours (RECIST).

Results Six out of ten SLTSs revealed a reduction of tumour size with a median of 14% according to RECIST. None of the ten SLTSs had a partial response (PR), whilst at distant metastatic sites one complete remission and two PRs occurred. Downsizing of SLTSs appeared most prominent in the first 2–4 months, which resulted in reconsidering cytoreductive nephrectomy in three patients. These three tumours invaded the liver on imaging and were reduced by 11, 18 and 20%.

Conclusions In this patient group with mRCC and surgically complex primary tumours or locoregional metastases, downsizing of SLTSs by neoadjuvant sunitinib was limited. Cytoreductive surgery was reconsidered in three patients. Given the overall reduction in tumour burden by sunitinib alone, further investigation to define the role of cytoreductive surgery is warranted.

Keywords Renal cell cancer · Primary tumour · Sunitinib · Nephrectomy

Introduction

Oral tyrosine kinase inhibitors targeting vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) receptors have altered the management of metastatic renal cell carcinoma (mRCC) [1]. Compared with cytokine therapy, sunitinib at a dose of 50 mg daily for 4 weeks on and 2 weeks off induces a high partial response (PR) rate of up to 40% at metastatic sites [2]. We have recently demonstrated that the same regimen of sunitinib leads to responses in the primary tumour [3] at a rate previously unknown for cytokine treatment [4]. Recently four cases have been described in which neoadjuvant-targeted therapy altered the surgical management in advanced RCC [5]. These and our results support to reevaluate the concept of neoadjuvant therapy to achieve downsizing of the
tumour that may facilitate debulking surgery of residual masses. In particular, metastatic or orthotopic sunitinib may change the surgical management of mRCC patients with bulky surgically complex primary tumours or locoregional metastases and an otherwise good or intermediate prognosis with a low distant metastatic load. Historically, cytokines had little effect on the primary tumour and cytoreductive surgery was often not an option in these particular patients. Within the frame of a sunitinib expanded access program, we offered upfront sunitinib to patients with mRCC to downsize surgically complex primary tumours and/or bulky locoregional metastases followed by cytoreductive surgery if feasible. We reviewed patient characteristics and our experience with this approach.

Patients and methods

Patient population

Medical records of patients treated with sunitinib for advanced RCC from December 2005 to November 2007 were reviewed. Most of the patients (n = 52) had been included in an expanded access program until September 2006 after which sunitinib was available through an extended registration protocol (n = 13). In the expanded access program, each participant signed an institutional review board-approved protocol-specific informed consent in accordance with national and institutional guidelines. Of 67 patients treated, 10 patients with primary metastatic RCC received sunitinib without prior nephrectomy due to surgically complex primary tumours or bulky locoregional metastases with the aim to downsize the tumour and allow cytoreductive surgery. Memorial Sloan-Kettering Cancer Center (MSKCC) criteria were evaluated based on the five risk factors: low Karnofsky performance status (<80), high LDH (≥1.5 times the upper limit of normal), low serum haemoglobin, high corrected serum calcium (≥10 mg/dL), and time from initial diagnosis to sunitinib treatment of less than 1 year [6].

Surgery-limiting tumour sites (SLT5s)

Surgically complex disease was defined as a primary tumour or retroperitoneal locoregional metastases for which removal was deemed technically not feasible or potentially associated with morbidity outweighing the benefit. This decision was exclusively based on imaging with computed tomography (CT) scans supported by magnetic resonance imaging (MRI) or ultrasound if required. This analysis does not contain patients in whom cytoreduction had been aborted because the lesions proved unresectable during explorative surgery. The lesions were summarised under the term SLT5s. The following imaging criteria applied: (a) direct and extensive invasion of the primary tumour to adjacent tissues (n = 7 with 4 liver and 1 soft tissue invasion) (Fig. 1a), (b) extensive retroperitoneal metastatic burden (n = 4 with bulky encasement of the caudal vein, aorta, coeliac axis or superior mesenteric artery) (Fig. 2a); and (c) mRCC with a primary tumour in a solitary kidney inaccessible to nephron-sparing surgery or other ablative procedures (n = 1). The decision to perform cytoreductive surgery after treatment with sunitinib was taken if Response Evaluation Criteria in Solid Tumours (RECIST) measurement showed a reduction of tumour size and imaging suggested a technically feasible resection.

Treatment and efficacy

Sunitinib was administered orally at a dose of 50 mg daily, consisting of 4 weeks on treatment followed by a 2-week rest period in cycles of 6 weeks. A dose reduction of sunitinib (to 37.5 mg and then to 25 mg) was allowed depending on the type and severity of adverse events. If patients had symptoms of progressive disease (PD) during the rest period, there was a possibility of continuous dosing of sunitinib at 37.5 mg/day.

At baseline, tumour burden was assessed according to RECIST by one-dimensional measurement of the largest diameter of the primary tumour and the sum of diameters of target lesions. Bone lesions were considered truly
non-measurable. To determine the overall metastatic load in relation to the primary tumour size, the sum of all measurable metastatic lesions was recorded additionally. Clinically, tumour response was assessed by CT scans every two to three cycles of treatment according to RECIST. To evaluate how a potential difference in response between primary tumour size and metastases may affect overall RECIST measurements, these lesions were calculated separately and together (overall). In addition, separate RECIST measurements were performed for the SLTSs to evaluate the effect of sunitinib on these unresectable lesions over time.

The progression-free survival (PFS) was the time between the first day of sunitinib and the date of PD on the CT scan or clear clinical evidence of PD. Overall survival (OS) was the time between the first day of treatment and the date of death or the date at which patients were last known to be alive. For PFS and survival analysis, data collection was closed on 1 November 2008. PFS and OS were calculated with the Kaplan-Meier method. PFS and OS of the ten patients treated with the primary in situ were compared to the PFS and OS of mRCC patients with nephrectomy prior to sunitinib treated at our institution (n = 54). Patients treated with sunitinib for extensive distant metastasis but a small resectable primary which was not removed because nephrectomy would have no substantial impact on the tumour burden were excluded from the analysis (n = 3).

Results

Patients and treatment

Patient characteristics are summarised in Table 1. Of ten patients the SLTSs were primary tumours in six patients and bulky locoregional metastases in four. All patients had histologically proven RCC of clear cell subtype. MSKCC criteria disclosed an intermediate risk for all but one patient with a poor prognosis. Four patients reduced sunitinib dosing: patient nos. 3 and 5 reduced to a continuous dose of 37.5 mg/day due to symptomatic progression in a 2-week rest period preceding the second cycle; patient nos. 8 and 9 reduced to 37.5 mg/day for 4 weeks on and 2 weeks off after, respectively, 10 months and 6 weeks due to grade III hand-foot syndrome and other grade III adverse events. Adverse events are summarised in Table 2.

Efficacy

According to RECIST, two out of ten patients reached an overall PR, six had stable disease (SD) and one had PD (Table 3). One patient could not be evaluated for response because of early termination due to PD. Excluding this patient, RECIST calculations were analysed for the primary tumour only, the metastases only and the overall response, resulting in a median reduction of, respectively, 10% (range −20 to +11%), 22% (range −100 to +50%) and 14% (range −32 to +18%). Between 1.2 and 3.9 months, the median reduction of the SLTSs was 11% (range −20 to +46%). Six out of nine patients had a reduction of the SLTS (median −14%, range −20 to −9%) (Figs. 1, 2, 3). None of the reduction in SLTSs was qualified as PR. Downstaging of the SLTSs appeared to be most prominent in the first 2–4 months. Median PFS was 6 months (range 0.2–15 months) and the median OS was 15 months (range 2–22 months). The PFS and survival of the individual patients are shown in Table 3. In addition, the median PFS for patients with a previous nephrectomy (n = 54) was 9 months (range 1–30 months, P = 0.031 (Fig. 4). Currently, the median OS has not reached patients with previous nephrectomy (range 0.5–32 months).

Cytoreductive nephrectomy

In three patients, cytoreductive nephrectomy was performed following downsizing by sunitinib and response at metastatic sites. Patient no. 3 had been diagnosed with lung, bone and subcutaneous metastases of RCC. Surgery of the primary
tumour was expected to be complex because of liver invasion on imaging. The patient experienced a PR on sunitinib treatment with a 56% decrease of the metastases. The primary tumour decreased by 18% on one-dimensional measurements according to RECIST which was accompanied by an increase in necrosis. During sunitinib, the performance status improved from 2 to 0. Sunitinib was discontinued 3 weeks before surgery. Nephrectomy was feasible although the tumour invaded the liver capsule. Pathology revealed vital tumour cells and large areas with necrosis, but no direct invasion into the liver parenchyma. Sunitinib was restarted, but 6 months thereafter patient had PD.

Patient no. 4 was diagnosed with lung, liver and lymph node metastases of RCC. Cyoreductive nephrectomy was considered complex because of liver invasion (Fig. 1). Initially, interferon alpha was started which was discontinued after 2 months because of a mixed response. Sunitinib was initiated which induced an overall PR (gPR). Encasement of the metastases was reduced on imaging with a 56% decrease of the metastases. The primary tumour was exised to be cII. Liver invasion was reduced on imaging. During sunitinib the performance status improved from 2 to 1. After 10 months the patient underwent nephrectomy and partial liver resection after 1 week off sunitinib. Pathology revealed a large necrotic tumour with remnants of direct liver and inferior

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**Table 1.** Patient’s characteristics at baseline

<table>
<thead>
<tr>
<th>Pt no.</th>
<th>Age</th>
<th>Gender</th>
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<td>Primary</td>
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<td>M</td>
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<td>10</td>
<td>BLRM</td>
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<td>12</td>
<td>BLRM</td>
<td>6</td>
<td>Encasement blood vessels</td>
</tr>
</tbody>
</table>

2: intermediate, 3: poor. PS Eastern Cooperative Oncology Group (ECOG) performance status. SLTs surgery-limiting tumour site. BLRM macro metastatic metastases
* Risk groups according to Memorial Sloan-Kettering Cancer Center (MSKCC) criteria associated with shorter survival (based on five risk factors: high Karnofsky performance status (>90%), high LDH (>1.5 times the upper limit of normal), low serum haemoglobin, high corrected serum calcium (>10 mg/dL), and time from initial diagnosis to treatment of less than 1 year)[12]

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**Table 2.** Adverse events during sunitinib treatment

<table>
<thead>
<tr>
<th>Pt no.</th>
<th>Adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Thrombocytopenia gr II, lymphocytopenia gr II, anemia gr III, fatigue gr I, stomatitis gr I, nausea gr II, vomiting gr II, hand-foot syndrome gr I</td>
</tr>
<tr>
<td>2</td>
<td>Anemia gr I, anemia gr II, nausea gr II, vomiting gr II, hand-foot syndrome gr I</td>
</tr>
<tr>
<td>3</td>
<td>Stomatitis gr II, nausea gr I, vomiting gr I, diarrhoea gr I, hand-foot syndrome gr IV, dislocation hand gr I</td>
</tr>
<tr>
<td>4</td>
<td>Taste alteration gr I, nausea gr I, vomiting gr I, diarrhoea gr I, oedema gr I</td>
</tr>
<tr>
<td>5</td>
<td>Nausea gr I, vomiting gr I, hand-foot syndrome gr I</td>
</tr>
<tr>
<td>6</td>
<td>Thrombocytopenia gr I, lymphocytopenia gr I, fatigue gr II, anemia gr II, nausea gr I, vomiting gr I, proteinuria gr I</td>
</tr>
<tr>
<td>7</td>
<td>Thrombocytopenia gr II, taste alteration gr I, stomatitis gr II, hypertension gr I</td>
</tr>
<tr>
<td>8</td>
<td>Hand-foot syndrome gr III</td>
</tr>
<tr>
<td>9</td>
<td>Thrombocytopenia gr II, stomatitis gr III, nausea gr III, vomiting gr III, diarrhoea gr III, hand-foot syndrome gr III</td>
</tr>
<tr>
<td>10</td>
<td>Fatigue gr I</td>
</tr>
</tbody>
</table>

* Pt no. 8 had a dose reduction because of grade III hand-foot syndrome

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stable disease

RECIST performed for metastases only.

D performance status.

RECIST performed for metastases only. RECIST overall survival.

RCC. The primary tumour invaded the liver on imaging. Sunitinib as first-line treatment resulted in complete response of the metastases and a decrease of the primary tumour of 20% with an increase of the necrotic area. The performance status improved from 2 to 1. The patient underwent a successful nephrectomy after sunitinib was discontinued 2 days before surgery. The tumour invaded the liver capsule, but not the parenchyma, which was confirmed histologically. Based on the excellent response at the metastatic sites, sunitinib was withheld. Follow-up CT scans, 6 months after surgery, demonstrated PD of the small lung lesions.

<table>
<thead>
<tr>
<th>No.</th>
<th>RECIST SLTS (%)</th>
<th>Primary (%)</th>
<th>Metastases (%)</th>
<th>Overall (%)</th>
<th>Overall</th>
<th>PFS (months)</th>
<th>Survival (months)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>9</td>
<td>4</td>
<td>1</td>
<td>SD</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>1</td>
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</tr>
<tr>
<td>3</td>
<td>-18</td>
<td>-18</td>
<td>-96</td>
<td>-32</td>
<td>PR</td>
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<td>15</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>11</td>
<td>48</td>
<td>32</td>
<td>PR</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>+46</td>
<td>+40</td>
<td>+77</td>
<td>+41</td>
<td>PD</td>
<td>0.2</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>-20</td>
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<td>-100</td>
<td>-20</td>
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</tr>
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<td>+50</td>
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<td>SD</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>+13</td>
<td>1</td>
<td>+11</td>
<td>+46</td>
<td>SD</td>
<td>4</td>
<td>15</td>
</tr>
</tbody>
</table>

Fig. 3 Change in the longest diameter of SLTS during sunitinib treatment. Patient nos. 3, 4 and 6 underwent cytoreductive surgery (solid triangle).

caval vein invasion. Four months after surgery, patient had PD and sunitinib was resumed.

Patient no. 6 was diagnosed with metastases to the lungs, mediastinal and locoregional lymph node metastases of RCC.

Discussion

This retrospective analysis suggests that downsizing SLTSs of RCC by neoadjuvant sunitinib may be of limited benefit for the surgical management. Although in none of the ten SLTSs a PR was reached, cytoreductive surgery was reconsidered in three after a reduction of the longest diameter of SLTS.
11–20%. It cannot be concluded, however, that the successful resection was technically facilitated by sunitinib pre-treatment in those patients. The decision to treat patients with surgically complex primary mRCC was based on imaging and not on resectability at explorative surgery. Despite downsizing, histology suggests that local downsizing does not occur. It appears that the effect of downsizing is most prominent in the first 2–4 months (Fig. 3). Remarkably, in none of the four patients with retroperitoneal encasement of major blood vessels, such as the caval vein, aorta, coeliac axis and superior mesenteric artery, did this approach led to reconsidering resection. Although reduction in size was observed, it was too limited to safely attempt surgery. Sunitinib was given at the standard dose of 50 mg/day, though it was reduced to 37.5 mg in four patients. It was noticed recently that by starting the dose at 37.5 mg/day, a similar time to progression could be achieved at the cost of half of the response rate [7]. Whether the increase of the dose of sunitinib over 50 mg for a short upfront period will lead to a higher rate of PR still needs to be investigated. In one patient, sunitinib was discontinued 48 h before surgery, suggesting that even a short-time interval may be sufficient to safely perform surgery. The half-life of sunitinib is relatively long and is approximately 40 h, whereas that of the active metabolite S313262 is approximately 80 h [8]. It is known that after discontinuing sunitinib, some patients, especially those with an initial tumour response, may experience a rapid clinical deterioration. This rebound phenomenon may be due to early regrowth of the tumour vascularisation [9] or to tumoral oedema [10]. Maintaining an interval as short as possible between withdrawal of sunitinib and surgery may be of importance in view of the observed rebound phenomenon with accelerated tumour growth in the rest period.

Within the community of urological surgeons, there is no uniformly accepted definition of unresectable tumour lesions. A tumour that may be deemed unresectable in one centre may be judged accessible in another. This reflects different levels of expertise and often multidisciplinary approaches are required. To account for this lack of definition, we chose the term of complex surgery. Some common criteria to define surgically complex tumours hold for the majority of centres performing cytoreductive surgery [11, 12]. These are direct extensive invasion of the liver in right-sided renal masses or involvement of the coeliac axis or superior mesenteric artery in the case of left-sided renal masses or bulky retroperitoneal lymph node metastases [13, 14]. If not revealed at explorative surgery, these criteria are usually based on clinical imaging. For the majority of urological surgeons, they represent hallmarks of unresectability or resectability leading to excessive morbidity in the setting of mRCC. Generally, the acceptance of morbidity associated with surgery is closely related to the overall outcome of the disease. As an example, in testicular cancer, complete post-chemotherapy excision of bulky retroperitoneal metastases including complex surgery with resection of the caval vein or aorta often results in definite cure [15, 16]. In patients with mRCC and synchronous distant metastases, however, the morbidity of such extensive surgery, even if technically feasible, appears imbalanced against the limited survival benefit that may be achieved [12, 17–20]. Assessment of resectability may be further biased by the fact that invasion on imaging does not always reflect invasion at pathological examination [21]. We are aware that in our series, the decision whether or not a lesion was resectable was based on these clinical criteria rather than explorative surgery. This may bias the interpretation of true unresectability, although the decision at explorative surgery may in turn be biased by a difference in surgical skills. However, this reflects current clinical practice. It is important to note that the conclusion of limited downsizing following neoadjuvant therapy remains unaffected by the definition of resectability.

There is an ongoing discussion whether the current response assessment by RECIST is sufficient to decide whether treatment with targeted therapy is beneficial [22]. From a surgical endpoint, however, reduction of the longest diameter on imaging is relevant for the assessment of downsizing and ultimately for the resectability of a primary tumour or large metastatic lesion. In this analysis, the reduction in tumour size was more profound in the metastatic lesions than in the primary tumour. Whilst two PRs and one CR according to RECIST were observed at metastatic sites, none of the primary tumour sites or bulky retroperitoneal lesions qualified as PR.

There have been very recent observations that neoadjuvant-targeted therapy may reduce the extent of surgery in advanced RCC cases with thrombosis in the caval vein [23], nodal involvement, renal fossa recurrence and tumour within a solitary kidney [24]. In line with these positively selected cases, this analysis of a consecutive series of patients suggests that some may benefit from neoadjuvant therapy to reconsider surgery. However, it remains unknown whether this approach will alter the outcome for patients with distant metastases. Neoadjuvant sunitinib may therefore have a limited future role in selected patients with advanced disease of doubtful resectability in whom a subsequent surgically complete resection might be achieved. Ultimately, only few patients presenting with mRCC will have primary tumours or locoregional metastases of doubtful resectability. In a series reported by Kassoul et al. [13] in 498 patients who underwent cytoreductive surgery for primaries in the case of mRCC, only 23 patients were identified with cT4N1M1 disease. The median survival of these 23 patients was poor, being 7.1 months for those receiving
additional systemic therapy, but only 2.5 months for those who had not. Although these data have been collected retrospectively in the pre-sunitinib era, surgery seemed only justifiable in those few with symptomatic tumours.

Therefore, for patients presenting with primary mRCC, the fundamental question remains if survival will be improved by removing a partially responding asymptomatic primary tumour in the face of partially and temporarily responding systemic disease [24]. In fact, high-level evidence from randomised trials only supports cytoreductive nephrectomy in conjunction with interferon alpha [25, 26]. Neoadjuvant sunitinib may have impact on surgical management. However, it is premature to conclude that cytoreductive surgery is required either prior to or after sunitinib in these patients. Randomised studies investigating cytoreductive surgery and its sequence in combination with sunitinib or other targeted therapies are needed.

Conflict of interest statement There is no conflict of interest.

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Chapter 3B

Progression of a caval vein thrombus in two patients with primary renal cell carcinoma on pretreatment with sunitinib

Axel Bex, Astrid A.M. van der Veldt, Christian Blank, Martijn R. Meijerink, Epie Boven, John B.A.G. Haanen

LETTER TO THE EDITOR

Progression of a caval vein thrombus in two patients with primary renal cell carcinoma on pretreatment with sunitinib

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To the Editor

Oral tyrosine kinase inhibitors (TKIs) and the monoclonal antibody bevacizumab have significantly changed the management and perspectives of patients with metastatic renal cell cancer (mRCC). Especially patients with a clear cell histological subtype benefit from therapies targeted against signalling of the vascular endothelial growth factor (VEGF) and platelet-derived growth factor. Sunitinib has been registered as first- and second-line therapy for mRCC. This TKI can induce high partial response (PR) rates of up to 40% at metastatic sites and can prolong progression-free survival (PFS) and overall survival (OS) as compared to interferon-alpha (11 and 26 months versus 5 and 22 months, respectively) [1]. In primary tumours, sunitinib-induced responses as well as responses induced by the VEGF-directed monoclonal antibody bevacizumab have been described at a rate previously unknown for cytokine-based treatment [2–4]. Therefore, presurgical targeted therapy might facilitate nephrectomy by downsizing primary tumours. In addition, responses have been reported in bulky retroperitoneal lymph nodes and even in tumour thrombi with caval vein extension, which may improve the surgical management of these tumour sites [5–11].

In contrast to these promising reports, we describe two patients with mRCC of a clear cell subtype who developed a progressive caval vein thrombus during sunitinib, which resulted in a negative impact on surgical management.

Case reports

Two patients were treated in accordance with a phase II trial in which sunitinib-induced responses in primary tumours were investigated. The main inclusion criteria of this trial were histologically confirmed metastatic mRCC with a clear cell subtype and a resectable asymptomatic primary tumour. Extensive metastatic disease defined as non-resectable metastases in case of one metastatic site or two metastatic sites, a World Health Organization (WHO) performance status of 0 or 1, an intermediate risk profile according to Memorial Sloan-Kettering Cancer Center (MSKCC) criteria, and no prior systemic treatment with biological response modifiers, TKIs, monoclonal antibodies or chemotherapy. Patients were treated with sunitinib 50 mg/day for four weeks on and two weeks off for two cycles. At completion of the second treatment cycle, a computed tomography (CT) scan was performed for response evaluation and patients were admitted for surgery within one week.

Case 1

A 49-year-old male patient presented with a primary RCC tumour in the right kidney with thrombus extension into the infrarenal caval vein up to the liver. The patient had metastatic disease in lungs and para-aortic lymph nodes. Metastatic burden was limited and the sum of the longest diameter of all
metastatic lesions was only 2.8 cm. Resection of the primary and thrombus was feasible, but it was decided to include the patient in the protocol and take advantage of potential downsizing of the thrombus. The patient started sunitinib 50 mg/day (four weeks on and two weeks off) as first-line treatment. During the first week of the second cycle the patient was admitted with fatigue grade 3, dyspnoea and ascites. The CT scan revealed extension of the thrombus into the right atrium with liver congestion. The size of the primary tumour and metastases, however, had remained stable (Figure 1). The performance score deteriorated rapidly from WHO 0 to 3 and nephrectomy could not be performed anymore. The patient died two months after the start of sunitinib due to thrombus-induced backward and liver failure.

**Case 2**

A 55-year-old male patient was diagnosed with resectable primary RCC in the left kidney, one liver metastasis and bone metastases. After pain-attenuating radiotherapy for metastases in spine and hip, the patient initiated first-line sunitinib treatment at 50 mg/day (four weeks on and two weeks off). At response evaluation in the last week of the second cycle, the liver metastasis showed a minor reduction of 19% in size, while the primary tumour had not changed. In addition, the CT scan revealed a newly developed thrombus in the caval vein (Figure 2). Nephrectomy was performed in a trans-abdominal approach requiring extension of the surgical field to safely perform a cavotomy. The adjacent tissues to

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**Figures**

1. CT scans of the first patient at baseline (A) and after (B) one cycle of sunitinib A1 and B1: The primary tumour has decreased in size. A2 and B2: The caval venous thrombus has increased in size (A2) and extends from orthotopic (A3) towards the right atrium (B3). Note the absence of the thrombus in the right atrium before treatment (A3).

2. CT scan of the primary tumour of the second patient at baseline (A) and after two cycles of sunitinib demonstrating a newly formed thrombus in the caval vein (arrows) (B).
the renal vein were adhesive due to a reaction of the thrombotic material. Histopathological examination demonstrated a primary tumour of 1.0 cm diameter with clear cell histology and a necrotic centre. The thrombus contained tumour cells as well (Figure 3). The patient recovered and continued on sunitinib until progressive disease (PD) in liver and bone lesions five months thereafter. He died from disease after a survival period of 12 months from the time of diagnosis.

Discussion
We here describe two mRCC patients on presurgical sunitinib to facilitate secondary nephrectomy who developed a progressive caval vein thrombus while on treatment. In the first patient, an initially resectable caval vein thrombus progressed into the right atrium and surgery could not be performed due to rapid clinical deterioration as a result from backward and liver failure. In the second patient, a tumour thrombus developed with extension into the caval vein which required extended surgery, while the liver metastasis demonstrated regression. It cannot be excluded that progression of the thrombus in the first patient may have been related to venous thrombotic events [12], but histology revealed a tumour thrombus in the second patient.

In the cytokine era, responses in primary tumours and caval vein thrombs were rare; mRCC patients with primary tumours in situ were more likely to die from distant metastases than from local progression [13]. Therefore, initial cytokine therapy was used to select patients for nephrectomy that showed a response in metastases. For two reasons, however, this strategy may be less logical in mRCC patients treated with targeted agents, such as sunitinib. First, mixed responses in the primary tumour and metastases may occur, indicating the risk of progression of initially resectable primaries to inoperable tumours despite a sunitinib-induced response in metastases. Second, after development of PD during sunitinib and subsequent discontinuation of this drug, a resectable primary tumour may rapidly progress or may cause severe symptoms requiring palliative treatment.

The effect of downstaging primary tumours is most prominent in the first three months, suggesting that a few cycles of sunitinib may be sufficient to facilitate the subsequent surgical procedure. In previous reports on sunitinib, however, the PD rate in primary tumours varied from 0 to 47% [4,14]. The study by Thomas et al. [14] and the present cases indicate that initially resectable primaries and tumour thrombs can progress to inoperable tumours within a few months. The wide range of the PD rate may reflect a high variability in sunitinib sensitivity of primary tumours and indicates that biomarkers are warranted to early identify mRCC patients with failure of downsstaging primaries. Since a 24 hour discontinuation period of sunitinib appears to be safe to perform surgery [15], the management plan can be changed rapidly in case patients develop PD in resectable primaries or tumour thrombs in the presence of stable disease in metastases.

Combined therapy of presurgical sunitinib followed by nephrectomy in mRCC patients might improve the quality of life and even prolong PFS and OS. Several patients have been described in whom sunitinib could be discontinued during a disease-free period after this treatment procedure [16]. Since there is no evidence from randomized controlled trials, the role and sequence of cytoreductive surgery in combination with targeted therapy in mRCC are open for debate. Currently, a number of trials have been initiated to investigate the efficacy of presurgical sunitinib in mRCC patients with a primary tumour in situ. The present two cases illustrate, however, that not each patient benefits from this approach. What caused my thrombotic events or true tumour growth, caval vein thrombs can develop and progress under targeted therapy. Physicians should be aware of progressive primary tumours and tumour thrombs to avoid missing an opportunity for nephrectomy.

Figure 3. Histology of the primary tumour (A) (H&E, 200x) and the caval vein thrombus which contains end tumour cells of clear cell histology (B) (H&E, 100x).
Progressive caval vein thrombus of renal cell cancer during sunitinib


[References]


Chapter 4

Brain metastases in patients with renal cell cancer receiving new targeted treatment

Helgi H. Helgason, Henk A. Mallo, Helga Droogendijk, John B.A.G. Haanen, Astrid A.M. van der Veldt, Alfons J.M. van den Eertwegh, Epie Boven

Brain Metastases in Patients With Renal Cell Cancer Receiving New Targeted Treatment

In December 2005, a 45-year-old man with progressive metastatic renal cell cancer (mRCC) started palliative treatment with sunitinib malate (Sutent; Pfizer, New York, NY) 50 mg daily oral dosing for 4 weeks followed by a 2-week rest period in cycles of 6 weeks. Right-sided nephrectomy had been performed for stage III clear cell carcinoma in March 2001. In October 2002, he was diagnosed with symptomatic mediastinal lymphadenopathy and lung metastases. He received fluorouracil, interleukin-2a, and interferon alfa, later followed by anti-interleukin-6, according to two different trial protocols. Both treatments resulted in a relatively long period of disease stabilization. At initiation of sunitinib, he was dyspneic on exertion and had a cough, but was otherwise in good general health. He was not using any medications. During the first five treatment cycles, he developed National Cancer Institute Common Terminology Criteria of Adverse Events (version 3.0) grade 2 skin rash, itch, fatigue, and stomatitis not requiring supportive medications or dose...
Disease evaluation confirmed previously described stable disease according to Response Evaluation Criteria in Solid Tumors (Figs 1A, before treatment; and 1B: after 6 months of sunitinib). During the 2-week rest period of the ninth cycle, he presented with right-sided headache and pain at the back of his right eye, accompanied by nausea and vomiting and sensory neuropathy in the left arm. All vital signs were normal. A magnetic resonance imaging scan of the brain showed three brain lesions with perilesional edema (Fig 2) suggestive of brain metastases. Neurologic symptoms disappeared promptly after initiation of dexamethasone, which was followed by whole-brain radiotherapy (five times; 4 Gy). Dexamethasone was tapered but not discontinued, despite possible drug interaction through cytochrome P450 isoenzyme 3A4 metabolism, because of relapsing headache. Disease evaluation confirmed previously documented stable disease (Fig 1C, after 12 months of sunitinib and at the time of brain metastases). Therefore, sunitinib treatment was reinstated shortly after radiotherapy. Stable disease was maintained as demonstrated on a computed tomography scan 3 months after resuming sunitinib (Fig 1D). Unfortunately, 3 weeks later, he deteriorated with signs of brain herniation, which did not respond to corticosteroids, and died.

Sunitinib, a novel oral tyrosine kinase inhibitor (TKI) targeting multiple receptors involved in angiogenesis, is approved for the treatment of mRCC. It is not known whether sunitinib can prevent the occurrence of brain metastases. The incidence of brain metastases as the only metastatic site in mRCC is less than 1%.7,8 and occurs as part of the first presentation of mRCC in approximately 3% of the patients.9-11 The cumulative incidence is approximately 10% in mRCC.4,6-8 and the median overall survival is 3 to 6 months, although a longer survival has been reported.12 We performed a retrospective analysis of 91 mRCC patients treated with sunitinib on a compassionate-use basis in our centers during the last 2 years and identified nine patients (10%; age 45 to 77 years) who developed symptomatic brain metastases. This incidence confirms previous results and suggests that sunitinib does not influence the incidence of brain metastases. Seven patients developed stable disease and two had partial response lasting 2 to 9 months until progression within the CNS. In all patients, CNS disease was the first sign of progression, but in six patients, including our patient described here, this was the only progressive metastatic site. After radiotherapy or surgery, the sunitinib was effectively continued in three patients. This suggests a difference in the pharmacokinetic behavior and less activity of sunitinib within the brain, compared with another registered TKI for mRCC, sorafenib, which has been suggested to reduce the incidence of brain metastases.13 No CIE measurements of sunitinib concentration have been performed in humans, but animal studies indicate that sunitinib penetrates the blood-brain barrier to some extent.14 Sunitinib could also be a substrate for P-glycoprotein–mediated efflux, similar to imatinib, which would limit its distribution within the brain.15 Of interest, six of nine patients developed CNS symptoms during the 2-week rest period of the treatment cycle. One explanation for this observation could be that sunitinib has antiedema activity, as observed for the multitargeted TKI,14 thereby masking pre-existent brain metastases. Our case series suggests that sunitinib is inadequate for control of brain metastases and may temporarily mask their existence.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST
The author(s) indicated no potential conflicts of interest.

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Chapter 5

Genetic polymorphisms associated with a prolonged progression-free survival in patients with metastatic renal cell cancer treated with sunitinib


* Authors contributed equally

Genetic Polymorphisms Associated with a Prolonged Progression-Free Survival in Patients with Metastatic Renal Cell Cancer Treated with Sunitinib

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Abstract

Purpose: The objective of this study was to identify genetic polymorphisms related to the pharmacokinetics and pharmacodynamics of sunitinib that are associated with a prolonged progression-free survival (PFS) and/or overall survival (OS) in patients with clear-cell metastatic renal cell cancer (mRCC) treated with sunitinib.

Experimental design: A retrospective multicenter pharmacogenetic association study was performed in 136 clear-cell mRCC patients treated with sunitinib. A total of 30 polymorphisms in 11 candidate genes, together with clinical characteristics were tested univariately for association with PFS as primary and OS as secondary outcome. Candidate variables with P < 0.1 were analyzed in a multivariate Cox regression model.

Results: Multivariate analysis showed that PFS was significantly improved when an A-allele was present in CYP3A5 (P = 0.001), and a TCG copy was present in the NR1I3 haplotype (5719C/T, 7738A/C, 7837T/G; HR, 1.76; P = 0.017) and a TCG copy was present in the ABCG2 haplotype (2677G/T, 2677G/T; HR, 0.52; P = 0.033). Carriers with a favorable genetic profile (n = 95) had an improved PFS and OS as compared with noncarriers (median PFS and OS: 13.1 versus 7.5 months and 19.9 versus 12.3 months). Next to the genetic variants, the Memorial Sloan-Kettering Cancer Center prognostic criteria were associated with PFS and OS (HR, 1.99 and 2.27; P < 0.001).

Conclusions: This exploratory study shows that genetic polymorphisms in three genes involved in sunitinib pharmacokinetics are associated with PFS in mRCC patients treated with this drug. These findings advocate prospective validation and further elucidation of these genetic determinants in relation to sunitinib exposure and efficacy.

Introduction

For decades, the treatment options of metastatic renal cell cancer (mRCC) have been limited and systemic treatment primarily consisted of immunotherapy with cytokines. Increasing knowledge of the underlying biology of renal cell cancer (RCC), in particular the clear-cell subtype, has expanded the treatment options for patients with mRCC. (1) RCC is characterized by an inactivated von Hippel–Lindau (VHL) tumor suppressor gene. Inactivated VHL leads to elevated protein levels of hypoxia-inducible factors which upregulates VEGF and plasminogen-derived growth factor (PDGFR) genes and proteins. The development of targeted therapy against signaling of these proteins has significantly improved the perspectives of patients with mRCC.

Currently, sunitinib is the most widely prescribed drug for the treatment of mRCC, and has been registered as first-line and second-line therapy. Sunitinib is an oral tyrosine kinase inhibitor (TKI) which targets several receptors including VEGF receptors-1, -2, -3, PDGFR (PDGFR)-b and -c, c-KIT, and FLT-3. In a randomized controlled trial, sunitinib significantly prolonged the progression-free survival (PFS) and overall survival (OS) as compared with interferon (2, 3). Although sunitinib can achieve partial response rates of up to 40% (3–5), approximately 35% of mRCC patients do not benefit from sunitinib treatment (4, 5). Because sunitinib treatment may also result in...
Translational Relevance

Currently, sunitinib is the most widely prescribed drug for the treatment of metastatic renal cell cancer. Unfortunately, only a part of treated patients will benefit from sunitinib therapy, despite the implementation of clinical prognostic criteria in the choice of therapy. As multiple systemic treatment modalities arise, a further refinement is needed to identify renal cell cancer patients who predispose to benefit from sunitinib treatment and those who do not. One of the possible options is to study the differential response to sunitinib treatment in order to identify genetic polymorphisms related to the pharmacokinetics and pharmacodynamics of this drug. In the future, genetic variants may be added to the current prognostic criteria, enabling physicians to predict benefit from sunitinib in individual patients.

Therefore, expression levels and functional activity of these drug transporters may have important consequences for the efficacy of sunitinib.

The cytochrome P450 (CYP) 3A (CYP3A) family is the predominant drug metabolizing enzyme and CYP3A4 is thought to be the key enzyme for the biotransformation of sunitinib (11). CYP3A4 is predominantly found in the liver and its expression is regulated by the ligand-activated nuclear receptors NR1I2 [pregnane X receptor (PXR)] and NR1I3 [constitutive androstane receptor (CAR)] (12). In addition, other enzymes of the cytochrome P450 family (CYP3A5, CYP1A1, and CYP1A2) may metabolize sunitinib, as these enzymes are known to be involved in the metabolism of otherTKIs (13).

Besides pharmacokinetic factors, pharmacodynamic factors may determine the efficacy of sunitinib. In RCC, sunitinib is thought to exert its major therapeutic effect by inhibition of the VEGFR on tumor-associated endothelium, leading to reduced tumor angiogenesis (14). In addition, inhibition of the PDGFR might increase the antiangiogenic effects of sunitinib by targeting pericytes, which are able to protect endothelial cells from apoptosis (15). As the main targets for sunitinib are thought to be located in the microenvironment of tumor cells, the efficacy of sunitinib treatment may be related to the genetics of the surrounding microenvironment (16). Particularly, genetic variation in VEGFR-2 may affect sunitinib activity, because VEGFR-2 is expressed in the normal endothelium (17) and the tumor vasculature may develop from processes of the host (18).

Single nucleotide polymorphisms (SNP) in genes encoding for efflux transporters, metabolizing enzymes, and drug targets may affect the efficacy of sunitinib in mRCC, as SNPs in specific genes have previously been associated with sunitinib-induced toxicities in patients with mRCC and gastrointestinal stromal tumors (19, 20). Therefore, SNPs may be useful markers for personalized treatment planning and may be candidate markers for selecting mRCC patients for sunitinib treatment. The objective of the current study was to identify SNPs involved in the pharmacokinetics and pharmacodynamics of sunitinib that are associated with a prolonged PFS and/or OS in mRCC patients.

Patients and Methods

Study population

In our previous study, 219 sunitinib-treated patients with various malignancies were included to investigate the association between SNPs and sunitinib-induced toxicities (19). In the present study, a subset of patients with histologically proven clear-cell RCC was selected for the analyses. A total of 116 consecutive mRCC patients who initiated sunitinib treatment between December 2005 and May 2008 were included. Sunitinib was administered orally at a dose of 50 mg daily, consisting of 4 weeks of treatment followed by a 2-week rest-period in cycles of 6 weeks. Dose reductions of sunitinib were allowed if necessary toxicities (6, 7), pretreatment markers to identify mRCC patients with a favorable outcome to sunitinib therapy, despite the implementation of clinical prognostic criteria in the choice of therapy. As multiple systemic treatment modalities arise, a further refinement is needed to identify renal cell cancer patients who predispose to benefit from sunitinib treatment and those who do not. One of the possible options is to study the differential response to sunitinib treatment in order to identify genetic polymorphisms related to the pharmacokinetics and pharmacodynamics of this drug. In the future, genetic variants may be added to the current prognostic criteria, enabling physicians to predict benefit from sunitinib in individual patients.

Therefore, expression levels and functional activity of these drug transporters may have important consequences for the efficacy of sunitinib.

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depending on the type and severity of adverse events according to the current guidelines (21). The study was approved by the medical ethics review board.

Study design
Demographic and clinical data of patients were reported on case record forms designed for data collection for this study. Patient characteristics considered relevant for PFS and OS analysis were age, gender, Eastern Cooperative Oncology Group (ECOG) performance status, prior systemic therapy, prior radiotherapy, the number of metastatic sites, and the risk factors according to Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic criteria, which is based on 3 risk factors including low Karnofsky performance status (< 80%), high lactate dehydrogenase (LDH; > 1.5 times the upper limit of normal), low hemoglobin level, high corrected serum calcium (≥ 10 mg/dL), and time from initial diagnosis to treatment < 1 year (22). Residual blood or serum samples taken for routine patient care were stored at −20°C in the local hospital laboratory. Of each patient one whole blood or serum sample was collected from the participating hospitals. All samples were anonymized by a third party, according to the instructions stated in the Codes for Proper Use and Proper Conduct in the Self-Regulatory Codes of Conduct (www.federa.org).

Genetic polymorphisms
Nineteen polymorphisms in 7 genes involved in the pharmacokinetics and 11 polymorphisms in 4 genes involved in the pharmacodynamics of sunitinib were selected (Supplementary Table S1). Selection criteria for the polymorphisms were a minor allele frequency > 0.2 in Caucasians and an assumed clinical relevance based on previously reported associations or the assumption that nonsynonymous amino acid change leads to changed protein functionality.

The 11 candidate genes were selected on their potential relation with the pharmacokinetics and pharmacodynamics of sunitinib (Fig 1). First, the candidate genotypes were selected by literature review. If there was no available data in the literature review, we referred to the SNPs from the dbSNP database (http://www.ncbi.nlm.nih.gov/sites/entrez). ABCB1, ABCG2, NR1D1, NR1D3, CYP3A5, CYP3A4, and CYP1A2 were selected for the pharmacokinetic pathways, whereas VEGFR-2, VEGFR-3, PDCER2, and FLT3 were selected for the pharmacodynamic pathways. The most common functional SNPs in human ABCB1 are the synonymous S453C, > T; 1286C > T changes and the nonsynonymous 2677G > T change. As functional studies have shown that the haplotype of these three SNPs is a silent mutation and alters the function of the efflux transporter including its substrate specificity (23), the haplotypes of ABCB1, instead of the three individual SNPs, was included in the analysis. Although VEGFR-1 is a target of sunitinib, and CYP3A4 is an important enzyme for metabolism of sunitinib (11), no polymorphisms of VEGFR-1 and CYP3A4 were analyzed, as no functional polymorphisms met the criteria for SNP selection.

Methods for genotyping assay validation and haplotype estimation have been described previously (19). Briefly, genomic DNA was isolated from 1 ml of serum or EDTA-blood with the MagNaPure LC (Roche Diagnostics). Polymorphic sites in genomic DNA were analyzed with TaqMan assays (Applied Biosystems).

Statistical design and data analysis
For PFS and OS, data collection was closed on August 31, 2009. The primary outcome measure of this study, PFS, was defined as the time between the first day of sunitinib and the date of progressive disease (PD) according to Response Evaluation Criteria in Solid Tumors (24), clear clinical evidence of PD or death due to PD within 12 weeks after the last response evaluation. If a patient had not progressed, PFS was censored at the time of the last follow-up. If the PD date was unknown or a patient died due to PD later than 12 weeks after the last response evaluation, PFS was censored at the last adequate tumor assessment. OS was the secondary outcome and was defined as the time between the first day of sunitinib treatment and the date of death or the date at which patients were last known to be alive.

All patient characteristics were tested univariately against the primary outcome using Kaplan–Meier and Cox-regression analysis, depending on the tested variables. The polymorphisms and haplotypes were tested univariately against PFS and OS using the Kaplan–Meier method. For this initial analysis, the general model was used. Given the exploratory nature of this study, variables with a P ≤ 0.1 were selected as candidate variables for multivariate Cox-regression analyses. Data were fitted to the most appropriate model (multiplicative, dominant or recessive) and tested in the multivariate Cox-regression analysis with PFS and OS as depending variables. Additional patient characteristics were introduced in the multivariate analyses based on univariately tested results if P < 0.1. Hazard ratios (HR) were generated considering patients with the most common clinical factor or genotype as the reference group. Missing data were kept as missing except for factors in the MSKCC score and the ECOG performance status. Patients with missing performance status (n = 2), LDH (n = 2), hemoglobin (n = 1), and baseline calcium values (n = 2) were assumed to be part of the worse prognostic score. Accordingly, MSKCC scores were increased with one risk factor in 5 patients and with two risk factors in 1 patient.

As a result only 5 patients were categorized into the intermediate risk group, whereas 3 other patients were categorized into the poor risk group. Patients with missing ECOG performance statuses (n = 2) were scored as ECOG = 1. To test these assumptions, the multivariate analyses were performed with and without the replacement of the patients with missing factors in the MSKCC score. Similar results were generated, indicating that the replacement was legitimate. All statistical analyses were performed using SPSS 16.0 software. A 20% improvement (HR, 0.44) in PFS...
at ~1 year in patients with sunitinib was judged to be clinically meaningful by the investigators designing the study. Forty-six events with disease progression were estimated to be needed to detect such an improvement using a two-sided, unstratified log-rank test with an overall significance level of 0.05 and power of 0.80. All results from the multivariate analyses with \( P < 0.05 \) were considered significant. Because this is an explorative study, no correction for multiple testing was made.

### Results

#### Study population

The main patient characteristics are presented in Table 1. Thirty-one (22.9%) patients had one metastatic site, 47 (34.6%) patients had two metastatic sites, and 58 (42.6%) patients had at least 3 metastatic sites. According to the MSKCC prognostic criteria most patients (59.6%) were categorized into the intermediate risk group, whereas 24.3% and 15.2% of the patients were categorized into the favorable and poor risk group, respectively.

At the time of the analysis, 47 (34.6%) patients were alive and 92 (67.6%) patients had disease progression. Overall, the median PFS time was 10.0 months (range, 7.6–12.4 months) and the median OS time was 16.5 months (range, 13.5–19.2 months). The baseline characteristics entered into the multivariate Cox models included the MSKCC risk factors, the number of metastatic sites, and age for PFS analyses, and the MSKCC risk factors and the number of metastases for OS analysis. The ECOG performance status was excluded from the multivariate analyses due to collinearity with the MSKCC prognostic criteria (22).

#### Pharmacogenetic factors for sunitinib and progression-free survival

Among the 30 studied polymorphisms, only polymorphisms related to the pharmacokinetics of sunitinib were predictive of PFS (Table 2). A prolonged PFS was found in the univariate analysis of patients with presence of the A-allele in CYP3A5*6A/G (\( P = 0.017 \)), absence of a CAT copy in the NR1I2 haplotype (\( P = 0.021 \)), presence of the Callele in NR1I2*0505C/T (\( P = 0.025 \)), absence of the C-allele in ABCG2*842A/G (\( P = 0.072 \)), and presence of a TCG copy in the ABCG2 haplotype (\( P = 0.019 \), 0.047, and 0.049, respectively), whereas only the ECOG performance status and the number of metastatic sites were also prognostic for OS (\( P = 0.004 \) and 0.058, respectively).

### Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
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<th>%</th>
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</tr>
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<td></td>
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<tr>
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<td></td>
</tr>
<tr>
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<tr>
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<tr>
<td>Arab</td>
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<td>0.7</td>
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<tr>
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<td>Previous nephrectomy</td>
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<td>Previous radiation therapy</td>
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<td>1</td>
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<td>2.9</td>
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<tr>
<td>Liver</td>
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<td>24.3</td>
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<tr>
<td>Bone</td>
<td>130</td>
<td>95.6</td>
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<tr>
<td>Lymph nodes</td>
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<td>44.9</td>
</tr>
<tr>
<td>Brain</td>
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<td>3.7</td>
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<td>MSKCC risk factors*</td>
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<tr>
<td>0 (favorable)</td>
<td>33</td>
<td>24.3</td>
</tr>
<tr>
<td>1–2 (intermediate)</td>
<td>81</td>
<td>59.0</td>
</tr>
<tr>
<td>3 (poor)</td>
<td>22</td>
<td>15.6</td>
</tr>
</tbody>
</table>

### Risk groups according to Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic criteria (based on the 5 risk factors: sex, Karnofsky performance status (<60%), high LDH (>1.5 times the upper limit of normal), low serum hemoglobin, high corrected serum calcium (<10 mg/dL), and time from initial diagnosis to treatment <1 year [23])

*MSKCC risk factors: sex, Karnofsky performance status (<60%), high LDH (>1.5 times the upper limit of normal), low serum hemoglobin, high corrected serum calcium (<10 mg/dL), and time from initial diagnosis to treatment <1 year [23].
Multivariate analysis confirmed the following factors as significant (<0.05) predictors of improved PFS: the MSKCC risk factors (HR: 1.988; 95% CI, 1.394–2.837), the number of metastatic sites (HR: 1.400; 95% CI, 1.042–1.880), age (HR: 1.031 per year increase; 95% CI, 1.003–1.060), presence of the A-allele in CYP3A5 6986A/G (HR: 0.266; 95% CI, 0.079–0.892), absence of a CAT copy in the NR1I3 haplotype (HR: 1.758; 95% CI, 1.108–2.790), and the presence of a TCG copy in the ABCB1 haplotype (HR: 0.522; 95% CI, 0.287–0.950).

### Table 2. Univariate and multivariate analyses of progression-free survival in mRCC patients treated with sunitinib

<table>
<thead>
<tr>
<th>Factors</th>
<th>No.</th>
<th>Univariate analyses</th>
<th>Multivariate analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median PFS (mo)</td>
<td>95% CI</td>
<td>HR</td>
</tr>
<tr>
<td><strong>Clinical factors</strong></td>
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<tr>
<td>MSKCC risk factors</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>33</td>
<td>24.3</td>
<td>11.3–37.2</td>
</tr>
<tr>
<td>&gt;1</td>
<td>81</td>
<td>8.7</td>
<td>6.3–11.2</td>
</tr>
<tr>
<td>&gt;3</td>
<td>22</td>
<td>7.0</td>
<td>0.0–14.4</td>
</tr>
<tr>
<td>No. of metastatic sites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>31</td>
<td>19.4</td>
<td>7.8–31.0</td>
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<tr>
<td>&gt;1</td>
<td>47</td>
<td>11.0</td>
<td>5.1–18.8</td>
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<tr>
<td>&gt;3</td>
<td>58</td>
<td>8.1</td>
<td>7.4–9.6</td>
</tr>
<tr>
<td>Age (HR = 1.024 per year increase)</td>
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<tr>
<td>1</td>
<td>1,000–1,046</td>
<td>0.047</td>
<td>1.031 per year increase</td>
</tr>
<tr>
<td>&gt;1</td>
<td>1,023–1,060</td>
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<tr>
<td><strong>Genetic factors</strong></td>
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<td>Pharmacokinetic pathway</td>
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<tr>
<td>CYP3A5 6986A/G</td>
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<tr>
<td>GG versus AG = AA</td>
<td>117</td>
<td>9.3</td>
<td>6.9–11.8</td>
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<tr>
<td>NR1I3 haplotype^a</td>
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<tr>
<td>Other-other versus CAT-CAT</td>
<td>75</td>
<td>13.3</td>
<td>7.8–18.8</td>
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<tr>
<td>NR1I2-8055C/T</td>
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<tr>
<td>CC = CT versus TT</td>
<td>119</td>
<td>10.8</td>
<td>8.0–13.6</td>
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<tr>
<td>NR1I2-25385C/T</td>
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<tr>
<td>CC = CT versus TT</td>
<td>17</td>
<td>6.7</td>
<td>3.6–9.9</td>
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<tr>
<td>ABCB1 haplotype^b</td>
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<tr>
<td>Other-other versus TCG-other</td>
<td>100</td>
<td>8.4</td>
<td>7.0–9.7</td>
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<td>ABCG2 345/A</td>
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<tr>
<td>GG versus AG</td>
<td>124</td>
<td>9.0</td>
<td>5.9–11.2</td>
</tr>
<tr>
<td>TT</td>
<td>12</td>
<td>19.4</td>
<td>0.0–40.8</td>
</tr>
</tbody>
</table>

*a Only factors with P < 0.1 level are presented; factors with P < 0.1 in the univariate analyses were selected for multivariate analyses.

^b HR < 1.0 indicates that the factor associates with improved PFS, HR > 1.0 associates with worse PFS.

^c Risk groups according to Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic criteria (based on the 5 risk factors: low Karnofsky performance status (<80%), high LDH (>1.5 times the upper limit of normal), low serum hemoglobin, high corrected serum calcium (>10 mg/dL), and time from initial diagnosis to treatment <1 year) (22).

^d Multiplicative model, HR per increase in MSKCC class or number of metastatic sites class.

Description haplotypes: | NR1I3 5719C/T, 7738A/C, and 7837T/G; | ABCB1 3435C/T, 1236C/T, and 2677G/T.

**Multivariate analysis confirmed the following factors as significant (<0.05) predictors of improved PFS: the MSKCC risk factors (HR: 1.988; 95% CI, 1.394–2.837), the number of metastatic sites (HR: 1.400; 95% CI, 1.042–1.880), age (HR: 1.031 per year increase; 95% CI, 1.003–1.060), presence of the A-allele in CYP3A5 6986A/G (HR: 0.266; 95% CI, 0.079–0.892), absence of a CAT copy in the NR1I3 haplotype (HR: 1.758; 95% CI, 1.108–2.790), and the presence of a TCG copy in the ABCB1 haplotype (HR: 0.522; 95% CI, 0.287–0.950).**
polymorphisms, presence of the C-allele in NR1I2-25385C/T (P = 0.017), presence of a TCG copy in the ABCB1 haplotype (P = 0.097) and presence of the A-allele in ABCG2 34G/A (P = 0.072) were associated with a prolonged OS. In addition, univariate analyses identified two pharmacodynamic polymorphisms including two GCGT copies in the PDGFR-\(\alpha\) haplotype and presence of the A-allele in VEGFR-2 1718T/A as factors for a prolonged OS (P = 0.002 and 0.022, respectively).

Multivariate analysis confirmed the MSKCC risk factors (HR: 2.273; 95% CI, 1.595–3.238) and the presence of the A-allele in VEGFR-2 1718T/A (HR: 2.907; 95% CI, 1.224–6.903) as significant (<0.05) predictors of a prolonged OS.

In multivariate analysis, there was a trend toward an improved OS for patients with a TCG copy in the ABCB1 haplotype (HR: 0.593; 95% CI, 0.332–1.061; P = 0.078) or presence of the A-allele in ABCG2 34G/A (HR: 0.416; 95% CI, 0.162–1.070; P = 0.069).

Table 3. Univariate and multivariate analyses of overall survival in mRCC patients treated with sunitinib

<table>
<thead>
<tr>
<th>Factors</th>
<th>No.</th>
<th>Median OS (mo)</th>
<th>95% CI</th>
<th>P</th>
<th>HR</th>
<th>95% CI</th>
<th>P</th>
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<tr>
<td>0</td>
<td>33</td>
<td>Not reached</td>
<td>-</td>
<td>&lt;0.001</td>
<td>2.737</td>
<td>1.535–4.038</td>
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<tr>
<td>1–2</td>
<td>81</td>
<td>14.8</td>
<td>11.8–17.7</td>
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<td></td>
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<tr>
<td>≥3</td>
<td>23</td>
<td>19.9</td>
<td>13.1–26.7</td>
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<td>28.8</td>
<td>15.3–42.2</td>
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<td>2</td>
<td>47</td>
<td>15.6</td>
<td>13.5–17.7</td>
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<td>≥3</td>
<td>58</td>
<td>13.2</td>
<td>9.5–16.9</td>
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<tr>
<td>NR1I2-25385C/T</td>
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<td>CC – CT versus TT</td>
<td>118</td>
<td>17.1</td>
<td>12.9–21.2</td>
<td>1</td>
<td>0.178</td>
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<td>TT</td>
<td>18</td>
<td>10.2</td>
<td>7.4–13.1</td>
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<td>Other-other versus TCG-other</td>
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<td>GG versus AG</td>
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<td>12.5–18.3</td>
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<td>0.416</td>
<td>0.162–1.070</td>
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<td>AA – AT versus TT</td>
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<td>16.3</td>
<td>12.4–20.2</td>
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<td>1</td>
<td>2.907</td>
<td>1.224–6.903</td>
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*Only factors with P < 0.1 level are presented; factors with P < 0.1 in the univariate analyses were selected for multivariate analyses.

**HR** < 1.0 indicates that the factor associates with improved OS, HR > 1.0 associates with worse OS.

Risk groups according to Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic criteria (based on the 5 risk factors: low Karnofsky performance status (<80%), high LDH (>.5 times the upper limit of normal), low serum hemoglobin, high corrected serum calcium (>10 mg/dL), and time from initial diagnosis to treatment <1 year) (22).
had an improved PFS and OS as compared with noncarriers (median PFS: 13.1 versus 7.5 months, \( P = 0.001 \) and median OS: 19.9 versus 12.3 months, \( P = 0.009 \)). Multivariate analysis including the clinical factors showed consistent predictive value of the model for PFS and showed a trend for OS (HR: 0.541; 95% CI, 0.340–0.860, \( P = 0.009 \) and HR: 0.667; 95% CI, 0.420–1.058, \( P = 0.085 \), respectively; Fig. 2).

Discussion

In mRCC patients treated with sunitinib, the MSKCC risk groups and the number of metastatic sites are clinical factors that are usually associated with PFS and OS (2,3,7). However, these clinical factors are prognostic criteria that are associated with the extent of the disease and do not necessarily predict antitumor efficacy of a specific drug. As an increasing number of drugs is currently available for the treatment of mRCC (25), tools are needed to identify patients who predispose to benefit from sunitinib treatment and to select individual mRCC patients for treatment with this drug. The efficacy of sunitinib may be influenced by multiple genes encoding for enzymes, efflux transporters and targets related to the pharmacokinetics and pharmacodynamics of sunitinib. Therefore, we analyzed whether SNPs in the pharmacokinetic and pharmacodynamic pathways of sunitinib were predictive of PFS and OS in patients with clear-cell mRCC. Our study showed that next to 3 clinical characteristics (MSKCC prognostic criteria, number of metastatic sites, and age), 3 genetic variants in the CYP3A5, NR1I3, and ABCB1 genes were predictive factors for PFS. In addition, a role of an A-allele in VEGFR-2 1718T/A for a prolonged OS as a secondary outcome was found.

Clinical benefit from sunitinib treatment may depend on systemic exposure to sunitinib. Sunitinib is metabolized primarily to the active N-de-ethylated metabolite SU12662, which reaches similar plasma concentrations and has equivalent biochemical activity as the parent compound (26). Thereafter, SU12662 undergoes a second N-de-ethylation step, which occurs at a slower rate, to the inactive metabolite SU14335. Recently, a meta-analysis of pharmacokinetic data in 443 patients treated with sunitinib, showed that higher plasma levels of sunitinib and its active metabolite SU12662 were associated with a prolonged time–to–tumor progression and OS (27). Currently, it is not clear which underlying factors account for the observed interindividual differences in plasma levels of sunitinib and its active metabolite SU12662. Interindividual differences in sunitinib exposure may be the result of variations in sunitinib absorption, metabolism, distribution, and excretion through metabolizing enzymes and transporter proteins. Concerning the pharmacokinetics of sunitinib, the present study identified variants in three genes (CYP3A5, NR1I3, and ABCB1) as predictive factors for PFS in mRCC patients treated with sunitinib. Although these polymorphisms were not predictive of OS, there was a trend toward a prolonged OS for patients with a TCG copy in the ABCB1 haplotype. Additional treatment after discontinuation of sunitinib treatment may explain the discrepancy between the results of the PFS and OS analyses, as 29% of patients were subsequently treated with at least one other agent, including sorafenib (22%), temsirolimus (2%), and everolimus (4%).

Figure 2. Favorable (+) and unfavorable genetic profile (−) in mRCC patients treated with sunitinib for progression-free (A) and overall survival (B) using multivariate Cox regression analysis. Patients were categorized as carriers of the favorable genetic profiles when they had all six A-alleles in CYP3A5, a TCG copy in the ABCB1 haplotype or a missing CAT copy in the NR1I3 haplotype.
The ABC transporters may contribute to multidrug resistance in tumors by actively extruding drugs from cancer cells. In RCC, an increase in ABCR expression [35, 34] and activity [35] has been reported, suggesting a contribution of ABCR to the resistance of RCC to some antineoplastic drugs. Although polymorphisms in ABCR1 and ABCG2 may be associated with the development of RCC [36, 37], it is currently not known whether polymorphisms in ABCR and ABCG2 are associated with the expression and function of these transporters at the somatic level in renal cancer cells [38]. Nonetheless, the role of efflux transporters in tumor cells may be limited for acquired resistance to sunitinib, which may develop after an initial response to sunitinib, as acquired resistance to sunitinib may be more related to physiological changes in the tumor microenvironment of tumors, allowing reestablishment of angiogenesis during sunitinib treatment [16].

Clinical efficacy of treatment with TKIs may also be related to specific mutations in drug targets, as was shown for imatinib and gefitinib [39–40]. Currently, it is not known which targets in RCC predict response to sunitinib or whether the somatic polymorphisms of targets in RCC correlate with genetic polymorphisms obtained from germline cells. Of the studied pharmacodynamic polymorphisms of sunitinib, only a polymorphism of VEGFR-2 1714E/A was associated with a decreased OS in multivariate analysis, whereas the presence of two GCGT copies in the PDGFR-β haplotype was associated with a prolonged OS in univariate analysis. However, no significant association between these polymorphisms and PFS was found. These findings may suggest that polymorphisms in VEGFR-2 and PDGFR-β may be associated with the nature of the disease and may therefore be prognostic instead of predictive. However, prospective validation in an independent mRCC cohort that is not treated with sunitinib is necessary to determine whether the associated polymorphisms of the present study are predictive markers of sunitinib activity or prognostic markers of mRCC disease.

In our previous study, several polymorphisms in genes involved in the pharmacokinetic and pharmacodynamic pathways of sunitinib were associated with sunitinib-induced toxicity [19]. Polymorphisms of NR1I2 [absence of a CAG copy in the haplotype], ABCB1 [presence of a TTT copy], and VEGFR-2 (T allele in 1191 C/T) were signifi-
cantly related to specific mutations in drug targets, as was shown for imatinib and gefitinib [39–40]. Currently, it is not known which targets in RCC predict response to sunitinib or whether the somatic polymorphisms of targets in RCC correlate with genetic polymorphisms obtained from germline cells. Of the studied pharmacodynamic polymorphisms of sunitinib, only a polymorphism of VEGFR-2 1714E/A was associated with a decreased OS in multivariate analysis, whereas the presence of two GCGT copies in the PDGFR-β haplotype was associated with a prolonged OS in univariate analysis. However, no significant association between these polymorphisms and PFS was found. These findings may suggest that polymorphisms in VEGFR-2 and PDGFR-β may be associated with the nature of the disease and may therefore be prognostic instead of predictive. However, prospective validation in an independent mRCC cohort that is not treated with sunitinib is necessary to determine whether the associated polymorphisms of the present study are predictive markers of sunitinib activity or prognostic markers of mRCC disease.
with plasma levels of sunitinib and its active metabolite SU12662. If future studies reveal a relation between sunitinib pharmacogenetics and response to sunitinib, the sunitinib starting dose may be adjusted to dose-escalation of sunitinib ≥ 50 mg daily for patients without these genotypes and haplotypes. The nonpharmacogenetic profile may be used to select patients who may be eligible for alternate dosing schedules with immediate monitoring of plasma levels of sunitinib and its active metabolite SU12662. Before this genotypic profile can be implemented, prospective validation in an independent patient population is necessary. In conclusion, pharmacokinetic but not pharmacodynamic polymorphisms were independent predictive factors for FFS in patients with clear-cell mRCC who were treated with sunitinib. Patients with an A allele on CYP3A5 6986A/G, absence of a CAT copy in the NR1I3, and presence of a TCG copy in the CYP3A5 3557 T/C haplotype, the sunitinib haplotype, had a prolonged FFS. These polymorphisms may be valuable factors to identify patients with reduced exposure to sunitinib in order to improve treatment strategies in these patients. The findings of this study advance more pharmacogenetic studies in patients treated with sunitinib to further elucidate the role of these genetic determinants in sunitinib exposure and efficacy.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Chapter 6

Predictive factors for severe toxicity of sunitinib in unselected patients with advanced renal cell cancer

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Predictive factors for severe toxicity of sunitinib in unselected patients with advanced renal cell cancer

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AJM van den Eertwegh1 and JH van Haanen1,2,3

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Sunitinib has been registered for the treatment of advanced renal cell cancer (RCC). As patient inclusion was highly selective in previous studies, experience with sunitinib in general oncological practice remains to be reported. We determined the efficacy and safety of sunitinib in patients with advanced RCC included in an expanded access programme. ECOG performance status > 1, histology other than clear cell and presence of brain metastases were no exclusion criteria. Eighty-two patients were treated: 23% reached a partial response, 10% had stable disease, 20% progressed and six patients were not evaluable. Median progression-free survival (PFS) was 12 months and median overall survival (OS) was 15.5 months. Importantly, 47 patients (57%) needed a dose reduction, 15 (19%) because of treatment-related adverse events, 10 (12%) because of continuous dosing, and two because of both. Stomatitis, fatigue, hand-foot syndrome and a combination of grade 1–2 adverse events were the most frequent reasons for dose reduction. In 40 patients (49%), there was severe toxicity, defined as dose reduction or permanent discontinuation, which was highly correlated with low body surface area, high age and female gender. On the basis of age and gender, a model was developed that could predict the probability of severe toxicity.


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Clinical Studies

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Keywords: renal cell cancer; sunitinib; toxicity; dose reduction; non-clear cell histology

Advanced renal cell cancer (RCC) has been recognised as a cytokine-based disease. Increasing knowledge of the underlying biology of RCC, and more specifically, the clear cell subtype, has recently changed the treatment options. Clear cell carcinomas, which account for 75% of all RCC subtypes, appear to contain an inactivated von Hippel–Lindau (VHL) tumour suppressor gene in at least 60% of these tumours (Brugarolas, 2007). Von Hippel–Lindau gene alterations lead to elevated protein levels of hypoxia-induced factor-1α, which upregulates vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) genes and proteins (Brugarolas, 2007). The overexpression of these growth factors results in blood vessel formation which may account for the high vascular density of these tumours. Consequently, tumour angiogenesis has become an interesting therapeutic target in patients with metastatic RCC (mRCC). Antiangiogenic agents, such as bevacizumab (Escudier et al., 2007b), sorafenib (BAY 43–9006) (Escudier et al., 2007a) and sunitinib (Srivastava et al., 2007) have demonstrated significant antitumour activity in advanced RCC preferentially of the clear cell type excluding patients with poor prognosis. In a phase III clinical trial in mRCC, bevacizumab, a neutralising antibody against VEGF, in combination with interferon-α prolonged progression-free survival (PFS) with 4.8 months as compared to interferon-α alone (Escudier et al., 2007b). Sunitinib and sorafenib are oral tyrosine kinase inhibitors of the VEGF and PDGF receptors. In comparison with placebo, sorafenib prolonged PFS in cytokine-pretreated mRCC with almost 3 months (Escudier et al., 2007a). Sunitinib demonstrated a significantly prolonged PFS (11 v 5 months) as well as a higher objective response rate than treatment with interferon-α (51% vs 43%) (Motzer et al., 2007). Temsirolimus, an inhibitor of mammalian target of rapamycin (mTOR) kinase, has demonstrated to improve the overall survival (OS) in RCC patients with unselected cancer histology and poor prognosis in comparison with interferon-α (11 vs 7 and 8 months for, respectively, single-agent temsirolimus vs single-agent interferon-α and the combination) (Studier et al., 2007). In the pivotal trials on sunitinib, patients had to fulfil prespecified criteria. Eastern Cooperative Oncology Group (ECOG) performance status > 1, brain metastases, uncontrolled hypertension or clinically significant cardiovascular events or disease during the preceding 12 months were exclusion criteria (Motzer et al., 2006a, b, 2007). In addition, only patients with clear cell histology were allowed for entry in two out of the three previous studies (Motzer et al., 2008b, 2008). Nowadays, sunitinib can be prescribed widely to patients with advanced RCC, but the experience with this drug in an unselected patient population that does not meet the above-described criteria has yet to be revealed. Here, we report on a first experience with sunitinib treatment in a

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large advanced-stage RCC patient population reflecting general oncological practice and show that clinical benefit is comparable to that observed in the earlier phase III trials. An unexpectedly high number of patients, however, required dose reductions to maintain an acceptable quality of life.

PATIENTS AND METHODS

Patient population

From December 2005 to September 2006, patients with histologically confirmed advanced RCC were enrolled in a global expanded access programme (EAP) for treatment with sunitinib. Results are reported for patients treated in two centres in Amsterdam (VU University medical center and the Netherlands Cancer Institute).

Until May 2006, patients were included only after cytotoxic-based therapy had failed and, thereafter, the drug was also available first-line. Inclusion criteria were as follows: age 18 years or age or older, adequate organ function (total serum bilirubin ≤2 × upper limit of normal (ULN), serum transaminases <5 × ULN, serum creatinine ≤2 × ULN, absolute neutrophil count >1.5 × 10^9/l, platelets >75 × 10^9/l, haemoglobin >9.0 g/dl) and resolution of all toxic effects of prior systemic therapy, radiotherapy or surgical procedure according to National Cancer Institute-Common Toxicity Criteria (NCI-CTC) version 3.0 grade ≤1. Before entry into the programme, each participant had to sign an institutional review board-approved protocol-specific informed consent in accordance with the national and institutional guidelines, which strictly adhere to the principles of the Declaration of Helsinki and its subsequent amendments.

Exclusion criteria were as follows: pregnancy or breast feeding, concurrent treatment in another therapeutic trial, previous treatment with sunitinib, catheter placement in the arterial system, severe or unstable angina, any unstable arrhythmia requiring medication or another severe acute or chronic medical or psychiatric condition or laboratory abnormality that would make the patient inappropriate for entry in this EAP.

Treatment, efficacy and adverse events

Sunitinib was administered orally at a dose of 50 mg daily, consisting of 4 weeks of treatment followed by a 2-week rest period in cycles of 6 weeks. A dose reduction of sunitinib (to 37.5 or 25 mg) was allowed depending on the type and severity of adverse events. If patients had symptoms of progressive disease (PD) during the rest period, there was the possibility for continued dosing of sunitinib at 37.5 mg per day.

Patients underwent physical examination on day 1 of every cycle. Complete blood cell count and serum chemistry tests were carried out on day 1 and 28 of every treatment cycle. Complete blood cell count was performed on day 14 of the first cycle. Electrocardiography was performed at baseline and on day 28 of the first cycle.

Haematological and non-haematological toxic effects were graded according to NCI-CTC version 3.0. Tumour evaluation was conducted on day 1, 14 and 28 of the first treatment cycle and on day 1 and 28 of each treatment cycle thereafter. If grade 3 haematological toxicity was recorded, the treatment was withheld until the recovery grade ≤2 or blood counts had returned to baseline after which sunitinib was resumed at the same dose level. In case of grade 4 haematological toxicity and grade 3 and 4 non-haematological toxicity, treatment was delayed until side effects had recovered to grade ≤2 or grade 1, respectively, or had returned to baseline after which the dose was reduced by one level at the discretion of the treating physician. In the case of grade 4 non-haematological toxicity, treatment was discontinued.

Computed tomography (CT) or magnetic resonance imaging (MRI) was performed at baseline and every two to three cycles of treatment to assess clinical response according to Response Evaluation Criteria in Solid Tumours (RECIST) (Therasse et al., 2000).

Data analysis

Specific case report forms were used for data entry. For response evaluation and toxicity, the cutoff date for data analysis was 1 March, 2007. For survival analysis, data collection was closed on 1 September, 2007. Patients were classified according to two prognostic classification systems for mRCC: (1) the Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic criteria (based on five risk factors low Karnofsky performance status (<80%), high lactate dehydrogenase (LDH), >1.5 times the ULN, low serum albumin and high corrected serum calcium (>10 mg per 100 ml) and time from initial diagnosis to treatment of less than 1 year) (Mekler et al., 2002) and (2) the prognostic criteria for VEGF-targeted therapy according to Choueiri et al. (2007) (based on the following 5 risk factors: time from diagnosis to treatment ≤2 years, baseline platelet count >300 × 10^9/l, baseline neutrophil count >4.5 × 10^9/l, baseline corrected calcium >8.5 mg per 100 ml or >10 mg per 100 ml and initial EGCG performance status >0).

Efficacy parameters were best response, time-to-treatment failure (TTF), PFS and OS. The TTF was defined as the time between the first day of treatment and the date of the first event considered as failure of treatment. Such events could be disease progression, early discontinuation (owing to unacceptable toxicity, patient's request and lost to follow-up) or death. The PFS was the time between the first day of sunitinib and the date of PD on CT or MRI, clear clinical evidence of PD or death owing to PD within 12 weeks after the last response evaluation. If a patient had not progressed, PFS was censored at the time of the last follow-up. If the PD date was unknown or a patient died owing to PD later than 12 weeks after the last response evaluation, PFS was censored at the last adequate tumour assessment. Overall survival was the time between the first day of treatment and the date of death or the date at which patients were last known to be alive. Progression-free survival and OS were calculated with the Kaplan–Meier method.

Severe toxicity was defined as dose reduction or permanent discontinuation of sunitinib because of treatment-related adverse events. The following clinical characteristics were analysed for a possible relation with severe toxicity: gender, age, body surface area (BSA), ECOG performance status, tumour type, presence of primary tumour, time of diagnosis to treatment, prior cytotoxic-based therapy, previous radiation therapy, number of tumour sites, liver metastases, MSKCC risk groups, Choueiri risk groups and baseline biochemical parameters. Baseline biochemical parameters (haemoglobin, LDH, albumin, creatinine, alkaline phosphatase and corrected calcium) were untransformed as a factor of the ULN. Statistical analysis was carried out using SPSS software (SPSS for Windows 15.0, SPSS Inc., Chicago, IL, USA).

Univariate logistic regression was performed to explore associations between the separate clinical characteristics and outcome. Subsequently, the variables with a significance of P<0.05 were used for multivariate logistic regression analysis.

RESULTS

Patients and treatment

Eighty-two patients with advanced RCC were registered in the EAP. Patient characteristics are depicted in Table 1. Fourteen patients had non-clear cell histology, 17 patients had a
16 patients had a concurrent primary tumour in situ and five patients had concurrent brain metastases. All patients received continuous for a period of at least 1 week. At the time of the analysis, 18 patients were still on study and 64 had discontinued treatment. Reasons for termination were performance status of ECOG > 1, in situ.
reduction because of both toxicity and continuous dosing (time to dose reduction 1.4 and 4.2 months).

Female gender, high age, low BSA and to a lesser extent also high LDL were significantly related with severe toxicity (univariate logistic regression; P = 0.006, P = 0.006, P = 0.035 and P = 0.035, respectively). There was no significant relation between severe toxicity and the separate prognostic risk groups according to the MSKCC criteria (Motzer et al, 2002) as well as the criteria of Choueiri et al (2007). In multivariate logistic regression, gender and age had a significant effect (P = 0.013 and P = 0.024, respectively) and the combination of these two variables was highly predictive for severe toxicity (P = 0.001). On the basis of gender and age, a model was developed to predict the probability of severe toxicity in male patients and female patients (Figure 2).

**DISCUSSION**

We have described the efficacy and safety of sunitinib treatment in an unselected mRCC patient population and can be found in general oncological practice. In our mRCC patients, the SD rate (39%) resembled that observed in the large phase III clinical trial in which sunitinib was compared with interferon-α, but the PR rate was slightly lower (23 vs 35%; Motzer et al, 2007). The PR rate in patients with clear cell histology (39%), however, was similar to that in the phase III study in patients with clear cell mRCC only (Motzer et al, 2007). The mRCC risk groups (Motzer et al, 2002) appropriately predicted PFS and OS in this patient population, which indicates that the Blom-pretreated factors model is still valid to predict survival in mRCC in the sunitinib era. The prognostic criteria of Choueiri et al (2007) designed for patients with clear cell histology receiving VEGF-targeted therapy, however, did not discriminate a difference in OS between risk groups 1 and 2 in our patient population. An explanation may be that we have treated a large number of cytokine-pretreated patients (68%) as well as patients with non-clear cell histology (17%).

In the non-clear cell histology patient population, 10 out of 14 patients had SD, whereas no PB was observed. Recently, Choueiri et al (2008) have reported their experience with sunitinib and sorafenib in patients with non-clear cell histology. During either sunitinib or sorafenib treatment, 5 out of 15 patients with non-clear cell histology, either papillary or chromophobe tumours, reached a PR, whereas 56 patients had SD of more than 4 months.
The present data and the study of Choueiri et al (2008) indicate that patients with non-clear cell histology may benefit from sunitinib. Furthermore, patients with poor performance status (ECOG >1) and brain metastases also experienced benefit from sunitinib-treatment in 65 and 40% of cases, respectively.

Treatment-related adverse events were mostly grade 1 or 2 and only five grade 3 toxicities were observed. The incidence rates of the most common grade 3 adverse events requiring dose discontinuation and/or reduction, such as hand–foot syndrome, stomatitis, diarrhoea, fatigue and hypertension were greatly similar to the rates reported in previous trials (Demetri et al, 2006, Motzer et al, 2006a, b, 2007). In this patient population, we observed a relatively lower incidence of thrombocytopenia and leucopenia than that reported in the largest trial on sunitinib so far (Motzer et al, 2007). Although thyroid function was not measured consistently, only five patients experienced hypothyroidism grade 1–2 (data not shown).

More than half of our patients needed a dose reduction of sunitinib and 35 out of 82 patients (43%) because of treatment-related adverse events. In comparison, only for 25% of the patients treated with sunitinib in the large randomised phase III trial of sunitinib vs IFN-α a dose reduction was reported, which might partially be explained by a higher number of patients with ECOG >1 in our population. The remarkably high number of dose reductions, however, was not only based on grade 3 toxicity, but also on the accumulation of a series of grade 1 and 2 adverse events. Those toxicities were palliated in every possible way.

Some adverse events, however, interfered excessively with daily life, such as stomatitis and taste alteration requiring changes in food habits, hand–foot syndrome limiting walking and the urgent pattern of diarrhoea with risk for soiling. In this respect, the NCI-CTC grading system is inadequate to express the impact of particular toxicities of sunitinib for the well-being of the patient.

Our findings are indicative that the sunitinib dosing schedule is not optimal for unselected mRCC patients and that a number of patients are initially overtreated resulting in unnecessary adverse events. On the other hand, patients who do not experience any toxicity may be undertreated. Therefore, dosing on the basis of BSA might be meaningful, as BSA was highly correlated with severe toxicity. In the previous phase I study in patients with solid tumours, the simulated interpatient variability in drug plasma levels between BSA-normalised and fixed dosing was comparable on days 1 and 20 for both sunitinib and its major plasma metabolite SU012622 (Farrar et al, 2006). It was concluded that no or minimal improvement in variability could be expected from calculating the dose on the basis of BSA. With respect to our data, it should be reconsidered to administer initial doses on the basis of BSA and taper off to tolerable doses if required, or increase the dose if no toxicity is observed. Alternatively, population-based sunitinib SU012622 plasma levels could be of help to develop better algorithms for optimal sunitinib dosing.

With the use of the fixed dosing regimen, we not only found a highly significant correlation between severe sunitinib-related toxicity and patient characteristics BSA, but also with female

Figure 1  Kaplan–Meier curves for progression-free survival and overall survival of mRCC patients treated with sunitinib for risk groups 1 (– – ) according to the MSKCC criteria (Motzer et al, 2002) (A and C) and the criteria according to Chuseiri et al (2007) (B and D).
gender and high age. We developed a model to predict the probability of severe toxicity based on gender and age in which BSA was not additive. Although the model requires external validation, it might be helpful to closely monitor patients at risk to develop invalidating adverse events on sunitinib given in the currently proposed schedule. It can also be proposed to dose patients on BSA and determine, whether female gender and high age remain prognostic factors for severe toxicity. Any grade 3 toxicity was also significantly related to gender and BSA, but not to age. The occurrence of any grade 3 adverse event was the reason for dose reduction or discontinuation of sunitinib in 79% of these patients.

Ten (12%) patients required continuous dosing at a lower dose of 37.5 mg daily owing to objective disease progression or recurrence of disease-related symptoms in the 2-week period of rest of the treatment cycle. Two phase II studies have demonstrated that the safety of a continuous dosing schedule of 37.5 mg per day in patients with RCC and gastrointestinal stromal tumours (GIST) was similar to that of the intermittent schedule (George et al).


Table 3 Non-haematological adverse events

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Grade 1 n (%)</th>
<th>Grade 2 n (%)</th>
<th>Grade 3 n (%)</th>
<th>All %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaemia</td>
<td>34 (5)</td>
<td>17 (2)</td>
<td>7 (9)</td>
<td>71</td>
</tr>
<tr>
<td>Nausea</td>
<td>31 (6)</td>
<td>9 (1)</td>
<td>4 (5)</td>
<td>54</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>13 (2)</td>
<td>17 (2)</td>
<td>4 (5)</td>
<td>34</td>
</tr>
<tr>
<td>Hand-foot syndrome</td>
<td>14 (2)</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>20</td>
</tr>
<tr>
<td>Fatigue</td>
<td>12 (2)</td>
<td>14 (2)</td>
<td>5 (6)</td>
<td>32</td>
</tr>
<tr>
<td>Fatigue grade 1</td>
<td>8 (1)</td>
<td>7 (0)</td>
<td>5 (0)</td>
<td>16</td>
</tr>
<tr>
<td>Fatigue grade 2</td>
<td>4 (0)</td>
<td>7 (0)</td>
<td>5 (0)</td>
<td>16</td>
</tr>
<tr>
<td>Fatigue grade 3</td>
<td>1 (0)</td>
<td>4 (0)</td>
<td>5 (0)</td>
<td>16</td>
</tr>
<tr>
<td>Taste alteration</td>
<td>22 (4)</td>
<td>6 (1)</td>
<td>0 (0)</td>
<td>32</td>
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<tr>
<td>Hypertension</td>
<td>5 (1)</td>
<td>17 (1)</td>
<td>5 (0)</td>
<td>27</td>
</tr>
<tr>
<td>Hypertension grade 1</td>
<td>4 (0)</td>
<td>17 (1)</td>
<td>5 (0)</td>
<td>27</td>
</tr>
<tr>
<td>Hypertension grade 2</td>
<td>1 (0)</td>
<td>17 (1)</td>
<td>5 (0)</td>
<td>27</td>
</tr>
<tr>
<td>Hypertension grade 3</td>
<td>0 (0)</td>
<td>17 (1)</td>
<td>5 (0)</td>
<td>27</td>
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<tr>
<td>Anorexia</td>
<td>4 (0)</td>
<td>12 (1)</td>
<td>5 (0)</td>
<td>22</td>
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<tr>
<td>Headache</td>
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<td>6 (0)</td>
<td>2 (0)</td>
<td>15</td>
</tr>
<tr>
<td>Yellow skin</td>
<td>12 (2)</td>
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<td>12</td>
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<tr>
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<td>4 (0)</td>
<td>0 (0)</td>
<td>11</td>
</tr>
<tr>
<td>Haematological adverse event*</td>
<td>92</td>
<td>37</td>
<td>0</td>
<td>132</td>
</tr>
</tbody>
</table>

Table 4 Haematological adverse events

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Grade 1 n (%)</th>
<th>Grade 2 n (%)</th>
<th>Grade 3 n (%)</th>
<th>All %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaemia</td>
<td>10 (12)</td>
<td>9 (11)</td>
<td>1 (1)</td>
<td>24</td>
</tr>
<tr>
<td>Leucocytopenia</td>
<td>7 (10)</td>
<td>3 (4)</td>
<td>1 (1)</td>
<td>11</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>7 (7)</td>
<td>3 (4)</td>
<td>1 (1)</td>
<td>11</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>7 (7)</td>
<td>3 (4)</td>
<td>1 (1)</td>
<td>11</td>
</tr>
<tr>
<td>Haematological adverse event*</td>
<td>92</td>
<td>37</td>
<td>0</td>
<td>132</td>
</tr>
</tbody>
</table>

Figure 2 Probability of severe toxicity from sunitinib (50 mg per day 4 weeks on and 2 weeks off) in patients with advanced RCC based on the following model: Probability of severe toxicity in male patients = exp(-2.750 + 0.059*age)/(exp(-2.750 + 0.059*age) + 1); Probability of severe toxicity in female patients = exp(-2.750 + 0.059*age)/(exp(-2.750 + 0.059*age) + 1); Grey lines represent confidence intervals.

Table 5 Severe toxicity causing change of sunitinib dosing

<table>
<thead>
<tr>
<th>Reason for dose reduction</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutropenia grade 3</td>
<td>6</td>
</tr>
<tr>
<td>Fatigue grade 3</td>
<td>5</td>
</tr>
<tr>
<td>Hand-foot syndrome grade 2 – 3</td>
<td>5</td>
</tr>
<tr>
<td>Combination of several grade 1 – 2 toxicities</td>
<td>5</td>
</tr>
<tr>
<td>Diarrhoea grade 3</td>
<td>2</td>
</tr>
<tr>
<td>Diarrhoea grade 2 – 3</td>
<td>1</td>
</tr>
<tr>
<td>Esophagitis grade 2 – 3</td>
<td>1</td>
</tr>
<tr>
<td>Hypertension grade 3</td>
<td>1</td>
</tr>
<tr>
<td>Rash mouth grade 3</td>
<td>1</td>
</tr>
<tr>
<td>Cognitive disorder grade 2</td>
<td>1</td>
</tr>
<tr>
<td>Transient ischaemic attack grade 3</td>
<td>1</td>
</tr>
</tbody>
</table>
| Ten (12%) patients required continuous dosing at a lower dose of 37.5 mg daily owing to objective disease progression or recurrence of disease-related symptoms in the 2-week period of rest of the treatment cycle. Two phase II studies have demonstrated that the safety of a continuous dosing schedule of 37.5 mg per day in patients with RCC and gastrointestinal stromal tumours (GIST) was similar to that of the intermittent schedule (George et al).
et al, 2007; Srinivas et al, 2007). In addition, preliminary results suggest a comparable PFS and OS for the two dosing schedules (Faries et al, 2007), although the objective response rate appears to be lower. In mRCC, the 4 weeks on and 2 weeks off schedule is the most preferred as a direct relation between the exposure to sunitinib (area under the plasma concentration-time curve) which is the highest during the 4 weeks on 50 mg per day, and a higher probability of PK, longer time-to-tumour progression, longer OS, and greater decrease in tumour volume have been observed (Hook et al, 2007).

In conclusion, sunitinib demonstrates clinical benefit in unsellected mCRC patients, including patients with non-clear cell histology, brain metastases and an ECOG performance status > 1.

The need for dose reduction owing to adverse events in this unsellected mCRC patient population is rather high. Gender, age and BSA are highly predictive of severe toxicity. Attempts to optimise the dosing schedule of sunitinib in unsellected mCRC patients are warranted.

ACKNOWLEDGEMENTS

We thank Elinor Kleeks and Lisevina Weyer for help with data management.

REFERENCES


Taylor AM van der Veldt et al, 2007; Srinivas et al, 2007). In addition, preliminary results suggest a comparable PFS and OS for the two dosing schedules... van der Veldt et al, 2007; Srinivas et al, 2007). In addition, preliminary results suggest a comparable PFS and OS for the two dosing... van der Veldt et al 265


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Chapter 7

Reversible cognitive disorders after sunitinib for advanced renal cell cancer in patients with preexisting arteriosclerotic leukoencephalopathy

Astrid A.M. van der Veldt, Alfons J.M. van den Eertwegh, Klaas Hoekman, Frederik Barkhof, Epie Boven

Reversible cognitive disorders after sunitinib for advanced renal cell cancer in patients with preexisting arteriosclerotic leukoencephalopathy

In 2006, the Food and Drug Administration has approved sunitinib (sunitinib malate, SU11248; Sutent®) for the treatment of advanced renal cell cancer and imatinib-resistant gastrointestinal stromal tumors (GIST). Sunitinib is a novel oral tyrosine kinase inhibitor that targets multiple receptors, e.g. the vascular endothelial growth factor receptors (VEGFR-1 and VEGFR-2), platelet-derived growth factor receptors α and β (PDGFRs) and c-Kit and Flk-3 [1, 2]. Blocking VEGF and PDGF receptors will inhibit tumor angiogenesis.

In the past years, the standard treatment of metastatic renal cell cancer (mRCC) has been on the basis of cytokines, such as interferon-α (IFN-α), but only <20% of patients have a response. A summarized analysis of two phase II studies on second-line sunitinib in mRCC has revealed a partial response in 42% of patients and stable disease for >3 months in 24% of patients [3, 4].

A recently completed, large randomized phase III trial has shown a significant increase of the median progression-free survival time in patients treated with sunitinib when compared with that in patients on IFN-α (11 versus 5 months) [5]. In advanced GIST after failure on imatinib, sunitinib has also demonstrated significant clinical benefit with a progression-free survival time of 22 weeks as compared with 6 weeks in patients on placebo [6].

Sunitinib is tolerated reasonably well. In general, side-effects graded according to National Cancer Institute-Common Toxicity Criteria do not exceed grade 2 and are reversible upon dose reduction or discontinuation of the drug. The most important side-effects are fatigue, diarrhea, nausea, stomatitis, hypertension, hand–foot syndrome, leukopenia and thrombocytopenia [5–6]. In the previous trials, patients included had to fulfill prespecified criteria, but currently the drug can be prescribed widely to patients in the general oncology practice.

Here, we describe three elderly patients with mRCC who developed cognitive and behavioral changes during sunitinib treatment. In all three patients, brain metastases were excluded. The neurological symptoms disappeared after discontinuation of sunitinib.

An 84-year-old female patient was known since 2002 with a local recurrence and liver metastases of renal cell cancer after a nephrectomy in 1997. For aggravating trigeminal neuralgia, she received gabapentin 300 mg three times per day. For one episode of atrial fibrillation in 2006, she received metoprolol. Additional medication consisted of risodronate for osteoporosis, diclofenac/interaprox for arthritis and pantoprazole to prevent gastritis. In 2004, she was on treatment of mRCC with IFN-α for 6 months. For one episode of atrial fibrillation in 2006, she received metoprolol. Additional medication consisted of risodronate for osteoporosis, diclofenac/interaprox for arthritis and pantoprazole to prevent gastritis. In 2004, she was on treatment of mRCC with IFN-α for 6 months.
letters to the editor

without clinical benefit. Because of progressive disease in 2006, the patient started sunitinib (50 mg daily oral dosing for 4 weeks, followed by a 2-week rest period in a cycle of 6 weeks). On a regular visit on day 12, her blood pressure had risen as compared with the baseline value (from 128/80 to 142/72 mmHg as measured by ambulatory 24-h blood pressure monitoring). On day 14, the patient visited the outpatient clinic because of periods of confusion and disorientation. She also had word-finding difficulties and a walking disorder. Furthermore, the pain of the trigeminal neuralgia had increased. At that time, sunitinib was temporarily interrupted. The next day the patient was admitted to the hospital because of increased confusion. The neurologist diagnosed cognitive disorders consisting of disorientation for time, expressive aphasia, perseveration and a gait disorder. A computed tomography (CT) scan of the brain demonstrated precocious leukoencephalopathy consistent with subcortical arteriosclerotic encephalopathy (SAE), but excluded brain metastasis (Figure 1A). Of interest, a magnetic resonance imaging (MRI) scan carried out in 2004 for trigeminal neuralgia had already demonstrated SAE of sufficient severity to fulfill criteria for vascular dementia according to the radiological criteria of the National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherche et l’Enseignement en Neurosciences (NINDS-ARIEEN)

(Figure 1B) [7]. Vascular dementia, however, had never been a clinical diagnosis in this patient. Three days after discontinuation of sunitinib, she completely recovered. Recovery from excessive pain of the trigeminal neuralgia did not occur until stereotactic radiotherapy. Sunitinib was not restarted. Thus, 7 months later, the neurological symptoms did not recur.

A 74-year-old male patient was diagnosed in 2006 with intracranial metastases, lung metastases and bone metastases of renal cell cancer after a nephrectomy in 2000. The patient also suffered from coronary artery disease with pectoral angina for which he received isosorbide mononitrate, calcium carbonate and nitroglycerine. Furthermore, he received metoprolol for hypertension, pravastatin for hypercholesterolemia, esmepram for hearthburn and almidem with fluticasone for emphysema. He likely had beginning dementia because of episodes of amnesia and hallucinations, but brain imaging had never been carried out. For progressive mHCC, the patient started in 2006 with sunitinib (50 mg daily 4 weeks on, 2 weeks off). On a regular visit on day 13, the blood pressure had raised as compared with the baseline value (from 110/60 to 122/71 mmHg as measured by ambulatory 24-h blood pressure monitoring). On day 18, the patient was admitted to the hospital because of a change in behavior suspected for brain metastases. Neurological examination showed a mask face, severe apathy, moderate bradypnoea, moderate hypokinesia and moderate rigidity with cog wheeling. Retrospectively, the patient’s family had noticed these changes since the start of sunitinib treatment. CT and MRI scans excluded brain metastases, but demonstrated SAE with ischemic deficits fulfilling the radiological criteria of NINDS-ARIEEN (Figure 2) [7]. Sunitinib was discontinued and 3 days thereafter the neurological symptoms disappeared. After a rest period of 2 weeks, sunitinib was restarted at a dose of 37.5 mg. Nine days later the patient was admitted to the hospital again because of recurrent bradypnoea, hypokinesia and rigidity. At this second admission, the symptoms were less severe and the blood pressure had not changed. It was decided to permanently discontinue sunitinib, after which the patient recovered promptly within 4 days. Seven months later the neurological symptoms have not recurred. Since 2006, an 82-year-old male patient was known with lung metastases of renal cell cancer. In 1992, the patient had a durable complete remission of the lung metastases after nephrectomy and one dose of interlaken-2. In 2002, he received an endoprosthesis for an abdominal aortic
annou.y. The patient did not have a history of cognitive problems. His medication consisted of diclofenac and paracetamol/codeine for pain in the chest and right hip, lansoprazole for constipation, esomeprazole for heartburn and tamsulosin to treat impotence. In 2006, the patient started with sunitinib (50 mg daily 4 weeks on, 2 weeks off) for progressive mRCC. CT scan evaluation after two cycles demonstrated stable disease. At the end of the fourth cycle, the patient visited the outpatient clinic because he experienced visual hallucinations and word-finding difficulties. Other neurological symptoms were absent. The blood pressure had raised as compared with the baseline value (110/59 versus 160/75 mmHg). A CT scan excluded brain metastases, but visualized age-related SAH (Figure 3). The severity was not sufficient to fulfill the NINDS-AIREN criteria [7]. During the rest period, the cognitive symptoms recovered promptly. Because the neurological symptoms were possibly related to sunitinib, the fifth cycle was started at a reduced dose of 37.5 mg. The complaints did not recur during the following cycles.

We describe three elderly patients with mRCC and accompanying cerebral vascular changes, who developed cognitive disorders on treatment with sunitinib. Since all patients recovered within 1 week, which is in line with the elimination half-life of sunitinib of 1–1.8 h [8], a causal relationship with the drug seems evident. Rechallenge with a reduced dose was successful in the third patient. The recurrent symptoms upon dose reduction in the second patient are also indicative for a causal relation.

In our patients, we did not detect brain metastases, and cerebrovascular accident or delirium caused by a metabolic disorder or co-medication could be excluded.

Since sunitinib is metabolized in the liver by cytochrome P450 isoenzyme 3A (CYP3A), attention should be paid to a drug interaction caused by co-medication. Inhibitors (e.g. clarithromycin or vorapaxar) and inducers (e.g. ketoconazol or terfenadine) of CYP3A can affect the plasma concentration of sunitinib and should be avoided. Our patients did not use co-medication that requires CYP3A for metabolism. Recovery from neurological symptoms also occurred in the patient on continuous gabapentin for trigeminal neuralgia.

Cognitive disorders during sunitinib treatment have not been described before. There is a case report, however, on a transient ischemic attack upon bevacizumab treatment [9]. Further, reversible neurological symptoms during treatment with bevacizumab and sorafenib (BAY 43–9006) have been reported [10–13]. In these cases, a reversible posterior leukoencephalopathy syndrome (RPLS) was the underlying...
cause which was accompanied by an elevated blood pressure. According to the product information, RPLS has also been described during treatment with sunitinib (>1% of cases). In our patients, however, there was absolutely no suspicion of a treatment-induced attack or evidence of posterior leukoencephalopathy on CT and MRI scans, while their blood pressure was only mildly increased.

Sunitinib and other inhibitors of VEGF signaling, such as sorafenib and bevacizumab, can induce hypertension to a variable degree [3, 4, 14, 15]. VEGFR-2 plays a role in the regulation of the vascular tone, since inhibition of the VEGFR-2 signaling route may cause vasoconstriction [16]. The effect of VEGF(R) inhibitors on the cerebral vasculature is unknown. Sunitinib and its metabolite are known to penetrate the brain up to 30%–40% of plasma concentrations in animal studies [17]. Since the neurological symptoms in our patients were reversible, sunitinib might reduce the cerebral blood flow due to vasoconstrictive effects.

Vascular dementia is known to be associated with a reduced cerebral blood flow, especially in the frontal lobes [18]. It is conceivable that sunitinib decreases the cerebral blood flow by vasoconstriction which might become symptomatic in elderly patients with preexisting cerebral vascular disease, such as microangiopathy. Ideally, future studies should investigate the cerebral blood flow before and during sunitinib treatment.

Positron emission tomography utilizing 18O-labeled H2O and O2/O is a valuable imaging modality to quantify regional blood flow and volume [19].

These three case reports strongly indicate a relationship between cognitive disorders and sunitinib. All three patients appeared to have preceding arteriosclerotic leukoencephalopathy which most likely has contributed to the development of those side-effects. Treating physicians should be aware of the occurrence of cognitive disorders in elderly patients using this new antitumor agent. Fortunately, the neurological symptoms seem to be reversible upon discontinuation or a dose reduction of sunitinib. Additional investigations of the effects of VEGF(R) inhibitors on the cerebral vasculature are warranted.

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letters to the editor

References


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Chapter 8

Pharmacogenetic pathway analysis for determination of sunitinib-induced toxicity


Pharmacogenetic Pathway Analysis for Determination of Sunitinib-Induced Toxicity


ABSTRACT

Purpose
To identify genetic markers in the pharmacokinetic and pharmacodynamic pathways of sunitinib that predispose for development of toxicities: thrombocytopenia, leukopenia, mucosal inflammation, hand-foot syndrome, and any toxicity according to National Cancer Institute Common Toxicity Criteria higher than grade 2.

Patients and Methods
A multicenter pharmacogenetic association study was performed in 219 patients treated with single-agent sunitinib. A total of 31 single nucleotide polymorphisms in 12 candidate genes, together with several nongenetic variants, were analyzed for a possible association with toxicity. In addition, genetic haplotypes were developed and related to toxicity.

RESULTS
The risk for leukopenia was increased when the G allele in CYP1A1 2455A/G (odds ratio [OR], 6.24; P = 0.029) or the A allele in CYP3A5 (OR, 2.8; P = 0.016) were present. The risk for mucosal inflammation was increased in the presence of the G allele in CYP1A1 2455A/G (OR, 4.03; P = 0.046) or a copy of TT in the ABCB1 1236C/T, 11034C/T, and ABCG2 694C/T haplotypes (OR, 2.56; P = 0.021) and the prevalence of hand-foot syndrome was increased when a copy of TT in the ABCB1 1236C/T, 11034C/T, and ABCG2 694C/T haplotypes (OR, 2.56; P = 0.021) was present.

CONCLUSIONS
This exploratory study suggests that polymorphisms in specific genes encoding for metabolizing enzymes, efflux transporters, and drug targets are associated with sunitinib-related toxicities. A better understanding of genetic and nongenetic determinants of sunitinib toxicity should help to optimize drug treatment in individual patients.

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INTRODUCTION

The oral, multigeneric tyrosine kinase inhibitor sunitinib (sunitinib malate; Sutent, Pfizer Pharmaceuticals Group, New York, NY) is known to inhibit vascular endothelial growth factor receptors (VEGFRs) 1, 2, and 3, platelet-derived growth factor receptor (PDGFR) α and β, and several tyrosine kinase 3 receptor (FLT3), and the receptor encoded by the ret proto-oncogene (RET) 7. Sunitinib is approved for first-line treatment of metastatic renal cell carcinoma (mRCC) and imatinib-resistant metastatic gastrointestinal stromal tumors (GIST). 7,13 Targeted cancer therapies are generally considered to be less toxic than conventional chemotherapy since they specifically inhibit tyrosine kinase receptors that are frequently overexpressed or mutated in various types of tumor cells. 7

Tyrosine kinases, however, are also present in normal tissues and toxic effects are therefore difficult to eliminate. The 4 weeks on 2 weeks off dosing schedule of sunitinib was selected for the first phase I study on request of the health authorities to allow patients to recover from potential bone marrow and adrenal toxicity observed in animal models, indicating that toxicity was regarded as a serious problem. 7,8 Although the proportion of patients with grade 3 or 4 adverse events was relatively low in the recent phase III studies, a dose interruption appeared to be necessary in 34% of patients with mRCC and in 28% of patients with GIST whereas a dose reduction was required in 32% and 11%, respectively. Similar
A total of 219 patients from five Dutch medical centers were analyzed in this study. This study was approved by the medical ethics review board. Patients were treated at the Erasmus University Medical Center (n = 51), the Nether- lands Cancer Institute (n = 51), Leiden University Medical Center (n = 47), Vrije University Medical Center (a = 36), and the University Medical Center Groningen (a = 35). This collection of DNA and patient data was performed between June 2006 and May 2008. A total number of 159 nRCC, 30 GIST, and 10 patients with other tumors were included in this study. Of these, 77 patients with nRCC and 26 patients with GIST were treated according to an expanded access program of sunitinib. Eligible patients were treated with single-agent sunitinib for at least one treatment cycle (6 consecutive weeks of 50 mg per day followed by a 2-week period of rest).

Study Design
Sunitinib toxicity was evaluated during the first treatment cycle by CTCAE version 3.0. Toxicity scores were assessed by analysis of adverse events, physical examination, and laboratory parameters in baseline (be-fore starting sunitinib), after 4 weeks of sunitinib therapy, and at 6 weeks (just before starting the second cycle). Demographic and clinical data of patients were reported on case report forms designed for data collection in this study. Patient characteristics considered relevant for experiencing toxicity were age, sex, ethnicity, body-surface area (BSA), Eastern Cooperative Oncology Group (ECOG) performance status, tumor type, renal, liver, and bone marrow function (serum creatinine, total bilirubin, albumin, ALT, AST, he- moglobin, leukocytes, and thrombocytes). Blood and/or serum samples taken for routine patient care were stored at -20°C at the local hospital laboratory. Of each patient one whole blood or serum sample was collected from the participating centers. All samples were anonymized by a third party, according to the instructions stated in the Codex for Proper Use and Proper Conduct in the SoR Regulatory Code of Conduct (www.lodex.org).

Determination of Toxicity
All adverse events were graded by independent physicians of the particip- ing medical centers. Four- and 6-week reported toxicities were compared in phase I study. The primary outcome measure of this study was thrombocytopenia, leucopenia, mucosal inflammation, hand-foot syndrome, and objective and toxicities. In case of any toxicity higher than grade 2, a dose interruption and, depending on the kind of toxicity, a reduced treatment with 25% dose reduction is advised in the drug label of sunitinib. Moreover, mucosal inflammation and hand-foot syndrome are frequently reported and poorly manageable and therefore dose reduction is not usually considered. In addition, dose reduction of at least 25% according to the drug label (data complete for 157 patients), which is applied because of safety or tolerability issues, after cycles 1 to 3, was related to the toxicity outcomes.

Statistical Design and Data Analysis
For the analysis of toxicity, we used dichotomous and points expressed as increased toxicity (yes/no) or any toxicity (yes/no). All demographic and clinical variables were tested univariately against the selected primary outcome using $r$ test, the Mann-Whitney $U$ test or the $\gamma$ test, depending on the tested variables. A $r$ test was also used to detect linkage Table 1. Polymerase Gene Variants in the Pharmacokinetic and Pharmacodynamic Pathways of Sunitinib

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency</th>
<th>No. of Genes</th>
</tr>
</thead>
<tbody>
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<td>PKG1</td>
<td>77%</td>
<td>130</td>
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<tr>
<td>CYP1A2</td>
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</table>

No. is number assigned yet.

**Table 1. Polymerase Gene Variants in the Pharmacokinetic and Pharmacodynamic Pathways of Sunitinib**

**PATIENTS AND METHODS**

**Toxicity**

Sunitinib is used as palliative therapy. It is time to toxicities were compared in phase I study. The primary outcome measure of this study was thrombocytopenia, leucopenia, mucosal inflammation, hand-foot syndrome, and any toxicity higher than grade 2. Toxicities were selected based on objective and toxicities. In case of any toxicity higher than grade 2, a dose interruption and, depending on the kind of toxicity, a reduced treatment with 25% dose reduction is advised in the drug label of sunitinib. Moreover, mucosal inflammation and hand-foot syndrome are frequently reported and poorly manageable and therefore dose reduction is not usually considered. In addition, dose reduction of at least 25% according to the drug label (data complete for 157 patients), which is applied because of safety or tolerability issues, after cycles 1 to 3, was related to the toxicity outcomes.

**Genetic Polymorphisms**

Nitro- or CYP3A4 polymorphisms in seven genes involved in the pharmaco-kinetic and -dynamic pathways of sunitinib were selected. Selection criteria for the polymorphisms were an allele frequency higher than 5% in whites and an essential clinical outcome based on previous reports of associations or the assumption that non-enzymatic renal acid changes lead to changed protein functionality. The selected poly- morphisms are listed in Table 1. Methods for genotyping assay validation and haplotype estimation are included in the Appendix (online only).

**Statistical Design and Data Analysis**

For the analysis of toxicity, we used dichotomous and points expressed as increased toxicity (yes/no) or any toxicity (yes/no). All demographic and clinical variables were tested univariately against the selected primary outcome using $r$ test, the Mann-Whitney $U$ test or the $\gamma$ test, depending on the tested variables. A $r$ test was also used to detect linkage...
discontinuation (LDI). The polymorphisms were initially tested with 294.16. 13.2. From ranked to P = .1, the polymorphisms were fitted and the most appropriate model (multiplicative, dominant, or recessive) was selected. The number of copies of each haplotype was used as a parameter in this analysis. The polymorphisms and haplotypes were tested univariately against the selected primary outcomes using a 4 test. Candidate variables with P = .1 were selected for the multiple logistic regression analyses with toxicity as depending variable. All multivariate logistic regression analyses were conducted for age, sex, and ECOG performance status. Additional patient characteristics were introduced in the multivariate analysis based on univariate tested results if P = .1. Missing data were kept as missing data except for BSA and ECOG performance status. Missing BSA values (n = 13) were replaced for the median BSA (2.38 m²) and missing ECOG performance status (n = 5) were replaced for the median ECOG performance status (1). To test this action, the multi- variate analyses were performed with and without the replacement of the patients with missing BSA and ECOG performance status. Similar results were generated, indicating that the replacement was legitimate. All statistical analy- ses were performed using SPSS version 16.0.1 (SPSS, Chicago, IL). With the sample size of our study, an increase in toxicity of 17% could be measured between two groups with a power of 80% and a CI of 95%. All results from the multivariate analyses with Fless than .05 were considered significant. Since this was an exploratory study, no correction for multiple testing was done.

RESULTS

Patients
Nineteen of 219 patients had to be excluded from analysis for several reasons including progressive disease (PD) during the first treatment cycle resulting in early death (n = 4), discontinuation of anumum in the first treatment cycle due to adverse events (hyperten- sion grade 3, headache grade 3, and rash grade 3, respectively; n = 3), and no acceptable genotyping access rates due to poor DNA quality (n = 12). For toxicity analyses, a total of 200 patients were assess- ed (Table 1). To test this action, the multi- variate analyses were performed with and without the replacement of the patients with missing BSA and ECOG performance status. Similar results were generated, indicating that the replacement was legitimate. All statistical analy- ses were performed using SPSS version 16.0.1 (SPSS, Chicago, IL). With the sample size of our study, an increase in toxicity of 17% could be measured between two groups with a power of 80% and a CI of 95%. All results from the multivariate analyses with Fless than .05 were considered significant. Since this was an exploratory study, no correction for multiple testing was done.

Toxicities
The hematoxicologic toxicities scored in this analysis were thrombo- cytopenia (48% any grade) and leukopenia (59% any grade). Nonle- morologic toxicities were primarily any toxicity higher than 2 (22%), mucosal inflammation (44%), and hand-foot syndrome (19%; Table 3). Dose reduction after cycles 1 to 3 was related to mucosal inflam- mation (P = .002) and any toxicity higher than grade 2 (P = .001).

Pharmacogenetic Risk Factors for Sunitinib-Induced Toxicity
The results of the multivariate logistic regression analysis for the selected end points thrombocytopenia, leukopenia, mucosal inflam- mation, hand-foot syndrome, and any toxicity higher than grade 2 are summarized in Table 4. For thrombocytopenia, an increase in age (P = .030) and ECOG performance status (P = .050) were indepen- dently significant in the multivariate logistic model. The factors asso- ciated with development of leukopenia were CYP1A2*2A5*A5, the presence of the G allele in an additive model was related to a 6.2-fold increase in the risk for leukopenia during the first treatment cycle (P = .029), the presence of the FE37 38C allele (dominant model) was related to a 2.8-fold reduction in the risk for leukopenia (P = .008), the absence of the NKL3 CAG haplotype was related to a

<table>
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<td>Median hemoglobin, mg/dL</td>
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Abbreviations: ECOG, Eastern Cooperative Oncology Group; GCT, gastroin- testinal stromal tumor; PD, progressive disease; PD, partial response; SD, stable disease; PD, progressive disease.

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1.7-fold increased risk for leukopenia ($P = 0.041$), and one grade increase in ECOG performance status, implicating a worse clinical condition, was related to a 1.8-fold reduction in the risk of leukopenia ($P = 0.016$). The presence of the V607E/T199T allele (additive model) was related to an increased risk of 2.4-fold for the development of any toxicity higher than grade 2 ($P = 0.048$), while the risk for this toxicity was 2.6-fold higher when 1 or 2 copies of T1 were in the ABCG2 haplotype versus present ($P = 0.016$). For mucosal inflammation, only CYP3A4 2A3/5A5 was independently related, the C allele (additive model) resulted in a 4.0-fold higher risk for mucosal inflammation ($P = 0.021$).

The occurrence of hand-foot syndrome was related to the ABCG2 haplotype; the absence of copies of the TTT haplotype was protective and was related to a 2.6-fold lower risk to experience hand-foot syndrome as compared to patients with copies of the TTT haplotype ($P = 0.032$). The explained variance ($R^2$) of the patient characteristics, without taking the polymorphisms into account, in the multivariate analysis was between the 2% to 10% of the total variance. After adding the selected polymorphisms the explained variance increased to 10% to 25% of the total variance.

**Discussion**

To the best of our knowledge, this is the first study exploring the relationship between drug-induced toxicity and genetic polymorphisms in genes encoding for enzymes, drug transporters, and targets involved in the pharmacokinetics and pharmacodynamics of sunitinib.

Sunitinib is metabolized by cytochrome P450 3A4 (CYP3A4) and CYP3A5. In addition, affinity of sunitinib for the ATP-binding cassette transporters ABCG2 and ABCB1 has also recently been reported.31 The transcription of CYP3A4 is regulated by members of the NR1I1 nuclear receptor subfamily.32 Metabolism through CYP3A4 and CYP3A2 is hypothesized since these enzymes appear to be involved in the metabolism of multiple tyrosine kinase inhibitors (eg, imatinib, sorafenib).33,34 Both genes encoding the sunitinib targets, as well as genes encoding the enzymes (except for CYP3A4, in which not functional polymorphisms have been identified) and drug transporters involved in sunitinib’s disposition and metabolism are highly polymorphic and may be related to the different toxicity responses in patients treated with sunitinib.

Although the nature and incidence of adverse events related to sunitinib are currently well recognized and described, data regarding determinants of toxicity are still scarce.35,36,37,38,39,40 So far, only one study has described factors (low BSA, high age, female sex) that are associated with the development of severe toxicities, defined as dose reduction or permanent discontinuation of sunitinib therapy.39 That study, however, was limited to patient characteristics and no genetic determinants were investigated. In our study, those patient characteristics, and another (performance status), were included as covariates in the data analysis. We should emphasize, however, that the definition of the end point severe toxicity is different in both studies as well as the observed study period (whole sunitinib treatment period vs first treatment cycle in our study).

To our knowledge, we report for the first time herein that the ABCB1 TTT haplotype was related to hand-foot syndrome. The TTT haplotype as well as the T allele at positions 3143C/T and the T polymorphism at 1298C/T separately have been associated with higher exposure to drugs transported by ABCB1 due to a decreased expression of the ABCB1 transporter.41,42 Also, for the other ABC transporters investigated, ABCG2, the TT haplotype was related to the development of increased toxicity (eg, any toxicity $\geq$ grade 2). This haplotype has been associated with increased ornitine-oximutase expression, a tyrosine kinase inhibitor that uses metabolic and predispiction pathways similar to those of sunitinib.43 Thus, our results concerning ABCB1 and ABCG2 are in line with previously reported functional consequences of the studied genetic variants and might lead to an increased systemic exposure to sunitinib resulting in dose-limiting toxicities. Certainly, to confirm our findings, further studies that relate pharmacogenetics to pharmacokinetics and pharmacodynamics are required.

Thus far, the cytochrome CYP3A1 enzyme has not been described as being involved in the metabolism of sunitinib. For other receptor tyrosine kinase inhibitors, such as erlotinib, imatinib, and gefitinib, affinity for CYP3A1 has been demonstrated in in vitro studies.44,45,46 Therefore, we also included genetic variants of CYP3A4 in this study. The polymorphism studied in CYP3A4 resulting in an amino acid change of isoleucine 462 valine was found to be related to the occurrence of mucosal inflammation and leukopenia. This suggests that CYP3A4 may also play a role in the metabolism of sunitinib in vivo.

In addition, we investigated genetic polymorphisms in the NR1I1 gene, encoding the constitutive androstane receptor. This nuclear receptor plays an important role in the regulation of multiple drug detoxification genes, such as CYP3A4. The functionality of polymorphisms in NR1I1 is not yet fully elucidated, however we found a relationship between the absence of the CAG haplotype in...
Table 1: Factors Relevant for Esudate-Related Toxicity, Defined as Thrombocytopenia, Leukopenia, Any Toxicity > Grade 2, Mucosal Inflammation, or Hand-Foot Syndrome

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<th>P</th>
<th>Multivariate OR</th>
<th>95% CI</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other other</td>
<td>0.39</td>
<td>0.26 to 0.58</td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Multivariate models included age = 1 and sex = 1.1, which are set, and interactions between the two groups of genotypes, and 1.2, a set, and included each included as and with numbers. Abbreviations: OR, odds ratio; ECOG, Eastern Cooperative Oncology Group; description haplotypes; T′′ = ARK2 -1922CT and 1942TT; T′ = −1922CT and −1942GT; T = −1922CT and −1942GG; G′′ = ARK2 -1922GG and 1942TC; G′ = −1922CT and −1942GT; G = −1922CT and −1942GG; R11 = R11C and 1143G; R2 = R11C and 1580T/C. All toxicity outcomes in the described are included for any toxicity > grade 2. Under the uncorrected data, only two genotypes are included in the multivariate analysis.
Pharmacogenetics of Sunitinib Toxicity

The functionality of VEGF and its receptors was previously found to be predictive for the development of coronary artery disease due to a lower binding efficiency of VEGF to the polymorphic VEGFR-2. In our study, those genotypes were related to the development of any toxicity higher than 2, which predominantly included fatigue, thrombocytopenia, and hypertension. The polymorphic receptor might therefore be involved in sunitinib-induced cardiac toxicity and the development of hypertension.

The importance of the FLT3 receptor has been described in relation to the development of several subtypes of leukemia such as acute myeloid leukemia, acute lymphocytic leukemia, and chronic myeloid leukemia, in which FLT3 is frequently overexpressed and/or mutated. However, the association between FLT3 polymorphism and a reduction in the risk of leukemia has not previously been described. Since sunitinib-induced leukemia could be regulated strongly by this polymorphic receptor the clinical relevance should be further investigated.

In our study, a large number of candidate polymorphic loci were evaluated and multiple analyses of each genetic polymorphism were performed. This introduces the potential problem of multiple testing which increases the risk to find false-positive relations. However, our study was designed to explore associations that should be confirmed in an independent group of patients. The presented odds ratios and CIs facilitate comparisons of replicate studies with our data.

The ECOG performance status was not consistently related to the occurrence of toxicities in our study. The quantified performance status is multifactorial and is dependent on subjective interpretation of the physician. Moreover, in our study patients with poor performance status had relatively high baseline thrombocyte and leukocyte counts resulting in a small number of reported leukopenia and thrombocytopenia in this group in the first treatment cycle.

Toxicities in the first treatment cycle of sunitinib were used as outcome measure. It is reasonable that signs of clinical deterioration from disease progression in later cycles could be misinterpreted and would interfere with the drug-induced toxicity outcome. We hypothesized that patients that suffer from relatively mild (grade 1 or 2) toxicities in the first treatment cycle were at risk for developing more severe toxicity during further treatment cycles because the 2 weeks of rest would not be sufficient for patients to recover to baseline conditions. This cumulative effect is underscored by measured blood cell counts and the observed dose reduction after cycles 1 to 3. Indeed, we found for leukocyte count, and to a lesser extent also for thrombocyte count, that 91% and 73%, respectively, of the patients had not returned to baseline values (defined as >90% of baseline counts) at cycle 2 day 1 (data not shown). In addition, we found that musosal inflammation and any toxicity higher than grade 2 were strongly related to a dose reduction after cycles 1 to 3, indicating that these toxicities are regarded as clinically relevant to the treating physicians.

Together, the genetic, clinical, and demographic determinants in this exploratory analysis explain between 10% and 23% of the total variance in toxicity response. Although it indicates that the major part of the variability is left unexplained, it also shows that pharmacogenetics may make a great contribution to explaining variability in sunitinib toxicity as compared to the nongenetic determinants in our study. From this study, we cannot conclude whether the genetic variants are prognostic or predictive markers, due to the absence of a placebo-treated control group of patients. However in the future, pharmacogenetics may help to select patients which need a prior dose reduction to prevent toxicities.

In conclusion, this study suggests a relationship between polymorphisms in the genes CYP1A1, ABCB1, ABCG2, NR11, VEGFR2, and FLT3 and the development of sunitinib toxicity. The next step will be to validate our data with the aim to better understand the determinants of sunitinib toxicity.

REFERENCES


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AUA Authors' Disclosures of Potential Conflicts of Interest

Although all authors completed the disclosure declaration, the following authors or their immediate family members indicated a financial interest which may be related to the subject matter under consideration in this article. Certain relationships marked with a “C” were compensated; those relationships marked with a “D” were disclosed to the responsible parties and were not found to be related to the value of the content or the significance of the content in the context of the material

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Data analysis and interpretation: Nolka P. van Erp, Karel Excoffon, Ron H.J. Matthiessen, Judith A.M. Wessels, Hans Gelderman


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Data analysis and interpretation: Nolka P. van Erp, Karel Excoffon, Ron H.J. Matthiessen, Judith A.M. Wessels, Hans Gelderman


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CORRECTIONS

Author Corrections


In Table 4, under Hand-foot syndrome, the ABCB1 CCT haplotype was given, whereas it should have been the TCG haplotype, as follows:

"TCG-TCG → TCG-other → other-other"

The authors apologize to the readers for the mistake.

DOI: 10.1200/JCO.2010.30.2380


In the Results section, under “QoL of Patients With Endometrial Cancer Assigned to Laparoscopy Versus Laparotomy,” the number of patients in the last sentence of the third paragraph was given as 273, whereas it should have been 237, as follows:

"Of 237 patients reporting the time to return to work, the median time to return to work was 42 days for the laparoscopy group compared with 45 days in the laparotomy group, a small but significant difference (Wilcoxon rank sum test, P /H11005 /H11005.04)."

The authors apologize to the readers for the mistake.

DOI: 10.1200/JCO.2010.30.2398

The March 20, 2010, article by Goldzweig et al, entitled “Meeting Expectations of Patients With Cancer: Relationship Between Patient Satisfaction, Depression, and Coping” (J Clin Oncol 28:1560-1565, 2010), contained an error in the spelling of the second author’s name. It was originally given as Amichai Meirowitz and should have been Amichay Meirovitz. The authors apologize to the readers for the mistake.

DOI: 10.1200/JCO.2010.30.2414

Journal Corrections


In Figure 1B, the labels for resistant and sensitive cell lines were inadvertently transposed.

In the legend of Figure 2A, the reference to “bottom, lung” as well as the corresponding P value (P /H11005 /H11005.03) should have been omitted.

Journal of Clinical Oncology apologizes to the authors and readers for the mistakes.

DOI: 10.1200/JCO.2010.30.2323

The February 1, 2010, Diagnosis in Oncology article by Zareifar et al, entitled “T-Cell Lymphoblastic Lymphoma of the Sternum” (J Clin Oncol 28:e51-e53, 2010), contained an error in the spelling of the third author’s name. It was originally given as Mehran Karim and should have been Mehran Karimi. Journal of Clinical Oncology apologizes to the authors and readers for the mistake.

DOI: 10.1200/JCO.2010.30.2364

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Chapter 9

Choi response criteria for early prediction of clinical outcome in patients with metastatic renal cell cancer treated with sunitinib

Astrid A.M. van der Veldt, Martijn R. Meijerink, Alfons J.M. van den Eertwegh, John B.A.G. Haanen, Epie Boven

Choi response criteria for early prediction of clinical outcome in patients with metastatic renal cell cancer treated with sunitinib

BACKGROUND: Because sunitinib can induce extensive necrosis in metastatic renal cell cancer (mRCC), we examined whether criteria defined by Choi might be valuable to predict early sunitinib efficacy.

METHODS: Computed tomography was used for measurement of tumour lesions in mm and lesion attenuation in Hounsfield units (HU). According to Choi criteria partial response (PR) was defined as at least 10% decrease in size or 15% increase in attenuation. The Response Evaluation Criteria in Solid Tumours (RECIST) is the most widely used measurement system in clinical trials and is not change the management of these patients. The Choi criteria (Choi et al., 2007) have defined a partial response (PR) as a 10% decrease in size or a 15% increase in attenuation on CT scan, whereas PD was defined as >10% increase in size without meeting PR criteria by change in attenuation. Because both Choi criteria correlated better with disease-specific survival in imatinib-treated GIST patients than RECIST, several studies have indicated that sunitinib can induce necrosis in metastatic renal cell cancer (mRCC). As Choi et al. (2007) have developed new response criteria to evaluate imatinib treatment in patients with GIST. The Choi criteria include changes in tumour attenuation on computed tomography (CT), which reflect tumour density (Benjamin et al., 2007; Choi et al., 2007). Choi et al. (2007) have defined a partial response (PR) as a >10% decrease in one-dimensional tumour size or a >15% increase in tumour attenuation on CT scan, whereas PD was defined as >10% increase in size without meeting PR criteria by change in attenuation. The Choi criteria correlated better with disease-specific survival in imatinib-treated GIST patients than RECIST.

RESULTS: A total of 55 mRCC patients treated with sunitinib were included. At first evaluation, according to the Response Evaluation Criteria in Solid Tumours (RECIST), 7 patients had PR, 38 stable disease (SD) and 10 progressive disease (PD), whereas according to Choi criteria 16 patients had PR, 6 SD and 13 PD. Median tumour attenuation decreased from 66 to 47 HU (P = 0.001). In patients with PR, Choi criteria had a significantly better predictive value for progression-free survival and overall survival (both P < 0.001) than RECIST (P = 0.665 and 0.191 respectively). The predictive value for RECIST increased (P = 0.001 and <0.001 respectively), when best response during treatment was taken into account.

CONCLUSION: Choi criteria could be helpful to define early mRCC patients who benefit from sunitinib, but the use of these criteria will not change the management of these patients.

Keywords: sunitinib; renal cell cancer; Choi criteria; RECIST; tumour attenuation; tumour response;


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Choi response criteria for early prediction of clinical outcome in patients with metastatic renal cell cancer treated with sunitinib.
Choi response criteria for sunitinib in RCC

<table>
<thead>
<tr>
<th>Response</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>Disappearance of all lesions</td>
</tr>
<tr>
<td>PR</td>
<td>A decrease in size of ≥ 50% or a decrease in tumour attenuation (HU) of ≥ 15% on CT, MRI, or FDG PET. No new lesions.</td>
</tr>
<tr>
<td>SD</td>
<td>No obvious progression of non-measurable disease. Minimal increase in size of ≤ 10% and does not meet criteria of PR or PD. No symptomatic deterioration attributed to tumour progression.</td>
</tr>
<tr>
<td>PD</td>
<td>An increase in tumour size ≥ 20% and does not meet criteria of PR or PD.</td>
</tr>
</tbody>
</table>

Abbreviations: CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease; CT = computed tomography; MRI = magnetic resonance imaging; FDG PET = positron emission tomography; HU = Hounsfield units.

usefulness of Choi criteria with RECIST in sunitinib-treated mRCC patients.

PATIENTS AND METHODS

Patients and treatment

Medical records of patients who were treated with sunitinib for advanced RCC in two centres in the Netherlands (VU University Medical Center and The Netherlands Cancer Institute) from December 2005 to October 2007. Most patients had been included in an expanded access programme (EAP) (Van der Veldt et al, 2008a) until September 2008 after which sunitinib was registered and available on director's prescription. In the EAP, each participant signed a protocol-specific informed consent approved by the institutional review board in accordance with national and institutional guidelines. For further analysis of CT scans according to Choi criteria, adequate safeguards to protect patient privacy were mentioned.

Sunitinib was administered orally at a dose of 50 mg daily, consisting of 6 weeks of treatment followed by a 2-week rest period in cycles of 6 weeks. Dose escalation of sunitinib to 50, 62.5, 75, 100, and 112.5 mg was allowed depending on the type and severity of adverse events. If patients had symptoms of PD during the rest period, there was the possibility for continuous dosing of sunitinib at 37.5 mg per day. For evaluation of sunitinib efficacy, CT scans were performed at baseline and during treatment to assess clinical response according to RECIST version 1.0 (Therasse et al, 2000). For RECIST, best response was also determined on subsequent CT scans during treatment. Progression-free survival (PFS) was the time between the first day of sunitinib treatment and the date of PD on the CT scan according to RECIST, clear clinical evidence of PD, or death due to PD within 12 weeks after the last response evaluation. If a patient had not progressed, PFS was censored at the time of the last follow-up. If the PD or death occurred before the patient died due to PD later than 12 weeks after the last response evaluation, PFS was censored at the last adequate tumour assessment. Overall survival (OS) was the time between the first day of treatment and the date of death or the date at which a patient was last known to be alive. For PFS and OS analyses, data collection was closed on 1 September 2009.

Image analysis

Patients were eligible for inclusion in the analysis, if they had CT scans at baseline and at first evaluation according to the same scan protocol in the same hospital and at least one tumour lesion at baseline ≥ 10 mm. Patients with bone metastasis (n = 2) or primary tumour (n = 1), as only evaluable lesion were excluded. Primary tumours were also excluded, as the overall response may be understimated due to their enormous size (Van der Veldt et al, 2008b; Rent et al, 2009). Permanent brachytherapy (Hadjipavlou et al, 2008) and bone metastases at baseline were excluded.

Routine helical CT scans of the thorax and abdomen were obtained with a scanning delay of 30 and 70 s after start of intravenous (i.v.) injection of a low-osmolar non-ionic contrast agent Omnipaque 300 (Amersham plc, Buckinghamshire, England) or Ultravist 300 (Bayer Shanghai Pharma, Berlin, Germany). For abdominal scans, Choi criteria were applied in the portal venous phase of contrast. All series were reconstructed in 5 mm contiguous axial slices. Scans were shown at standard soft tissue window (window centre, 20 Hounsfield units (HU); window width, 360 HU) to avoid pixel averages from surrounding lung parenchyma. Image viewing and manipulation were controlled with Carestream EAG 600 version 6.1 software (GE Healthcare Inc., Waukesha, WI, USA), which allows the radiologist to draw contours around the regions of interest. The software then automatically calculates the area enclosed by the perimeter and the mean attenuation of this area in HU. A specialised radiologist (MBM, 7 years of experience in radiology) masked to clinical history and patients' outcome and experinced in using the image viewing and manipulation software examined the CT scan images in the presence of a junior researcher (AAM vd V). To draw comparable contours over the tumours, we analysed CT scans at baseline and evaluation from one patient in the same session. Between the two observers, agreement on identification and delineation of the lesions was obtained in all cases. In addition, to evaluate the intra-observer variability for the determination of tumour attenuation, reproducibility of placing regions of interest over tumours was tested on 2 different days (≤3 months between the measurements). As to the intra-observer variability for tumour attenuation measurements, Spearman's correlation coefficients were ρ = 0.997 (P < 0.001) for the HU value of individual lesions as well as the mean HU value in the individual patients. For each patient, a maximum of 10 delineated tumour target lesions were identified (not more than 5 per organ). For RECIST measurements, the longest diameter of the tumour lesions was ≥ 10 mm, whereas for Choi criteria the diameter was ≥ 15 mm (Table 1; Choi et al, 2007). The attenuation on CT (density) of lesions ≥ 15 mm was determined in HUs by drawing a region of interest around the margin of the entire tumour. Then, the tumour attenuation measurements of all lesions were combined and a mean attenuation on CT was computed for each patient. Thereafter, the percentage of change in attenuation from the pretreatment scan to the first evaluation during sunitinib was calculated for each patient.

Statistics

Statistical analysis was performed using SPSS software (SPSS for Windows 15.0, SPSS Inc., Chicago, IL, USA). For testing possible correlations, the Spearman's correlation test was performed. The Wilcoxon's signed-ranks test was used to compare the changes in size and attenuation at baseline and at first evaluation. A two-tailed probability value of P < 0.05 was considered significant. For the analyses according to RECIST and Choi criteria, patients were categorised into response (CR + PR) vs no response (SD + PD). For RECIST, patients were also classified as having clinical benefit (CR + PR and SD ≥ 12 weeks) vs no clinical benefit (SD <12 weeks and PD). PFS and OS were calculated with the Kaplan–Meier method. Log-rank test was used to test the differences between survival curves.

RESULTS

Patients

A total of 35 mRCC patients treated with sunitinib were included in this study, of which 45 patients were participants in an EAP

<table>
<thead>
<tr>
<th>Table 1 Choi response criteria (Choi et al, 2007)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response</td>
</tr>
<tr>
<td>CR</td>
</tr>
<tr>
<td>PR</td>
</tr>
<tr>
<td>SD</td>
</tr>
<tr>
<td>PD</td>
</tr>
</tbody>
</table>

Abbreviations: CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease; CT = computed tomography; MRI = magnetic resonance imaging; FDG PET = positron emission tomography; HU = Hounsfield units.
of sunitinib. One patient was excluded due to evident differences in phases of i.v. contrast between the two subsequent CT scans. Table 2 presents the patients’ characteristics. The median age was 59 years (range: 20–81 years). Of 55, 48 patients had clear cell histology. Of 55 patients, 40 patients were cytokine-pretreated of whom 4 patients were also pretreated with other anti-angiogenic agents. The median time from the baseline CT scan and the initiation of sunitinib treatment was 0.5 months (range: 0–1.5 months). The median time from the start of sunitinib to the CT scan for first evaluation was 1.9 months (range: 1.1–3.4 months).

Response according to RECIST

For RECIST measurements, 225 tumour lesions were eligible. At first evaluation these lesions showed a median change in tumour size of −10% (range: −100 to +189%). Seven (13%) patients reached PR, 38 (69%) patients stable disease (SD), and 10 (18%) patients PD, resulting in 7 responders and 48 non-responders. Five out of ten patients with PD were categorised as PD based on the occurrence of new lesions, including two patients with symptomatic brain metastases. Ten (18%) patients had SD at first evaluation, but reached a PR at later time points (median time to PR: 3.9 months; range: 2.4–9.7 months), resulting in an overall PR rate of 31%. A total of 24 patients had SD after 12 weeks as best response.

Table 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total N: 55</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>34 (62)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>21 (38)</td>
<td></td>
</tr>
<tr>
<td>Median age, years (range)</td>
<td>58 (20–81)</td>
<td></td>
</tr>
<tr>
<td>ECOG performance status</td>
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</tr>
<tr>
<td>0</td>
<td>24 (45)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>22 (40)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5 (9)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (4)</td>
<td></td>
</tr>
<tr>
<td>Tumour type</td>
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<td></td>
</tr>
<tr>
<td>Clear cell</td>
<td>48 (87)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>7 (13)</td>
<td></td>
</tr>
<tr>
<td>Previous nephrectomy</td>
<td>45 (82)</td>
<td></td>
</tr>
<tr>
<td>Prior treatment</td>
<td></td>
<td></td>
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<tr>
<td>No</td>
<td>15 (27)</td>
<td></td>
</tr>
<tr>
<td>Cytokine based-therapy</td>
<td>40 (73)</td>
<td></td>
</tr>
<tr>
<td>Anti-angiogenic therapy</td>
<td>4 (7)</td>
<td></td>
</tr>
<tr>
<td>No. of disease sites</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>≥2</td>
<td>20 (36)</td>
<td></td>
</tr>
<tr>
<td>≥3</td>
<td>24 (45)</td>
<td></td>
</tr>
<tr>
<td>Sites of disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>49 (89)</td>
<td></td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>32 (59)</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>11 (20)</td>
<td></td>
</tr>
<tr>
<td>Local recurrence</td>
<td>7 (13)</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>4 (7)</td>
<td></td>
</tr>
<tr>
<td>MSKCC risk groups*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (favourable)</td>
<td>11 (20)</td>
<td></td>
</tr>
<tr>
<td>1–2 (intermediate)</td>
<td>26 (47)</td>
<td></td>
</tr>
<tr>
<td>3 (poor)</td>
<td>7 (13)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (2)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ECOG = Eastern Cooperative Oncology Group; MSKCC = Memorial Sloan-Kettering Cancer Center. ‘Risk groups according to MSKCC prognostic criteria. Criteria based on the 5 risk factors: low Karnofsky performance status (≤40%), high LDH (>1.5 times the upper limit of normal), low serum haemoglobin, high corrected serum calcium (>10 mg per 100 ml), and time from initial diagnosis to treatment of less than 1 year’ (Motzer et al. 2002).

Choi response criteria for sunitinib in RCC

Figure 1

An example of a renal cell cancer patient with lung metastases at baseline (A) and pulmonary cavitations at first evaluation during sunitinib (B, arrows). For the purpose of illustration the lung window setting is shown.
A

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Table 4 Change in tumour size and density for tumour lesions included in the Choi criteria for evaluation of sunitinib treatment in patients with mRCC

<table>
<thead>
<tr>
<th>Tumour site</th>
<th>Number of eligible lesions</th>
<th>Median pretreatment values (range)</th>
<th>Median values at first evaluation (range)</th>
<th>Median change (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (size/attenuation)</td>
<td>Size (mm)</td>
<td>Attenuation (HU)</td>
<td>Size (%)</td>
</tr>
<tr>
<td>Lung</td>
<td>52/41</td>
<td>25 (15–91)</td>
<td>59 (15–18)</td>
<td>10 (5–118)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>−24 (−75 to +21)</td>
<td>−31 (−95 to +184)</td>
<td></td>
</tr>
<tr>
<td>Lymph node</td>
<td>63/56</td>
<td>26 (15–225)</td>
<td>68 (0–118)</td>
<td>30 (18–151)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 (−25 to +190)</td>
<td>13 (−70 to +48)</td>
<td></td>
</tr>
<tr>
<td>User</td>
<td>54/46</td>
<td>31 (19–80)</td>
<td>83 (0–105)</td>
<td>22 (42–120)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>−19 (−25 to +140)</td>
<td>13 (−70 to +45)</td>
<td></td>
</tr>
<tr>
<td>Abdominal aorta</td>
<td>29/24</td>
<td>40 (15–165)</td>
<td>62 (15–125)</td>
<td>42 (18–200)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 (−100 to +100)</td>
<td>13 (−70 to +45)</td>
<td></td>
</tr>
<tr>
<td>Thoracic sites</td>
<td>123/104</td>
<td>24 (15–140)</td>
<td>64 (0–135)</td>
<td>23 (16–150)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 (−100 to +118)</td>
<td>13 (−70 to +45)</td>
<td></td>
</tr>
</tbody>
</table>

Total number of lesions: 173/149

Table 5 PFS and OS of mRCC according to Choi criteria

<table>
<thead>
<tr>
<th>Choi response criteria</th>
<th>PFS* Months (range)</th>
<th>OS* Months (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responders (n = 17)</td>
<td>Log-rank = 14.1, P &lt; 0.001</td>
<td>Log-rank = 20.0, P &lt; 0.001</td>
</tr>
<tr>
<td>Non-responders (n = 9)</td>
<td>14.5</td>
<td>13.4</td>
</tr>
<tr>
<td>RECIST at first evaluation</td>
<td>Log-rank = 0.6, P = 0.48</td>
<td>Log-rank = 1.7, P = 0.39</td>
</tr>
<tr>
<td>Responders (n = 7)</td>
<td>18.3</td>
<td>13.4</td>
</tr>
<tr>
<td>Non-responders (n = 4)</td>
<td>9.0</td>
<td>13.2</td>
</tr>
<tr>
<td>RECIST at best response</td>
<td>Log-rank = 11.2, P &lt; 0.001</td>
<td>Log-rank = 13.2, P &lt; 0.001</td>
</tr>
<tr>
<td>Responders (n = 10)</td>
<td>17.2</td>
<td>13.3</td>
</tr>
<tr>
<td>Non-responders (n = 7)</td>
<td>7.0</td>
<td>13.3</td>
</tr>
<tr>
<td>Clinical benefit† (n = 4)</td>
<td>Log-rank = 13.2, P &lt; 0.001</td>
<td>Log-rank = 13.2, P &lt; 0.001</td>
</tr>
<tr>
<td>No clinical benefit‡ (n = 10)</td>
<td>26.7</td>
<td>12.3</td>
</tr>
</tbody>
</table>

Abbreviations: PFS, Progression-Free Survival; OS, Overall Survival.

Response according to Choi criteria

For Choi criteria, less tumour lesions were eligible than for RECIST (Table 3; Figure 1), namely 173. Lesions most frequently excluded from the analysis had a tumour size <15 mm before treatment (n = 38). In 24 lesions the change in tumour attenuation could not be determined due to a tumour size <15 mm at evaluation. At baseline, the median tumour size was 26 mm (range: 15–140 mm) for all lesions, with a median attenuation of 66 HUs (range: 6–135 HUs). During sunitinib at first evaluation, the median size and attenuation had decreased to, respectively, 16 mm (range: 8–188 mm; Wilcoxon’s signed-ranks test, P < 0.001) and 47 HUs (range: 4–112 HUs; P < 0.001). A significant decrease in attenuation was measured at all tumour sites (Table 4; Figure 2).

Preliminary analysis did not show a significant difference in the change in attenuation between the seven patients with non-clear cell histology and the 48 patients with clear cell histology. Overall, a weak correlation was calculated between the percentage of change in tumour size and the percentage of change in attenuation (Spearman’s ρ = 0.187, P = 0.022).

For Choi criteria, lesions that reached PR, were defined as SD, had PR, or had PD, were each defined as ID according to Choi criteria. Patients were categorized as ID according to Choi based on decrease in tumour size ≥15% (n = 12), decrease in tumour attenuation ≥15% (n = 10) or both (n = 15). All six patients with SD according to Choi criteria had a PFS ≥12 weeks. Of note, 5 out of 38 patients defined as ID by RECIST had PD according to Choi criteria. Patients were categorized as ID according to Choi criteria based on decrease in tumour size ≥15% (n = 10). All six patients with SD according to Choi criteria had a PFS ≥12 weeks. Of note, 5 out of 38 patients defined as ID by RECIST had PD according to Choi criteria.

Figure 2 An example of a renal cell cancer patient on sunitinib treatment in which the lung lesion (arrow) showed a decrease in attenuation at first evaluation. (A) At baseline, the tumour attenuation was 107 Hounsfield units (HU) and the longest diameter 48 mm. (B) At first evaluation, the tumour attenuation was 65 HUs (−39%) and the longest diameter was 19 mm (−19%).
criteria. These three patients had a PFS > 12 weeks among whom was one patient that reached a PFS > 10 months.

Survival analysis

At first evaluation in patients with PR, Choi criteria had a significantly better predictive value for PFS and OS ($P < 0.001$ for both) than RECIST ($P = 0.085$ and 0.191 respectively) (Table 5; Figure 3). When best response during treatment was analysed according to RECIST, the predictive value of RECIST increased for both PFS and OS ($P < 0.001$ and 0.001 respectively). For clinical benefit (PR and SD X 12 weeks), the predictive value of RECIST for PFS and OS was also significant ($P < 0.001$ for both). When the two Choi criteria were analysed separately, in which patients with new lesions were categorised as PD, only a 15% decrease in attenuation was predictive for PFS ($P = 0.018$; log rank $= 5.6$) and OS ($P = 0.005$; log rank $= 7.8$).

DISCUSSION

In this study, we evaluated whether the new Choi criteria, which include changes in tumour attenuation, are of additional value to predict outcome in mRCC patients treated with sunitinib. The response rate of 31% as measured by RECIST was comparable with that reported previously (Motzer et al., 2007), indicating that the present study is representative for sunitinib treatment in
At first evaluation, Choi criteria of PR were able to define a large population with a long PFS and OS, whereas RECIST PR only identified seven patients with favourable clinical outcome. When patients with PR and SD 12 weeks during treatment were taken into account, the predictive value of RECIST substantially increased. The latter could have been expected, as patients with ID at first evaluation are likely to continue sunitinib treatment and a substantial number will eventually reach a PR or SD 12 weeks.

During sunitinib treatment, we observed tumour necrosis as illustrated by a reduction in tumour attenuation with a median decrease of 24% at first evaluation. The TKI sorafenib can also induce extensive necrosis (Flaherty, 2007). In comparison with placebo, sorafenib prolonged PFS in cytokine-pretreated mRCC with almost 3 months (Escudier et al., 2007), although the complete response (CR) and PR rates by RECIST were only < 1 and 10% respectively (Escudier et al., 2007). These data suggest that non-responders by RECIST may have benefited from sorafenib. Therefore, Choi criteria may also be valuable to early identify mRCC patients who will have a favourable outcome from sorafenib treatment. Response evaluation by Choi criteria should ideally be planned on a fixed day during treatment, i.e. day 28 of sunitinib administration in the 4 weeks on 2 weeks off schedule in MRC. The variability in timing of the first on-treatment CT scan in this study, however, could not be avoided because of its retrospective design.

Although Choi criteria could easily be applied on routine standardised contrast-enhanced CT scans, there were several limitations in the use of these criteria for evaluation of sunitinib-induced responses in mRCC. First, a large number of lesions (22%), especially long metastases, which could be measured by RECIST, had to be excluded due to a size < 15 mm at baseline. As mRCC patients can have rather small metastases, the reliability of Choi criteria may decrease when smaller lesions (<15 mm) significantly decrease in size or attenuation, but are ineligible for assessment. Second, the reliability of Choi criteria may also decrease when fewer lesions are included for analysis. For example, a 15% decrease in lesion attenuation in five lesions would be more reasonable than measured in one single lesion. Third, the attenuation of heterogeneous lesions may be assessed inaccurately, as the mean value is calculated in one region of interest in one slice. Fourth, measurements of relative hypodense lesions at baseline may be less reliable, because a 15% decrease in attenuation is less accurate than measurements in lesions with higher pretreatment attenuation values. For that reason, use of absolute change may be more precise than the percent change in attenuation. Fifth, attenuation measurements are not possible in lesions with sunitinib-induced cavitations, which was the case in eight long lesions (Figures 1). Sixth, although the intra-observer variability appeared to be rather low, the above-described limitations of Choi criteria imply a risk of high inter-observer variability and may even lead to a change in response in an individual patient. Therefore, mutual agreement on the delineation method should be achieved between the observers. Last, although i.v. contrast was administered according to the same scanning protocol and patients with clear differences in i.v. contrast were excluded, slightly different phases of scanning at subsequent time points may lead to incorrect changes in lesion attenuation. In that respect, it should also be mentioned that sunitinib-induced changes in cardiac output (Chu et al., 2007) might possibly influence the distribution of i.v. contrast. In mRCC patients, administration of i.v. contrast may be harmful in the presence of an impaired renal function.

The ultimate goal in the palliative treatment of mRCC is to prevent disease progression combined with acceptable quality of life. Sunitinib, however, is associated with a wide range of mild toxicities that can be cumbersome, and alternative treatments for mRCC, such as sorafenib and temsirolimus, are readily available. Therefore, surrogate markers for poor PFS and OS are warranted in patients with SD at first evaluation. Unfortunately, Choi criteria were not able to identify patients with clear-cut progression, because three patients defined as PD had a PFS > 12 weeks. Compared with RECIST, Choi criteria were not less optimal for identifying PD, probably due to the > 10% increase to the > 25% increase used by RECIST.

In conclusion, Choi criteria can be easily applied on contrast-enhanced CT scans of mRCC patients treated with sunitinib, but its reliability is limited, especially in patients with most lesions <15 mm, a small number of lesions, heterogeneous lesions or hypodense lesions at baseline. Although Choi criteria had a significantly better predictive value for PFS and OS than RECIST at first evaluation in patients with PD, its predictive value for outcome was similar to that of RECIST at later time points. Because Choi criteria were not able to easily identify patients with PD, these criteria may not change the management of sunitinib-treated mRCC patients.

ACKNOWLEDGEMENTS

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REFERENCES


Sunitinib-induced hemoglobin changes are related to the dosing schedule

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Sunitinib-Induced Hemoglobin Changes Are Related to the Dosing Schedule

To the Editor: We read with interest the correspondence by Alexandrescu et al4 reporting the occurrence of erythrocytosis in first of 21 patients treated with sorafenib or sunitinib. We also observed erythrocytosis during sunitinib treatment, although we detected that hemoglobin and erythrocyte changes occurred in a cyclic pattern. In an expanded access program, 82 patients with metastatic renal cell cancer were treated with sunitinib 50 mg daily, 4 weeks on, 2 weeks off. We measured hemoglobin levels on days 1 and 28 of each cycle and on day 14 of the first cycle. In 90% of patients, we observed a transient increase in hemoglobin during the first cycle. The median hemoglobin level of 7.5 mmol/L (range, 5.2 to 10.4 mmol/L) at baseline increased to 8.4 mmol/L (range, 6.0 to 10.9 mmol/L; Wilcoxon signed-rank test, P < .001) on day 14 and 8.0 mmol/L (range, 5.7 to 10.7 mmol/L; P < .001) on day 28 of the first cycle. After the 2-week rest period, the median hemoglobin level returned to baseline of 7.8 mmol/L (range, 5.2 to 10.4 mmol/L; P = .127). This transient increase in hemoglobin occurred in all subsequent cycles (Fig 1). In 69% of patients, the maximum level was reached during the first cycle (range, cycle 1 to cycle 10) with a median increase of 1.2 mmol/L. In 46 patients in the expanded access program, hemoglobin-associated variables, including hematocrit, erythrocytes, mean corpuscular volume, mean cellular hemoglobin, and mean corpuscular hemoglobin concentration, as well as vascular endothelial growth factor (VEGF), were available (Table 1). The incidence of erythrocytosis (erythrocytes above the upper limit of normal) was 20% during the first cycle, whereas a significant increase in erythrocyte numbers occurred in 81% of those patients.

The cyclic kinetics in hemoglobin values and erythrocyte numbers indicate that sunitinib scheduling is the cause of these changes. Thus far, its mechanism is not known. Tam et al3 have described that neutralization of VEGF in mouse and primate models can result in an increase in the secretion of erythropoietin from the liver, leading to erythrocytosis and erythrocytosis. Another study in mice has demonstrated that sunitinib can increase erythropoietin levels. Therefore, we measured erythropoietin at baseline and on day 14 of the first cycle in 20 unselected patients and indeed found an increase from a median of 11.2 U/L (range, 2.2 to 119.9) to 26 U/L (range, 12.9 to 54.2; P < .002). Although the difference in erythropoietin levels is significant, an erythropoietin-induced increase in erythrocytes is not expected to diminish rapidly within the 2 weeks of rest.

We propose another mechanism for the transient hemoglobin changes during sunitinib treatment. Like other inhibitors of VEGF signaling, sunitinib is known to raise blood pressure.5 In 39 of our patients, blood pressure was consistently monitored with a Dinamap浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦浦
and induced albumin escape, primarily in the lung, heart, liver, kidney, and GI tract. In 67 of 82 patients, baseline albumin levels were available; albumin decreased during sunitinib treatment from a median of 31 g/L on day 1 to a median of 30 g/L on day 28 (n = 67; P < 0.001) and increased after the rest period from 38 to 40 g/L (n = 67; P = 0.03). Loss of circulating plasma volume is a likely cause for the relative increase in hemoglobin, hematocrit, and erythrocytes by sunitinib.

In summary, we describe a zig-zag pattern in hemoglobin levels and erythrocyte numbers during sunitinib treatment in metastatic renal cell cancer patients. On the basis of previous studies and our findings, we hypothesize that the cyclic kinetics of hemoglobin and erythrocytes is the result of a temporary loss of intravascular fluid by sunitinib. 

REFERENCE


AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Chapter 11

Increased numbers of small circulating endothelial cells in renal cell cancer patients treated with sunitinib


* Authors contributed equally

Increased numbers of small circulating endothelial cells in renal cell cancer patients treated with sunitinib

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Abstract Mature circulating endothelial cell (CEC) as well as endothelial progenitor populations may reflect the activity of anti-angiogenic agents on tumor neovasculature or even constitute a target for anti-angiogenic therapy. We investigated the behavior of CECs in parallel with hematopoietic progenitor cells (HPCs) in the blood of renal cell cancer patients during sunitinib treatment. We analyzed the kinetics of a specific population of small VEGFR2-expressing CECs (CD45<sup>neg</sup>/CD34<sup>bright</sup>), HPCs (CD45<sup>dim</sup>/CD34<sup>bright</sup>), and monocytes in the blood of 24 renal cell cancer (RCC) patients receiving 50 mg/day of the multi-targeted VEGF inhibitor sunitinib, on a 4-week-on/2-week-off schedule. Blood was taken before treatment (C1D1), on C1D14, C1D28, and on C2D1 before the start of cycle 2. Also plasma VEGF and erythropoietin (EPO) were determined. Remarkably, while CD34<sup>bright</sup> HPCs and monocytes decreased during treatment, CD34<sup>bright</sup> CECs increased from 69 cells/ml (C1D1) to 180 cells/ml (C1D14; \( P = 0.001 \)) and remained high on C1D28. All cell populations recovered to near pre-treatment levels on C2D1. Plasma VEGF and EPO levels were increased on C1D14 and partly normalized to pre-treatment levels on C2D1. In conclusion, opposite kinetics of two circulating CD34<sup>bright</sup> cell populations, HPCs and small CECs, were observed in sunitinib-treated RCC patients. The increase in CECs is likely caused by sunitinib targeting of immature tumor vessels.

Keywords circulating endothelial (progenitor) cells · Renal cell cancer patients · Sunitinib · VEGF · Erythropoietin

Introduction

Anti-angiogenic compounds have shown efficacy in the clinic during recent years. In particular, the anti-vascular endothelial growth factor (VEGF) antibody bevacizumab [1] and the receptor tyrosine kinase inhibitors (TKIs) of the VEGF receptor family [2, sunitinib [3, 4] and sorafenib [5], have proven activity in a number of tumor types [6].

Sunitinib is an oral TKI of the VEGF receptors, platelet-derived growth factor (PDGF) receptors, Fl-3 and c-Kit, and has been approved for treatment of advanced renal cell
cancer (RCC) and imatinib-resistant gastrointestinal stromal tumors (GISTs). In a phase III trial in RCC patients, sunitinib has proven to be effective, albeit that a subset of RCC patients did not benefit from it [4]. Therefore, there is still a need for better understanding which conditions, factors, and cells facilitate or limit the beneficial effects of sunitinib on tumors.

In addition to immunohistochemical staining of tumor biopsies and imaging techniques that quantify tumor growth and perfusion [7], measurement of plasma circulating proteins, such as VEGF [8] or soluble VEGFRs [9], may reflect responsiveness to treatment. However, VEGF or sVEGFR2 plasma levels have not been shown to be predictive of response to sunitinib in GIST patients [10]. Alternatively, changes in the levels of circulating cells, such as newly recruited progenitor cells and monocytes or detached endothelial cells may be induced by anti-angiogenic treatment [7, 11].

Circulating endothelial progenitor (CEPs) cells have been suggested as potential pharmacodynamic or predictive biomarker in tumor patients [11]. CEPs were first described by Asahara et al. [12], who introduced the concept of circulating, bone marrow-derived endothelial progenitor cells, contributing to adult vasculogenesis. Later, Lyden et al. [13] have demonstrated that both VEGFR2+/– circulating endothelial cells as well as VEGFR1+/–/CD34+/CD133+ monocytes contributed to tumor vascularization. Recently, the source of highly proliferative endothelial outgrowth cells (EOCs) has been identified in CD34+/CD45+/CD133+ circulating cell populations [14, 15]. Besides CEPs, circulating endothelial cells (CECs) as thought to be shed from mature blood vessels may reflect the efficacy of anti-vascular treatment, as suggested in a number of studies [16–18]. At present, no studies have reported on changes in frequencies of CECs or CEPs in combination with hematopoietic progenitor cells (HPCs) during sunitinib treatment of RCC patients.

Previously, we have identified a rare population of small CD45+/CD133+/CD34+/CD133+/VEGFR2+/– cells in the peripheral blood (PB) of healthy volunteers, with increased numbers in cancer patients [19]. On the basis of endothelial marker expression these cells were indicated as “small-size EC-like cells” or CECs [20], because they are relatively small (<10 μm) when compared with mature CECs [21–23]. Also, these markers are the same as that of the source of highly proliferative late outgrowth endothelial cells present in umbilical cord blood or PB [15] and is clearly distinct from CD45+/CD34+/CD133+/VEGFR2+ cells in the peripheral blood of healthy volunteers, with increased numbers in cancer patients [19]. On the basis of endothelial marker expression these cells were indicated as “small-size EC-like cells” or CECs [20], because they are relatively small (<10 μm) when compared with mature CECs [21–23]. Also, these markers are the same as that of the source of highly proliferative late outgrowth endothelial cells present in umbilical cord blood or PB [15] and is clearly distinct from CD45+/CD34+/CD133+/VEGFR2+ cells in the peripheral blood of healthy volunteers, with increased numbers in cancer patients [19].

In a phase III trial in RCC patients, the date on which patients were last known to be alive. Data collection was closed on January 1st, 2008. HPCs, CECs, and plasma monitoring. At the time of blood sampling, the first 2 ml of blood was discarded and blood for flow cytometric enumerations was processed within 2–4 h. At each time-point, 7 ml of EDTA blood and 7 ml of citrate blood in a CPT tube (Becton Dickinson) were collected for measurement of circulating cell populations. One milliliter of full blood was used for the measurement of CECs and HPCs, based on CD45 and
CD34 marker expression and expressed as number per milliliter, as published in detail [19]. Analysis of the subsets of cells was performed with the antibodies CD45-FITC, CD34-APC, and IgG isotypes as has been described in detail [19]. For additional measurements of cell populations in patients, VEGFR2-APC and -PE antibodies were used. The viability marker 7-AAD was used to gate viable cells and annexin-V staining was used to determine early stages of apoptosis. To assure the gating of nucleated small CD34bright cells only, in a number of patients, we added extra analysis tubes using the dye styril-751 (LDS-751). Furthermore, we added tubes with 7-AAD plus 0.1% saponin to permeabilize the cells and allow access of the dye to nuclei of viable cells as described before [19, 25]. Flow cytometry was performed on a FACSCalibur (BD Biosciences) and data were analyzed using CellQuest Pro software. Subfractions of white blood cells (WBC) were calculated as number per milliliters of blood by using standard total WBC count on Sysmex [19]. The remaining EDTA blood was used for the preparation of plasma and stored at −80°C. Plasma VEGF levels and EPO were measured in duplicate with enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis). Albumin was determined using conventional methods in the department of clinical chemistry.

Human umbilical cord blood was obtained from full-term deliveries and was processed for flow cytometry, according to the patients PB samples and used as a reference to identify the CD45neg/CD34bright/CD133neg CEC population [15].

Statistics
Frequencies of circulating cell populations (numbers/ml), plasma levels of VEGF (pg/ml), and EPO (mIU/ml) were enumerated and expressed as median (range). Wilcoxon Signed Ranks test (SPSS for Windows 14.0, SPSS, Inc., Chicago, IL) was used to compare the biomarkers at pre-treatment and during treatment on C1D14, C1D28, and C2D1. Clinical benefit (CB) was defined as SD plus PR. PFS and OS were calculated with the Kaplan–Meier method and tested with the log rank test. Values of P ≤ 0.05 (two-sided) were considered statistically significant.

Results
Patient characteristics and response to treatment
Twenty-four RCC patients treated with sunitinib were enrolled in the study. One patient died on C1D14, due to early progression and was excluded from the analysis. The remaining patients (17 males and 6 females) had a median age of 63 years (range 40–84) at the start of treatment. For further patients characteristics, see Tables 1 and 2.

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<td>Female</td>
<td>6</td>
<td>26</td>
</tr>
<tr>
<td>Median age, years (range)</td>
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<td>Prior nephrectomy</td>
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<td>74</td>
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<tr>
<td>Prior cytokine-based therapy</td>
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<td>65</td>
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<tr>
<td>Site of metastatic disease</td>
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<tr>
<td>Lung</td>
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<tr>
<td>Liver</td>
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<tr>
<td>Bone</td>
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<tr>
<td>No. of disease sites</td>
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<tr>
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<tr>
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<tr>
<td>≥3</td>
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<td>MSKCC risk groups [15]</td>
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</tr>
<tr>
<td>Favorable risk</td>
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<td>Intermediate</td>
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<tr>
<td>Poor risk</td>
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<tr>
<td>Best response to sunitinib treatmenta</td>
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<tr>
<td>Partial response</td>
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<td>17 (19)</td>
</tr>
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<tr>
<td>Progressive disease</td>
<td>6</td>
<td>26 (29)</td>
</tr>
<tr>
<td>No evaluationb</td>
<td>2</td>
<td>7 (–)</td>
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<tr>
<td>Progression-free survivalc</td>
<td>8.0 (1.1–19.3)</td>
<td>–</td>
</tr>
<tr>
<td>Survivald</td>
<td>12.7 (1.4–25.2)</td>
<td>–</td>
</tr>
</tbody>
</table>

MSKCC, Memorial Sloan-Kettering Cancer Center
a CT or MRI was performed before treatment and after every two to three cycles to assess clinical response according to response evaluation criteria in solid tumors (RECIST) [24].
b Two out of 23 patients could not be evaluated for treatment response because of early discontinuation due to sunitinib-related side-effects.
c The PFS was the time between the first day of sunitinib and the date of progressive disease (PD) on CT or MRI or clear clinical evidence of PD.
d Survival was the time between the first day of treatment and the date of death or the date on which patients were last known to be alive.
patients, 4 patients (19%) achieved a PR as best response, 11 patients (52%) had SD, and 6 patients (29%) had PD. The median PFS of these 23 patients was 8.0 months (range 1.1–19.3) and the median OS was 12.7 months (range 1.4–23.2).

Blood cell counts during the first cycle of sunitinib

The median WBC count of the patients showed a decrease from 7.9 × 10^6 to 6.9 × 10^6 cells/ml on C1D14 (n = 23; P = 0.002) and a further decrease on C1D28 (from median pre-treatment 7.9 × 10^6 to 4.4 × 10^6 cells/ml, n = 15; P = 0.001). A similar pattern was seen for thrombocytes, neutrophils, and monocytes. The reduction of circulating monocytes and their partial recovery proceeded faster than the total WBC change, whereas the neutrophil decrease showed a more delayed effect. Erythrocytes and hemoglobin showed the reverse, i.e., a significant increase after 14 and 28 days, while the number of lymphocytes and basophils did not change during sunitinib treatment (Fig. 1).

Table 2 Patients characteristics and best response to sunitinib

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>RCC type</th>
<th>Prior treatment</th>
<th>Response</th>
<th>PFS (months)</th>
<th>Survival (months)</th>
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</thead>
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<tr>
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<td>PD</td>
<td>7.0</td>
<td>8.1</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>M</td>
<td>Papillary ca</td>
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<td>SD</td>
<td>10.1</td>
<td>11.0</td>
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<tr>
<td>4</td>
<td>76</td>
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<td>Second-line</td>
<td>SD</td>
<td>7.0</td>
<td>12.3</td>
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<td>57</td>
<td>M</td>
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<td>SD</td>
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<td>20.7</td>
</tr>
<tr>
<td>7</td>
<td>66</td>
<td>M</td>
<td>Papillary ca</td>
<td>Second-line</td>
<td>PD</td>
<td>1.2</td>
<td>1.4</td>
</tr>
<tr>
<td>8</td>
<td>60</td>
<td>M</td>
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<td>PR</td>
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<td>4.6</td>
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<td>11</td>
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<td>First-line</td>
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<td>9.1</td>
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<td>SD</td>
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<td>15.5</td>
</tr>
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<td>PD</td>
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</tr>
<tr>
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</tr>
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<td>15</td>
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<tr>
<td>16</td>
<td>59</td>
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<td>12.7</td>
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<td>17</td>
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<td>Clear cell</td>
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<td>9.7</td>
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<td>21</td>
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<td>PD</td>
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<td>15.4</td>
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<tr>
<td>22</td>
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<td>M</td>
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<td>5.1</td>
<td>9.0</td>
</tr>
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</table>

RCC, renal cell cancer; F, female; M, male; PR, partial response; SD, stable disease; PD, progressive disease; PFS, progression-free survival

a According to response evaluation criteria in solid tumors

Marker profile of two CD34bright populations: CECs and HPCs

Two populations of CD34bright circulating cells were evaluated: CECs and HPCs. The definitions of CECs and HPCs, according to CD45 and CD34 expression are visualized for a representative RCC patient (Fig. 2a) and for comparison from cord blood (Fig. 2b). CECs are CD45neg and CD133neg; HPCs are CD45dim and are largely CD133pos (Fig. 2a, b). Moreover, CECs have a slightly higher CD34 brightness than the majority of HPCs [19]. CECs are small in size being comparable with HPCs. CECs are viable cells, because they all exclude 7-AAD. We also checked in separate analysis tubes that both the CD34bright CD45neg and CD34bright CD45dim population had a similar positive 7-AAD/saponin staining as well as LDS-751 staining, confirming that both populations are nucleated cells. Other markers for which CECs are positive are...
CD31, CD105, CD146, and VEGFR2, as previously reported [19]. To confirm the VEGFR2 expression on CECs, we have measured VEGFR2 in parallel, in additional cancer patients. VEGFR2 positivity in CECs was high (median 65%), in contrast to the CD45<sup>dim</sup>/CD34<sup>bright</sup>/HPCs (1%). In addition, the EPO receptor was evaluated on CECs of five sunitinib-treated patients and was found present in 83.3% of the CECs (median range 66.7–93.3%). Plasma membrane VE-cadherin was undetectable in CECs in five treated patients (data not shown).

**Kinetics of CECs and HPCs during the first cycle of sunitinib**

A distinct difference in the kinetics of CECs (CD45<sup>neg</sup>/CD34<sup>bright</sup>/7-AAD<sup>neg</sup>) and HPCs (CD45<sup>dim</sup>/CD34<sup>bright</sup>/7-AAD<sup>pos</sup>)...
7-AAD<sup>neg</sup>) was observed during the first cycle of sunitinib (Fig. 3a). The median number of viable CECs before treatment (C1D1) was 69 cells/ml (range 8–472), much lower than the number of HPCs (1,350 cells/ml, range 305–5,351). The median of CECs increased from 69 on C1D1 to 180 cells/ml on C1D14 (n = 23; P = 0.001) and from pre-treatment 76 to 229 cells/ml on C1D14 (n = 23; P < 0.001). Both cell populations returned to values close to the pre-treatment levels after the 2-week period of rest (C2D1; Fig. 3a).

When the kinetic changes in circulating cells were expressed as percentage of pre-treatment values within individual patients, 102% increase in CECs numbers was observed after 2 weeks of treatment, whereas the HPCs showed a 65% decrease (Fig. 3b). A similar change was found on C1D28 (n = 14).

Plasma VEGF and EPO levels during the first cycle of sunitinib

Plasma levels of VEGF before treatment of sunitinib varied more than tenfold among individual patients and had a median value of 82 pg/ml (range 29–348, n = 19). These median levels increased from 82 to 185 pg/ml on C1D14 (n = 19, P = 0.001), from median pre-treatment 79 to 198 pg/ml on C1D28 (n = 12, P = 0.028) and returned to near pre-treatment levels on C2D1 (from 79 to 75 pg/ml, n = 12, P = 0.875; Fig. 3b). In a subgroup of patients, we assessed EPO levels and the median plasma EPO level on C1D1 was 12 mIU/ml, which increased with 63% after 14 days (median, n = 20; Fig. 3c). In six patients, EPO was measured during the complete cycle (Fig. 3d) showing increases of 60 and 216% at days C1D14 and C1D28, respectively, which remained above baseline level at C2D1.

Albumin concentrations determined in a larger group of patients did not change significantly over the 3-week period (data not shown).
RCC patients treated with sunitinib were unaltered at C1D28 (n = 67) in comparison with the initial values at C1D0, n = 81 (median of 38 μmol/l range 17–50 μmol/l and median of 41 μmol/l range 22–52 μmol/l, respectively).

Biomarkers and treatment outcome

Clinical benefit was observed in 15 out of 21 RCC patients. Seventeen of all patients had clear cell RCC, of which 14 showed CB. PD was observed in 6 patients; 3 clear cell RCC patients, 2 papillary carcinoma, and 1 chromophobe carcinoma indicating that the patients with a clear cell carcinoma had a good response to sunitinib. In the CB group, the change in CECs after 14 days was increased in 14 out of 15 patients and in the PD group 4 out of 6 patients showed an increase, while 2 had a decrease. An increased number of CECs (n = 18) after 14 days of sunitinib treatment, was associated with a longer PFS when compared with patients (n = 3) with a decreased number of CECs (log rank test; P = 0.034).

Discussion

We have investigated the changes in the frequency of circulating cells with specific emphasis on a population of small CD45<sup>−</sup>/CD34<sup>bright</sup> CECs, previously shown to be CD31<sup>pos</sup>/CD105<sup>pos</sup>/CD146<sup>pos</sup>/VEGFR2<sup>pos</sup>/CD133<sup>neg</sup> [19].
in advanced RCC patients during the first cycle of sunitinib treatment. CECs increased in parallel to plasma VEGF and EPO levels during the 4-week on and decreased during the 2-week off sunitinib period, while monocytes and HPCs displayed an opposite pattern of change.

Blood cell-based biomarker analysis related to sunitinib activity and clinical outcome has been studied only in GIST patients with the main conclusion that a smaller decrease in monocyte levels was seen in patients with clinical benefit compared to those with PD [10]. We observed a decrease in circulating monocyte number after sunitinib treatment in RCC patients in agreement with the GIST study; a correlation with response was not seen in our populations, possibly related to the limited number of patients with PD.

The number of HPCs decreased already maximally at C1/D4 in our patient group, in parallel to the monocytes, while the overall WBC count dropped more slowly, due to a more delayed change in circulating neutrophils (Fig. 1). The decrease in HPCs might be partly related to bone marrow suppression associated with the Flt3-salutary action of sunitinib, since Flt3-signaling is required for HPC proliferation [26, 27].

Despite intense interest in developing biomarker tests for response prediction [7, 28, 29], levels of CECs during sunitinib treatment of RCC patients have not yet been reported. Therefore, the most interesting and novel finding of our study was the increase in CD45neg/CD31pos CECs during sunitinib treatment. The CEC population in PB is a rare cell population [20], which is increased two to threefold in cancer patients [19]. In the present patient group, the median pre-treatment (C1D1) frequency of the CECs was 69 cells/ml (n = 23), which is well-comparable to the median of 81 cells/ml (range 32–132) in a mixed group of cancer patients [19]. The number of CECs approximately doubled in the RCC patient group by sunitinib treatment. Since we found a similar twofold increase in CEC levels (without decrease in HPC numbers) in a group of bevacizumab plus erlotinib, but not erlotinib-single agent treated NSCLC patients (L. Vroling et al., unpublished) [30], this increase is more likely related to inhibition of VEGFR signaling by sunitinib, rather than to inhibition of other targets or off-target effects of sunitinib. Being a most likely specific target-related effect of sunitinib, this increase in CECs remains an interesting cell population to be further investigated.

An important question regards the precise nature and function of the CEC population that is elevated after sunitinib treatment, in particular in the light of the current controversies on the identification and role in tumor angiogenesis of CECs or CEPs [11, 14, 25, 31]. A plausible explanation for the increased number of CECs is that they reflect endothelial cells, which became detached or shed from sunitinib-targeted immature (tumor) blood vessels. Although we have defined this population by the marker combination of CD45neg and CD31pos, which are both essential for discriminating these cells from the HPCs and all other MNCs, in theory, it may still be heterogeneous with regard to other EC markers. Importantly, we have assessed that this population has the highest VEGFR2 positivity (median 65% of all by defined cell populations in the PB, further supporting their endothelial nature. CECs are commonly characterized and defined by a heterogeneous, but rather large size and granularity, exceeding that of most mononuclear cell populations, typically >20 μm [22, 32, 33] and a high CD146 expression allowing selective extraction with immunobeads [34]. The median diameter of CD146+ PBMCs has been estimated 6.8 μm versus that of CD146+ CECs as 21.5 μm [22]. Our CECs are in the FSC/SSC range of the HPCs, which are <10 μm. This fits with the idea that these small CECs originate from a rather immature vasculature and/or are mobilized bone marrow or vascular wall resident EPCs. In support of this explanation, several data suggest that sunitinib might selectively prune immature nascent tumor neovessels not yet adequately stabilized by pericyte coverage [35, 36], while relatively saving mature vessels leading to vessel normalization [37].

A characteristic of endothelial cells in vitro is that they rapidly become apoptotic after detachment from their matrix [38]. However, in studies that measure CEC frequencies in PB, cell viability was either not assessed or the viability marker dye 7-AAD has been used to exclude dead cells, as in most flow cytometric approaches. While our CEC values are intact, CECs by the annexin-V (with ammonium chloride) protocol in several annexin-V staining or the dye SYTO-16, can detect early stages of apoptosis in cells that still exclude 7-AAD [39, 40]. We are not aware of studies reporting apoptotic CECs using annexin-V labeling, probably because this technique is not readily incorporated in most CEC protocols and also the use of frozen-thawed samples as used by some [10] precludes the reliable assessment of apoptotic cells [39]. Therefore, we have assessed the percentage of apoptotic CECs with annexin-V (with ammonium chloride) protocol in several RCC patients, separately from the main study protocol and found that the number of early apoptotic CECs was considerable (range 50–80% of CECs).

It should be noted that the endothelial cell marker VE-cadherin was virtually absent in most of our CEC subpopulations, while others reported it to be present on mature CECs circulating in PB [40]. The lack of event surface VE-cadherin expression may reflect the immature nature of these small CECs, or might also be explained by internalization during or after loss of endothelial junctions and detachment of the cells [41, 42].
An alternative possibility may be that our CD45neg/CD34bright CECs have endothelial progenitor (CEP) characteristics, such as those recently ascribed to CD45neg/CD34bright/CD133neg cells [14, 15, 43, 44]. A disturbed homing of VEGFR positive CEPs into the tumor vasculature caused by sunitinib might also contribute to the increase in CD45neg/CD34bright/CD133neg CEC population. It is important to note that the presence of a fraction of early apoptotic cells in the population of CECs does not exclude a priori the presence of endothelial progenitor cells, capable of highly proliferative outgrowth, since the CD45neg/CD34neg/CD133neg cell population from cold blood, which is the source of late EOCs, also contained up to 60% apoptotic cells (f. Timmemaann, personal communication). This lends support to the idea that the EPCs or EOC precursors circulating in human PB might be in majority rather resident cells from peripheral sites than from the bone marrow [45] and might exist in multiple states of differentiation [46].

In addition to the increase in CECs, the soluble growth factor VEGF increased during sunitinib exposure and partly normalized 2 weeks after cessation of drug intake. This finding is in accordance with previous findings on VEGF receptor inhibition studies in mice and man [10, 47, 48]. The mechanism for the VEGF increase is not known, but according to the study of Ebos et al. [49], may reflect a direct or indirect physiological response to receptor inhibition by sunitinib. Indeed, we found also a prominent increase in EPO during the first cycle of sunitinib, consistent with the findings of Ebos et al. [49] in sunitinib-treated mice. Functional consequences of increased plasma EPO levels in sunitinib-treated patients remain to be defined.

The rapid return of VEGF and CECs to the pre-treatment levels during the 2-weeks rest period is remarkable. Studies by McDornald et al. [50] have pointed to the rapid repopulation of neovascular casts after cessation of antiangiogenic treatment of tumor-bearing animals. The occurrence of a similar rapid resumption of vessel repair in the BCC patients might contribute to the rapid normalization of VEGF during the drug-free period.

The primary goal of this study was to investigate the presence and pattern of change of CD45neg/CD34bright/CD133neg CECs, separated from CD45neg/CD34neg/CD133neg HPCs in a cohort of sunitinib-treated RCC patients. The question, whether the observed changes in CECs or other circulating subsets of cells are just a pharmacodynamic marker of sunitinib-activity or might have a predictive value, needs to be addressed in a larger cohort of patients [51, 52].

In conclusion, this study shows that CD45neg/CD34bright/CD133neg HPCs counts change in opposite directions by sunitinib, monocytes and HPC decrease and CECs increase. CD45neg/CD34bright/CD133neg CECs might be detached ECs and reflect sunitinib anti-vascular effects or might include CEPs, which are potential targets.

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Chapter 12

Sunitinib-induced changes in circulating endothelial cell-related proteins in patients with metastatic renal cell cancer


* Authors contributed equally

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Sunitinib-induced changes in circulating endothelial cell-related proteins in patients with metastatic renal cell cancer

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* A.A.M. van der Veldt and L.V. contributed equally to this work

Vascular endothelial growth factor receptor (VEGFR) tyrosine kinase inhibitors are effective agents in the treatment of metastatic renal cell cancer (mRCC). We here investigated whether inhibition of VEGFR signals by sunitinib causes changes in plasma proteins associated with tumor endothelium. Forty-three patients with mRCC received sunitinib 50 mg/day in a 4-weeks on 2-weeks off schedule. Sequential plasma samples were obtained before treatment (C1D1), on C1D14, on C1D28, and on C2D0 before start of cycle 2. Plasma levels were assessed for VEGF, soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular cell adhesion molecule-1 (sICAM-1), von Willebrand factor (vWF), circulating angiopoietin-2 (Ang-2) and soluble Tie-2 (sTie-2). Total tumor burden was calculated at baseline and at first evaluation. Progression-free survival (PFS) and overall survival (OS) were determined. Tumor burden was positively associated with baseline circulating Ang-2 (Spearman’s rho (p) = 0.576, p = 0.026) and vWF (p = 0.417, p = 0.008). During sunitinib treatment, circulating Ang-2 and sTie-2 significantly decreased (p < 0.001 for both), plasma levels of sVCAM-1 and VEGF significantly increased (p = 0.022 and p = 0.001), whereas those of sICAM-1 and vWF remained stable. These protein changes had recovered on C2D0. The reduction in circulating Ang-2 levels on C1D28 was positively correlated with the percentage decrease in tumor burden (p = 0.655, p = 0.002). Baseline protein levels and subsequent changes were not associated with PFS or OS. In conclusion, sunitinib-induced changes in Ang-2, sTie-2, VCAM-1 and VEGF are related to the administration schedule, while reduction in Ang-2 is also associated with decrease in tumor burden.

Key words: sunitinib, renal cell cancer, angiogenesis, endothelial cell activation, vascular endothelial growth factor, soluble vascular cell adhesion molecule-1, soluble intercellular cell adhesion molecule-1, von Willebrand factor, circulating angiopoietin-2, soluble Tie-2

The development of antiangiogenic agents has significantly improved the perspectives of patients with metastatic renal cell cancer (mRCC), known as a disease resistant against standard chemotherapy. Currently, sunitinib is most widely prescribed for first-line treatment of mRCC. Sunitinib is an oral tyrosine kinase inhibitor (TKI) which targets several receptors including vascular endothelial growth factor receptor (VEGFR)-1, -2 and -3, platelet-derived growth factor receptor-α and β, c-KIT and FLT3. Among these receptors, VEGFR on tumor-associated endothelium is considered to be a relevant target for sunitinib in RCC. In the majority of RCC tumors, high levels of VEGF are being produced as a result from a defective function of the von Hippel-Lindau tumor suppressor gene.

VEGF is a potent angiogenic factor which plays a key role in tumor vascularization and is involved in proliferation, migration and tube formation of endothelial cells as well as endothelial cell survival. In response to VEGF, the endothelium is activated and several proteins are expressed by endothelial cells including vascular cell adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecule-1 (ICAM-1), von Willebrand factor (vWF), angiopoietin-2 (Ang-2) and Tie-2 (Tie-2). VCAM-1 and ICAM-1 are adhesion molecules and their soluble ectodomains (sVCAM-1 and sICAM-1) can be proteolytically released from the endothelial cell surface into the circulation. Next to upregulation of VCAM-1 and ICAM-1, VEGF is also known to activate exocytosis of intracellular secretory granules in endothelial cells, the so-called Weibel-Palade bodies, thereby releasing vasoactive substances, including von Willebrand factor (vWF) and Ang-2. The glycoprotein vWF is produced uniquely by endothelial cells upon endothelial cell activation and is essential for platelet adhesion to the subendothelial matrix after vascular injury. Ang-2 acts as a natural antagonist of its receptor Tie-2 and endothelial shedding of soluble Tie-2 (sTie-2) is stimulated by VEGF as well.

Inhibition of VEGFR signaling may affect endothelial cell activation and function, consequently leading to an
altered release of endothelial cell-related proteins. Measurement of these proteins in blood might be useful to monitor the effect of anti-VEGF therapy in cancer patients. Because sunitinib inhibits VEGFR signaling, we selected plasma VEGF, sVCAM-1, sICAM, vWF, circulating Ang-2 and sTie-2 in patients with mRCC to determine their value as potential biomarkers. We not only assessed the effect of sunitinib on these circulating proteins but also explored whether alterations in plasma protein levels were associated with the change in tumor burden as well as treatment outcome.

Patients and Methods

Patients, treatment and evaluation

A total of 43 consecutive mRCC patients treated with sunitinib in two Dutch medical centers were included in this study. Each patient signed a protocol-specific informed consent. Collection of data was part of three protocols,16–18 which were approved by the medical ethics review boards of the institutes.

Sunitinib was administered orally at a dose of 50 mg daily, consisting of 4 weeks on treatment followed by a 2-weeks rest period in cycles of 6 weeks. Dose reductions of sunitinib were allowed depending on the type and severity of adverse events.

Computed tomography (CT) or magnetic resonance imaging (MRI) was performed at baseline and every two to three cycles of treatment to assess clinical response according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0 (RECIST).19 At baseline and at first evaluation, total tumor burden was calculated, which was defined as the sum of the longest diameters of all lesions, including primary tumors.20

PFS was defined as the time between the first day of sunitinib and the date of progressive disease (PD) according to RECIST 1.1 or clear clinical evidence of PD. Overall survival (OS) was defined as the time between the first day of sunitinib treatment and the date of death or the date at which patients were last known to be alive.

Blood samples analyses

Sequential plasma samples were obtained before treatment with sunitinib defined as cycle 1 day 1 (C1D1), on cycle 1 day 14 (C1D14), on cycle 1 day 28 (C1D28), and after 2 weeks of rest on cycle 2 day 1 (C2D1). At the time of blood sampling, the first 2 mL blood was discarded before collection of 7 mL of EDTA blood. Within 30 min after collection, samples were centrifuged at 3,000 rpm for 10 min at 4°C. Plasma was removed and stored immediately at −80°C until analysis.

Concentrations of VEGF, sVCAM-1 and sICAM-1 were assessed with Quantikine enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems). A commercially available ELISA for vWF antigen was obtained from American Diagnostica (Greenwich, CT). The mean concentrations of circulating Ang-2 were measured with the human Ang-2 DuoSet ELISA Development kit (R&D Systems, Minneapolis, MN). According to the ELISA kits the range of protein levels in normal volunteers was below 115 pg/mL for VEGF, in the range of 140–991 ng/mL for sVCAM-1, 100–307 ng/mL for sICAM, 630–1,950 mU/mL for vWF21 and 21–280 pg/mL for circulating Ang-2.22

Samples from the same patient were pipetted into the same 96-well plate. Samples were run in duplicate and the mean value was recorded. In 20 patients, sTie-2 levels (Quantikine ELISA kit; R&D Systems) were measured on C1D1 and on C1D14. These patients were selected on the basis of the highest percentage change in plasma levels of circulating Ang-2 on C1D14 when compared to that on C1D1. According to the ELISA kit, the range of sTie-2 was in the range of 17–36 ng/mL in normal volunteers.

Of all 43 patients, blood samples were available on C1D1 and on C1D14, while on C1D28 and on C2D1 blood samples were available in 33 and 35 patients, respectively. Reasons for missing blood samples were a temporary or permanent discontinuation due to sunitinib-related adverse events or evident progression of disease. In 5 of 43 patients, the amount of plasma sample was limited and only VEGF and vWF levels could be measured.

Statistics

Statistical analysis was performed using SPSS software (SPSS for Windows 16.0, SPSS, Chicago, IL). Data were expressed as median with range. The Mann–Whitney U test and the Kruskal–Wallis test were used to examine the associations between plasma protein levels and patient characteristics. Changes in variables were expressed as the percentage change from baseline values. The Wilcoxon Signed Ranks test was used to compare plasma levels on C1D14, C1D28 and C2D1 with those at baseline. Correlations between continuous data sets were analyzed using Spearman’s correlation test. In addition, Fisher’s exact test was performed to determine associations between categorical variables. A two-tailed probability value of p < 0.05 was considered significant.

For PFS and OS, data collection was closed on August 1, 2010. PFS and OS were calculated using the Kaplan–Meier method. All patient characteristics were tested univariately against PFS and OS using Kaplan–Meier and Cox-regression analysis, depending on the tested variables. The plasma protein levels at baseline and their changes on day 14 were dichotomized by median splitting followed by univariate testing against PFS and OS using Kaplan–Meier analysis. Log-rank test was used to test the differences between survival curves. Candidate variables with p value ≤ 0.05 were selected for the multiple Cox-regression survival analysis with PFS and OS as dependent variables. Additional patient characteristics were introduced in the multivariate analyses based on univariately tested results if p value ≤ 0.05. All results from the multivariate analyses with p values < 0.05 were considered significant. The analyses were preplanned before the study started. Since this was an explorative study, no correction for multiple testing was done.
Results
Patient characteristics and treatment
Table 1 summarizes the baseline characteristics of the 43 patients. Thirty-eight (86%) patients had clear cell histology and 12 patients (28%) had a primary tumor in situ. Six (14%) patients had one metastatic site, 10 (23%) patients had two metastatic sites and 27 (63%) patients had at least three metastatic sites. According to the Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic criteria, most patients were categorized into the intermediate and poor risk group (each 44% of patients), whereas 12% of the patients were categorized into the favorable risk group.

According to RECIST, seven patients achieved a partial response (PR), 21 patients had stable disease (SD), 13 patients had PD and two patients could not be evaluated as a result of early termination due to side-effect related adverse events. At the time of the analysis, only three (7%) patients were still alive. Overall, the median PFS time was 7.0 months (range, 0.5–41.1 months) and the median OS time was 12.3 months (range, 0.5–41.1 months). In comparison with a previously published compassionate use cohort of sunitinib-treated mRCC patients, the median PFS and OS in this study were relatively short, which can be explained by a higher number of patients in the MSKCC poor risk group as well as a lower number of patients in the favorable risk group.

When all measurable lesions were taken into account (n = 40), the median tumor burden was 136 mm (range, 37–321 mm). Total tumor burden could not be measured adequately in three out of 43 patients, as the disease was mainly localized in the bone. In addition, six patients could not be evaluated for the effect of sunitinib on total tumor burden due to early termination of treatment. In 10 patients who were still alive at the time of the analysis, all measurable lesions were taken into account (n = 25). In two patients, one patient with PD and one patient with SD, the number of measurable lesions decreased by 9% (range, 8–23%) and 10% (range, 15–18%), respectively.

Of the clinical characteristics, the non-clear cell subtype and a higher number of metastatic sites were prognostic factors for a poor PFS (log rank = 0.832 and 11.115; ρ = 0.004 and 0.001, respectively) and OS (log rank = 14.600 and 7.351; p = 0.001 and p < 0.001, respectively). In addition, a higher number of MSKCC risk factors was prognostic for a poor OS (log rank = 7.840; p = 0.004).

Plasma protein concentrations at baseline
A considerable number of mRCC patients had higher plasma levels of VEGF, SV-CAM-1, sICAM-1, vWF and Ang-2 than those in healthy volunteers reported for the respective assays (see Patients and Methods section). The median level of VEGF was 97 pg/mL (range, 21–630 pg/mL). The median levels of the soluble adhesion molecules SV-CAM-1 and sICAM-1 were 762 ng/mL (range, 325–3,458 ng/mL) and 295 ng/mL (range, 114–450 ng/mL), respectively. The median levels of vWF and circulating Ang-2 were 2,281 μg/mL (range, 296–4,049 μg/mL) and 1,336 pg/mL (range, 353–1,503 pg/mL), respectively. Additional measurements of the soluble receptor of Ang-2, sTie-2, were performed in 20 patients with relatively high levels of Ang-2. The sTie-2 level at baseline (median, 48 ng/mL; range, 25–114 ng/mL) was elevated as compared to that in healthy volunteers.

### Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
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<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male: 27, Female: 16</td>
<td></td>
</tr>
<tr>
<td>ECOG performance status</td>
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<td></td>
</tr>
<tr>
<td>0</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>21</td>
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</tr>
<tr>
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<tr>
<td>3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>No. of metastatic sites</td>
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<td></td>
</tr>
<tr>
<td>0</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>10</td>
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</tr>
<tr>
<td>≥3</td>
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<td></td>
</tr>
<tr>
<td>MSKCC risk factors</td>
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</tr>
<tr>
<td>0 (favorable)</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>1 (intermediate)</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>2 (poor)</td>
<td>19</td>
<td></td>
</tr>
</tbody>
</table>

1ECOG, Eastern Cooperative Oncology Group. Risk groups according to Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic criteria (based on the five risk factors: low Karnofsky performance status (<90), high LDH (>1.5 times the upper limit of normal), low serum hemoglobin, high corrected serum calcium (>10 mg/dL), and time from initial diagnosis to treatment of less than 1 year).
Baseline plasma protein concentrations and patient characteristics

Baseline plasma protein levels were assessed for a possible relationship with patient characteristics. “Karnofsky” performance status (KPS), low serum hemoglobin, high corrected serum calcium (Ca), and time from initial diagnosis to treatment of less than 1 year were associated with higher levels of circulating Ang-2 (Spearman’s p = 0.67, p = 0.08, n = 39). There were no associations between tumor burden and plasma levels of VEGF, sVCAM-1, and sICAM-1. In further analysis, we determined whether there was a relationship between circulating Ang-2 levels and other proteins. It appeared that circulating Ang-2 levels were positively associated with most proteins, being levels of other proteins. It appeared that circulating Ang-2 levels were positively associated with most proteins, being levels of Ang-2 (Spearman’s p = 0.43, p = 0.07, n = 37), sICAM-1 (Spearman’s p = 0.57, p = 0.02; n = 37), and vWF (Spearman’s p = 0.92, p = 0.01; n = 36). In the separate analysis in 20 patients with relatively high levels of circulating Ang-2, there were also positively associated with Ang-2 levels (Spearman’s p = 0.40, p = 0.07, n = 20).

Effect of sunitinib on circulating plasma proteins

Figure 1 shows the alterations in plasma levels of the circulating proteins during sunitinib treatment in mRCC patients. The circulating levels of sICAM-1 and vWF did not significantly change during treatment with sunitinib. Sunitinib induced a significant rise in plasma levels of VEGF and sVCAM-1. The median VEGF levels increased from 97 pg/mL (range, 21–650 pg/mL) at baseline to 312 pg/mL (range, 57–1,722 pg/mL) on C1D14 (p < 0.001) and to 235 pg/mL (range, 53–1,499 pg/mL) on C1D28 (p < 0.001).

Table 2. Baseline plasma proteins vs. baseline characteristics

<table>
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<tr>
<th>Characteristic</th>
<th>No. of cases</th>
<th>Circulating plasma proteins at baseline*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td>VEGF (pg/mL)</td>
</tr>
<tr>
<td>Male</td>
<td>27</td>
<td>102 (21–450)</td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>91 (29–331)</td>
</tr>
<tr>
<td><strong>Histological category</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear cell</td>
<td>38</td>
<td>97 (21–360)</td>
</tr>
<tr>
<td>Nontumor cell</td>
<td>5</td>
<td>118 (37–191)</td>
</tr>
<tr>
<td><strong>Previous nephrectomy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>31</td>
<td>97 (29–450)</td>
</tr>
<tr>
<td>No</td>
<td>12</td>
<td>98 (21–360)</td>
</tr>
<tr>
<td><strong>No. of metastatic sites</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>11</td>
<td>64 (21–95)</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>118 (75–313)</td>
</tr>
<tr>
<td>≥2</td>
<td>27</td>
<td>91 (37–450)</td>
</tr>
<tr>
<td><strong>MSKCC risk factors</strong></td>
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</tr>
<tr>
<td>0 (favorable)</td>
<td>11</td>
<td>91 (29–318)</td>
</tr>
<tr>
<td>1 (intermediate)</td>
<td>19</td>
<td>102 (21–313)</td>
</tr>
<tr>
<td>≥2 (poor)</td>
<td>18</td>
<td>115 (29–650)</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01.

Data are reported as median (range). Mann-Whitney test was used to examine associations between plasma proteins and patient characteristics. Risk groups according to Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic criteria (based on the five risk factors: low–Karnofsky performance status (<20%), low serum hemoglobin, high corrected serum calcium (>10 mg/dL), and time from initial diagnosis to treatment of less than 1 year).
The median sVCAM-1 levels increased from 762 ng/mL (range, 325–3,458 ng/mL) at baseline to 931 ng/mL (range, 335–3,095 ng/mL) on C1D14 (p = 0.022) and to 994 ng/mL (range, 507–2,336 ng/mL) on C1D28 (p = 0.002). After the 2-weeks rest period (C2D1), both VEGF levels and sVCAM-1 levels grossly returned to baseline values, being 114 pg/mL (range, 34–412 pg/mL; p = 0.964) and 870 ng/mL (range, 417–1,988 ng/mL; p = 0.196), respectively.

Sunitinib induced a significant decline in levels of circulating Ang-2 and sTie-2. Two weeks after the start of treatment, the median level of circulating Ang-2 had significantly decreased from 1,336 pg/mL (range, 353–3,503 pg/mL) to 958 pg/mL (0–3,534 pg/mL; p < 0.001) and that of sTie-2 from 48 ng/mL (range, 25–114 ng/mL) to 37 ng/mL (range, 21–66 ng/mL; p < 0.001). On C1D28, the change in Ang-2 persisted (median, 884 pg/mL; range, 206–2,746 pg/mL; p < 0.001), but on C2D1 the levels of circulating Ang-2 had recovered and had grossly returned to baseline values (median, 1,232 pg/mL; range, 229–3,771 pg/mL; p = 0.178).

We next explored whether the significant changes in the concentration of one protein in the 4 weeks of sunitinib treatment were associated with that of another among patients. On C1D14, the percentage change in levels of circulating Ang-2 was negatively associated with the percentage change in the levels of VEGF (Spearman’s r = −0.457, p = 0.005, n = 36). Although changes in sTie-2 were also negatively associated with changes in VEGF (Spearman’s r = −0.456, p = 0.043, n = 20), there was no association between the percentage change in the levels of circulating Ang-2 and that of the other proteins, in particular not...
between circulating Ang-2 and sTie-2 (C1D14, Spearman's \( p = 0.080, p = 0.738, n = 20 \)).

Associations between changes in plasma proteins and tumor burden

We assessed the effect of sunitinib on total tumor burden at first evaluation in relation to changes in plasma proteins. There were no associations between changes in tumor burden and the increase in plasma levels of VEGF or sVCAM-1. Of interest, the percentage decrease in circulating Ang-2 on C1D28 was positively associated with the percentage decrease in tumor burden (Spearman's \( p = 0.065, p = 0.002; n = 24 \); Fig. 2). When the outlier with 92% increase in circulating Ang-2 was excluded, the association was still positive (Spearman's \( p = 0.531, p = 0.000; n = 23 \)). In addition, there was a trend on C1D14 (Spearman's \( p = 0.330, p = 0.067, n = 20 \)) and on C2D1 (Spearman's \( p = 0.367, p = 0.060, n = 27 \)). In patients with a >30% decrease in total tumor burden (\( n = 6 \)) the circulating Ang-2 levels on C2D1 had not recovered to baseline values. On C2D1, the medium absolute decrease in circulating Ang-2 in those patients was \(-365 \text{ pg/mL (range, } -668 \text{ to } 903 \text{ pg/mL)}\), while that in patients without clinically relevant tumor reduction (\( n = 21 \)) was \(-7 \text{ pg/mL (range, } -1,295 \text{ to } 414 \text{ pg/mL)}\).

Remarkably, one patient showed an absolute decrease of 2011 UICC

Discussion

In this study, we explored the effect of sunitinib on levels of circulating proteins involved in VEGF-regulated endothelial cell activation and function including VEGF, sVCAM-1, sICAM-1, vWF, Ang-2 and sTie-2. At baseline, mRCC patients showed elevated plasma levels of all proteins as compared with those reported in healthy volunteers. Most of these protein levels were related to factors associated with the extent of the disease. At baseline, total tumor burden was positively associated with vWF and circulating Ang-2. In addition, levels of circulating Ang-2 were positively associated with VEGF, sICAM-1, vWF and sTie-2. Treatment with sunitinib induced an increase in VEGF and sVCAM-1, a decrease in circulating Ang-2 and vWF, while levels of sICAM-1 and sTie-2 were not affected. After the 2-weeks rest period, changes in circulating proteins had grossly recovered. The change in circulating Ang-2 levels was positively associated with the percentage change in total tumor burden. Sunitinib-induced changes in plasma protein levels were not significantly associated with PFS or OS.

At baseline, plasma levels of all selected proteins were increased in patients with mRCC as compared with those in healthy volunteers. Elevated plasma levels of VEGF, sVCAM-1, sICAM-1 and sTie-2 were grossly comparable with those in patients with metastatic sites, and MSKCC risk factors. In multivariate analyses, pretreatment plasma levels of sVCAM-1 were no longer predictive of OS (hazard ratio: 1.835; 95% CI: 0.435–4.079; \( p = 0.136 \)).
reported in other mRCC cohorts.\textsuperscript{13,27–29} The high-plasma level of circulating Ang-2, however, is a new finding in mRCC. In RCC tumor tissue, expression of Ang-2 has been described predominantly on tumor-associated endothelium,\textsuperscript{30,31} but Ang-2 expression has been observed in RCC tumor cells as well.\textsuperscript{32} Currently, growing evidence indicates that Ang-2 plays an important role in tumor angiogenesis.\textsuperscript{32} Ang-2 can collaborate with VEGF to induce angiogenesis in

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image.png}
\caption{Examples of CT images in two patients with mRCC treated with sunitinib. (a–d) A responding patient with a primary tumor in situ (a, arrow), multiple lung and subcutaneous metastases (c, arrows) at baseline and at first evaluation during sunitinib (b, d). The patient showed a 71% decrease in circulating Ang-2 on C1D28 and a 33% decrease in total tumor burden at first evaluation. (e, f) A nonresponding patient with lung metastases at baseline (e, arrow) and at first evaluation during sunitinib (f). The patient showed a 92% increase in circulating Ang-2 on C1D28 and a 49% increase in total tumor burden at first evaluation.}
\end{figure}
a synergistic manner" and can facilitate the angiogenic process regulated by tumor-derived VEGF between existing host vessels that co-opt with tumor cells. In our study, circulating Ang-2 was the strongest factor associated with the extent of disease, which was characterized by a concurrent primary tumor in situ, a higher risk group according to mRCC and a larger total tumor burden. Nevertheless, levels of circulating Ang-2 or any other proteins were not independently associated with outcome in sunitinib-treated mRCC patients. In contrast to those findings, other studies have shown a correlation between high levels of circulating Ang-2 and a poorer prognosis in melanoma patients and in patients with advanced colorectal cancer treated with bevacizumab. Although baseline-circulating Ang-2 was not prognostic for outcome in our mRCC patients, a decrease in circulating Ang-2 appeared to be predictive for sunitinib-induced reduction in total tumor burden.

Levels of circulating Ang-2 and sTie-2 both declined after sunitinib treatment. Previous studies have shown that sunitinib and bevacizumab cause downregulation of Ang-2 mRNA in human tumor cells including glioma and rectal cancer xenografts. These reports and this study suggest that inhibitors of VEGF(R) signaling may decrease Ang-2 expression in tumors, which subsequently results in a decline of circulating Ang-2. In addition, inhibition of VEGF(R) signaling may reduce shedding of sTie-2, which is exclusively expressed on endothelial cells as described for RCC, thereby decreasing endothelial cell binding of Ang-2 to its receptor. As conflicting data have been published on tissue localization of Ang-2 expression in malignancies, we further explored whether the changes in circulating Ang-2 were possibly of endothelial origin. Hence, HUVECs were treated with sunitinib to determine changes in Ang-2 in supernatant. Although sunitinib had an inhibitory effect on HUVEC proliferation, secretion of Ang-2 by these cells was not affected (unpublished data). Since plasma levels of vWF, which is also stored in the Weibel–Palade bodies of endothelial cells, were not affected in our patients, these findings suggest that sunitinib mostly reduced Ang-2 production from tumor cells. In accordance, we found an association between the decrease in circulating Ang-2 levels and a reduction in total tumor burden in sunitinib-treated mRCC patients.

Apart from circulating Ang-2, sunitinib treatment resulted in changes in plasma levels of VEGF and sVCAM-1. As expected, VEGF levels increased after administration of sunitinib. A reactive rise in plasma VEGF is usually observed upon treatment with TKIs targeting VEGFR. In a preclinical study, sunitinib increased plasma VEGF levels in a dose-dependent manner, which was caused by a tumor-dependent response as well as a systemic tumor-independent response. Currently, the origin of the tumor-independent release of VEGF has not been identified. In this study, sunitinib treatment also induced a rise in sVCAM-1, while plasma levels of the other soluble adhesion molecules, sICAM-1, remained stable. Similarly, an increase in sVCAM-1 has been reported after bevacizumab-based therapy, while sICAM-1 levels were not notably affected. The increase of sVCAM-1 associated with inhibition of VEGF(R) has yet to be clarified. It has been described that nitric oxide (NO), which is produced signaling of VCAM-1 in human umbilical vein endothelial cells. This observation suggests that sunitinib may promote endothelial expression of VCAM-1 by inhibiting the repres-


Chapter 13A

Reduction in skin microvascular density and changes in vessel morphology in patients treated with sunitinib


* Authors contributed equally

Reduction in skin microvascular density and changes in vessel morphology in patients treated with sunitinib

Astrid A.M. van der Veldt, Michel F. de Boer, Epie Boven, Etto C. Eringa, Allons J.M. van den Eertwegh, Victor W. van Hinsbergh, Yvo M. Smulders and Erik H. Serré

Hypertension is a common side effect in cancer patients treated with inhibitors of vascular endothelial growth factor/vascular endothelial growth factor receptor-2 signaling and may represent a marker of clinical benefit. Functional rarefaction (a decrease in perfused microvessels) or structural rarefaction (a reduction in anatomic capillary density) may play an important role in the development of hypertension. We investigated whether sunitinib caused impairment of microvascular function and/or reduction of capillary density in patients with metastatic renal cell cancer (mRCC). Sixteen mRCC patients were treated with sunitinib (50 mg/day). Assessments of 24-h ambulatory blood pressure, microvascular endothelial function by laser Doppler fluxmetry, and capillary density by capillary microscopy were performed at baseline and days 14 and 28. Median blood pressure had increased on day 14 (systolic 10 mmHg, P<0.01 and diastolic blood pressure 8 mmHg, P<0.01). Capillary density had decreased from 69 to 61 capillaries/mm² (P<0.01). This decrease was related to the increase in systolic and diastolic blood pressure (r = –0.52, P<0.05 and r = –0.68, P<0.01, respectively). A more pronounced decrease in capillary density was associated with increased visibility of the subcapillary plexus (P=0.041). Preliminary findings indicated that median progression-free survival was significantly prolonged in patients with a greater than 6 capillaries/mm² decrease in density as compared with patients with a less pronounced decrease (P=0.049). In conclusion, reduction in skin capillary density is associated with a rise in blood pressure during sunitinib therapy and, by itself, might be useful as a predictive marker of clinical outcome. Anti-Cancer Drugs 21:439–446 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Key words: capillary density, hypertension, microcirculation, rarefaction, renal cell cancer, sunitinib

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Introduction

The central role of angiogenesis in promoting tumor progression and metastases formation is now well appreciated and is governed by a balance between stimulators and inhibitors of angiogenesis, a process by which new capillaries are formed from preexisting blood vessels [1]. Vascular endothelial growth factor (VEGF)/VEGF receptor-2 (VEGFR-2) signaling constitutes the predominant regulatory pathway for developmental and tumor angiogenesis. Therapeutic targeting of VEGF/VEGFR-2 signaling with bevacizumab, an anti-VEGF monoclonal antibody, or sunitinib and sorafenib, both receptor tyrosine kinase inhibitors targeting VEGFR-2, have provided survival benefit in specified cancer patients [1].

Arterial hypertension is a commonly reported side effect in trials with inhibitors of VEGF/VEGFR-2 signaling, such as bevacizumab and sunitinib [2,3]. Although conflicting data have been reported [4], the occurrence of antiangiogenic treatment-related hypertension seems to be a predictive marker for clinical outcome [5–8]. As an example, bevacizumab-induced hypertension was associated with favorable progression-free survival (PFS) in advanced colorectal cancer patients [8]. In addition, the occurrence of hypertension, particularly grade 3, was associated with better treatment response to sunitinib in metastatic renal cell cancer (mRCC) [7]. These findings suggest that the increase in blood pressure (BP) or underlying causative mechanisms may represent clinical biomarkers of treatment efficacy.

The mechanisms by which antiangiogenic therapy can increase BP are not yet fully understood. Proposed mechanisms include reduced formation of nitric oxide by endothelial cells, a reduced responsiveness of vascular smooth muscle cells to nitric oxide, an increased production of or reaction to vasoconstricting stimuli, and a reduction in microvascular density (rarefaction) [9–11]. Microvessels, that is, arterioles and capillaries, are a major contributor to total peripheral vascular resistance. Functional rarefaction (a decrease in perfused
microvessels) or structural rarefaction (a reduction in anatomic capillary density) may play an important role in the development of hypertension [12,13].

We investigated in mRCC patients whether sunitinib, a small molecule tyrosine kinase inhibitor of VEGFR-1, 2 and 3, platelet-derived growth factor receptor-α and β, c-KIT, and FLT3, impairs microvascular function and induces capillary rarefaction, which in turn might be associated with BP rise and clinical outcome in patients with mRCC.

**Methods**

**Patients, treatment, and evaluation**

Patients treated with sunitinib for mRCC from March 2006 to October 2007 were enrolled. Most patients had been included in an expanded access program until September 2006 [14] after which sunitinib was registered and available on doctor’s prescription. Each participant signed an institutional review board-approved protocol-specific informed consent in accordance with national and institutional guidelines. Sunitinib was administered orally at a dose of 50 mg daily, consisting of 4 weeks of treatment followed by a 2-week rest period in cycles of 6 weeks.

Computed tomography was performed at baseline and for every two to three cycles of treatment to assess clinical response according to Response Evaluation Criteria in Solid Tumors (RECIST) [15]. PPVs were the time between the first day of sunitinib and the date of progressive disease on computed tomography or clear clinical evidence of progressive disease. Overall survival (OS) was the time between the first day of treatment and the date of death or the date at which patients were last known to be alive.

**Blood pressure measurements**

At baseline, and days 14 and 28 ambulatory BP monitoring (SpaceLabs 90207, Redmond, Washington, District of Columbia, USA) was performed to obtain 24-h recordings of BP and heart rate [16]. Measurements were made at the nondominant arm with appropriately sized cuffs. The monitors measured BP and heart rate every 20 min from 7.00 to 22.00 h and every 30 min from 22.00 to 7.00 h. Hypertension was graded according to National Cancer Institute-Common Toxicity Criteria Version 3.0.

In patients with preexisting antihypertensive medication, the dose and schedule of antihypertensive treatment were not changed during the first 28 days of sunitinib treatment unless the patients developed grade 3 hypertension requiring antihypertensive treatment. The latter precluded patients from subsequent skin microvascular measurements.

**Skin microvascular measurements**

Baseline, days 14 and day 28 skin microvascular measurements were made as described earlier [16]. Microvascular studies were carried out in the morning in a temperature-controlled room (median: 22.9°C, range: 21.3–25.1°C) after 30 min of acclimatization. Patients had abstained from caffeine, alcohol, smoking, and meals overnight.

Nail fold capillaries in the dorsal skin of the third finger were visualized by a capillary microscope. Capillary density was defined as the number of erythrocyte-perfused capillaries per mm². Baseline capillary density represented the number of functional capillaries. The number of capillaries was counted off-line by an experienced investigator (MP&B) from a videotape. The investigator counting the capillaries was blinded to patients’ history and clinical outcome. The day-to-day coefficient of variation of baseline capillary density was 2.3 ± 1.8% [17].

After baseline measurements, venous occlusion was applied with the digital cuff inflated to 60 mmHg for 60 s, to expose a maximal number of perfused capillaries. Venous occlusion is supposed to reflect structural capillary density.

Using the same visual fields as those used during baseline measurements, the capillaries were counted in the 60 s recordings. The day-to-day coefficient of variation of peak capillary density during venous congestion was 9.3 ± 7.1% [12]. Venous occlusion at 60 mmHg for 120 instead of 60 s did not further increase the number of visible capillaries [12].

Endothelium-dependent and endothelium-independent vasodilatation of finger skin microcirculation were evaluated with laser Doppler fluxmetry combined with isthmophoresis of acetylcholine (Ach, for endothelium-dependent vasodilatation) and sodium nitroprusside (SNP, for endothelium-independent vasodilatation) as described earlier [16]. ACh (1%, Miochol; Novartis, Basel, Switzerland) mediates vasodilatation through the generation of nitric oxide and prostanoids in the endothelium. SNP (0.1%, Nipride; Roche, Basel, Switzerland) mediates vasodilatation through the generation of nitric oxide and prostans in the endothelium. SNP (0.1%, Nipride; Roche, Basel, Switzerland) is a nitric oxide donor, acting directly on smooth muscle cells to induce relaxation. Laser Doppler flux was measured on the middle phalanx of the second and fourth digits with the Periflux 4000 system (Perimed, Stockholm, Sweden) and expressed as arbitrary perfusion units. A protocol of multiple fixed doses (current intensity × delivery time) was used, resulting in an incremental dose-response curve. Skin temperature was monitored. Day-to-day reproducibility for isthmophoresis of Ach and SNP was 15.9 ± 8.4 and 13.9 ± 9.0%, respectively [16].

**Statistical analysis**

Data are expressed as median with range. Statistical analysis was performed using SPSS software (SPSS for Windows 15.0; SPSS, Inc., Chicago, Illinois, USA). The Wilcoxon signed-rank test was used to compare BP and microvascular variables on days 14 and 28 with baseline. The relationship between changes in these variables was
Patient characteristics were assessed by the Spearman correlation test. In addition, Fisher’s exact test was performed to determine associations between categorical variables. The association between BP rise and microvascular rarefaction on the one hand and PFS and OS on the other were calculated with the Kaplan-Meier method. For survival analysis, data collection was closed on 1 March 2009. For Fisher’s exact test and Kaplan-Meier analyses, changes in vascular parameters on day 14 were dichotomized by median splitting. A two-tailed probability value of \( P < 0.05 \) was considered significant.

**Results**

**Patients’ characteristics and treatment effects**

Patients’ characteristics and treatment effects are summarized in Table 1.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>(n=14) (%)</th>
<th>Median (range)</th>
</tr>
</thead>
</table>

**Concomitant medication**

- Cholesterol-lowering medication: 3 (19)
- Angiotensin-converting enzyme-inhibitor for nephropathy: 3 (19)
- Antiplatelet medication: 1 (6)
- Antidiabetic medication: 3 (19)

**Blood pressure increases during sunitinib treatment**

During sunitinib treatment, baseline capillary density decreased on days 14 and 28 (Table 2). Capillary density during venous occlusion significantly decreased on days 14 and 28 (\( P = 0.005 \) and 0.003, respectively). Endothelium-dependent (Ach mediated) and endothelium-independent (SNP mediated) vasodilatation did not change during treatment. Changes in blood pressure are related to changes in capillary density.

**Changes in blood pressure**

On day 14, there were significant inverse correlations between changes in BP and in capillary density at baseline and during venous occlusion (Fig. 3) whereas this was not the case for changes in microvascular endothelium-dependent and endothelium-independent vasodilatation. Analyses on day 28 were not substantially different, although differences were not statistically significant.

**Changes in vessel morphology**

An unexpected finding on day 14 was that capillary microscopy revealed prominent visibility of the sub-papillary plexus in eight out of 16 patients (Fig. 2). In all patients, the subpapillary plexus was not visible at baseline. Remarkably, seven out of eight patients with a visible subpapillary plexus had a more pronounced sunitinib-induced decrease in capillary density during venous occlusion. The association between the increased visibility of the subpapillary plexus and the more...
### Table 2: Blood pressure and microvascular measurements before and during sunitinib 50 mg/day

<table>
<thead>
<tr>
<th></th>
<th>Baseline median (range)</th>
<th>Day 14 median (range)</th>
<th>Day 28 median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood pressure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-h SBP (mmHg)</td>
<td>118 (98–141)</td>
<td>123 (108–170)**</td>
<td>123 (107–157)</td>
</tr>
<tr>
<td>24-h DBP (mmHg)</td>
<td>68 (55–86)</td>
<td>75 (63–107)**</td>
<td>75 (60–91)**</td>
</tr>
<tr>
<td>24-h MAP (mmHg)</td>
<td>87 (75–105)</td>
<td>93 (79–118)**</td>
<td>93 (80–115)**</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>84 (65–98)</td>
<td>75 (54–98)**</td>
<td>77 (63–90)**</td>
</tr>
<tr>
<td><strong>Capillary density</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (capillaries/mm$^2$)</td>
<td>50 (36–59)</td>
<td>46 (38–77)</td>
<td>44 (22–58)</td>
</tr>
<tr>
<td>Venous occlusion (capillaries/mm$^2$)</td>
<td>69 (51–99)</td>
<td>61 (44–88)**</td>
<td>65 (47–90)**</td>
</tr>
<tr>
<td>Temperature ($^\circ$C)</td>
<td>31.5 (30.7–32.3)</td>
<td>30.2 (29.8–32.3)</td>
<td>30.4 (29.8–32.3)</td>
</tr>
<tr>
<td><strong>ACh-mediated vasodilatation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline perfusion (PU)</td>
<td>14 (5–28)</td>
<td>13 (4–35)</td>
<td>13 (5–35)</td>
</tr>
<tr>
<td>Plateau (PU)</td>
<td>59 (16–191)</td>
<td>90 (70–160)</td>
<td>70 (70–160)</td>
</tr>
<tr>
<td>Percentage increase (%)</td>
<td>366 (71–1100)</td>
<td>396 (11–124)</td>
<td>449 (11–160)</td>
</tr>
<tr>
<td>Temperature ($^\circ$C)</td>
<td>30.2 (29.8–32.3)</td>
<td>30.3 (29.8–32.3)</td>
<td>30.4 (30.1–30.4)</td>
</tr>
<tr>
<td><strong>SNP-mediated vasodilatation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline perfusion (PU)</td>
<td>13 (4–25)</td>
<td>8 (5–87)</td>
<td>10 (3–28)</td>
</tr>
<tr>
<td>Plateau (PU)</td>
<td>63 (30–106)</td>
<td>69 (24–106)</td>
<td>69 (13–145)</td>
</tr>
<tr>
<td>Percentage increase (%)</td>
<td>468 (92–1838)</td>
<td>475 (34–1280)</td>
<td>483 (79–3482)</td>
</tr>
<tr>
<td>Temperature ($^\circ$C)</td>
<td>31.3 (30.0–32.0)</td>
<td>31.2 (30.1–32.5)</td>
<td>30.2 (30.0–32.2)</td>
</tr>
</tbody>
</table>

ACh, acetylcholine; bpm, beats per minute; DBP, diastolic blood pressure; MAP, mean arterial pressure; PU, arbitrary perfusion unit; SBP, systolic blood pressure; SNP, sodium nitroprusside.

*P < 0.05, compared with baseline value by the Wilcoxon signed-rank test.
**P < 0.01, compared with baseline value by the Wilcoxon signed-rank test.

Fig. 1

Relation between changes in blood pressure and changes in capillary density at baseline (a and c) and during venous occlusion (b and d) on day 14 of sunitinib 50 mg/day. DBP, diastolic blood pressure; SBP, systolic blood pressure.
pronounced decrease in capillary density during venous occlusion was significant (Fisher’s exact, \( P = 0.041 \)). At later time points, a livedo reticularis-like disorder on the fingers and arms could be observed in patients with an increased visibility of the subpapillary plexus (Fig. 3).

**Changes in capillary density and vessel morphology predict clinical outcome**

In our RCC patients treated with sunitinib we carried out a preliminary analysis to know whether the vascular changes were related to clinical outcome. On day 14, changes in BP did not have a significant predictive value for PFS and OS (SBP, \( P = 0.87 \) and 0.34, respectively; DBP, \( P = 0.30 \) and 0.22, respectively). Patients with a decrease in the number of capillaries greater than median (2 capillaries/mm\(^2\)) had a prolonged OS (\( P = 0.033 \)). Patients with a decrease in the number of capillaries during venous occlusion greater than median (6 capillaries/mm\(^2\)) had a prolonged PFS and OS (\( P = 0.044 \) and 0.008, respectively) (Fig. 4). In these patients median PFS and OS were 11 and 32 months whereas these values were 3 and 11 months for patients with capillary rarefaction \( \leq 6 \)/mm\(^2\). In addition, patients with an increased visibility of the subpapillary plexus on day 14 had a prolonged median PFS and OS compared with patients without this microscopic skin pattern (\( P = 0.015 \) and 0.013, respectively) (Fig. 4). There were no associations between changes in BP and microvascular function with tumor response according to RECIST.

**Discussion**

In this study we tested the hypothesis that the BP rise, known to be induced by sunitinib, is associated with a reduction in skin microvascular density. We report four novel observations. First, sunitinib treatment is indeed associated with capillary rarefaction, which in turn is directly related to an increase in BP. Second, sunitinib treatment is not associated with impaired microvascular endothelium-dependent and endothelium-independent vasodilatation. Third, the visibility of the subpapillary plexus increased during sunitinib, which was associated with a decrease in capillary density. Fourth, although preliminary, sunitinib-induced capillary rarefaction is predictive for PFS and OS.

Our study has several strengths. All measurements were performed at relatively early time points (day 14). Twenty-four-hour BP monitoring greatly improved the...

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Fig. 2

Skin capillaroscopy at baseline (a) and on day 14 (b) showing increased visibility of the subpapillary plexus after 2 weeks of sunitinib 50 mg/day. The skin fold in the left upper part of the figure (arrow) indicates that the same capillary field was visualized.

Fig. 3

Patient showing a reticular pattern on fingers (a) and arms (b) during sunitinib treatment.
reliability of BP recordings. In addition, our patient population was homogeneous with regard to tumor type and drug dose (50 mg sunitinib per day), and we excluded patients requiring anti-hypertensive treatment during the study. Finally, the long follow-up enabled us to evaluate possible associations between clinical outcome, BP and microvascular function.

A rise in BP can be found in most patients treated with sunitinib [19] with overt hypertension arising in approximately 20% of the patients [20]. Although sunitinib targets several receptor tyrosine kinases, VEGF/VEGFR-2 signaling seems to be essential for the rise in BP. The role for VEGF/VEGFR-2 in BP regulation is illustrated by the extremely high prevalence of hypertension (92%) during treatment with a combination of sunitinib and bevacizumab [21]. Two recent studies have suggested microvascular rarefaction as a mechanism leading to high BP when abrogating VEGF/VEGFR-2 signaling, but a possible relationship was not examined [10,11]. Microvascular rarefaction was described to occur in the skin of patients with advanced colorectal cancer receiving bevacizumab [10] and in the mucosal surface of the inner lip of patients with advanced solid tumors receiving telatinib, a small molecule tyrosine kinase inhibitor of VEGFR-2 and platelet-derived growth factor receptor-β, and c-KIT [11]. Here, we not only measured the development of capillary rarefaction during the inhibition of VEGF/VEGFR-2 signaling, but we were also able to show the presence of a direct association between the decrease in capillary density and the rise in BP.

Although the cause-and-effect relationships of rarefaction and hypertension are still under debate [9,13], mathematical modeling of in-vivo microvascular networks predicts an exponential relation between capillary and arteriolar number and vascular resistance. Total vessel rarefaction up to 42% can increase tissue vascular resistance by 21% [22]. Indirect evidence suggests that microvascular rarefaction, by affecting peripheral vascular resistance, may indeed initiate the pathogenic sequence in sunitinib-induced BP rise. Hypertension caused by an increase in vascular resistance is characterized by a slight decrease in circulating plasma volume, a decrease in
cardiac output and reduced sympathetic activity. In accordance, hematocrit values and erythrocyte numbers are increased in sunitinib-treated patients [19], possibly reflecting a decreased circulating plasma volume. Simultaneously, cardiac output is decreased [23] and heart rate is depressed [12], this study, possibly reflecting decreased sympathetic activity. Humoral factors, such as catecholamines, endotelin-1 and urotensin-II, seem to play a minor role in arterial hypertension during inhibition of VEGF/VEGFR-2-signaling [24]. Renal microvascular dysfunction, accompanied by a shift in the renal pressure–natriuresis relationship, is probably necessary to maintain the initial elevation of BP [25].

Capillary nonperfusion may merely represent the downstream consequence of impaired nitric oxide synthesis leading to reduced vasodilatation at the precapillary arteriolar level. Indeed, Mourad et al. [10] have reported a decrease in endothelium-dependent vasodilatation after 6 months of bevacizumab treatment. Endothelium-independent vasodilatation, however, was not assessed and, therefore, reduced formation of nitric oxide by endothelial cells could not be distinguished from a decreased responsiveness of vascular smooth muscle cells to nitric oxide. Moreover, these findings may be secondary to a chronic increase in BP instead of the cause of a rapid rise in BP [13]. In this study both microvascular endothelium-dependent and endothelium-independent vasodilatation were unaffected by sunitinib therapy. It is important to realize, however, that dermal vasodilatation in response to iontophoresis of Ach is mediated not only by nitric oxide, but also by prostanoids [26], which are unaffected by antiangiogenic therapy [27]. Nevertheless, the responsiveness of microvascular smooth muscle cells to nitric oxide seems to be intact. Our methodology does not preclude impaired endothelium-dependent and endothelium-independent vasodilatation at the level of the resistance vessels or conduit arteries. Impaired endothelium-independent vasodilatation in conduit arteries has been shown during telatinib therapy [11].

An unexpected, but related finding was the visibility of the subpapillary plexus during sunitinib treatment. The subpapillary plexus, which consists of an anastomosing network of arcades and venules and is located at a depth of 400–500 μm from the surface, is not visible in most healthy individuals (> 80%) [28]. At later time points during sunitinib treatment, a livedo reticularis pattern could be observed macroscopically on the fingers and toes of the patients with a visible subpapillary plexus. The visibility of the subpapillary plexus was associated with a more marked decrease in capillary density and, in addition, predictive of favorable clinical outcome. In general, a reticular pattern can be caused by several underlying factors that increase the visibility of the venous network in the skin [29]. Venous stasis of blood owing to slow flow in the draining veins secondary to reduced arterial inflow is a hallmark feature of vasodilatory livedo reticulums [30] and may have caused the change in vessel morphology during sunitinib treatment. In addition, the increase in hematocrit and erythrocyte numbers during sunitinib treatment [19,31] may have contributed to the observed livedo reticulums pattern, because this phenomenon is also associated with polycythemia vera [29].

Capillary density, as a possible direct biomarker of the antiangiogenic and BP increasing potential of sunitinib, might be useful as a biomarker of efficacy. Although preliminary, median PFS and OS were 11 and 32 months for patients showing sunitinib-induced capillary rarefaction greater than 6/mm² whereas these values were 3 and 11 months for patients with a capillary rarefaction ≤6/mm². As expected, three patients who developed ≥ grade 2 hypertension showed capillary rarefaction greater than 6/mm² associated with a better clinical outcome. Whether sunitinib-induced capillary rarefaction may indeed constitute an early indicator of antitumor activity needs to be confirmed in a larger series of mRCC patients. In addition, it needs to be investigated whether sunitinib-induced capillary rarefaction may indeed constitute an early indicator of antitumor activity.

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Chapter 13B

Sunitinib-induced reduction in skin microvascular density is a reversible phenomenon

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letters to the editor

Sunitinib-induced reduction in skin microvascular density is a reversible phenomenon

Arterial hypertension is a well-recognized adverse event in cancer patients treated with inhibitors that target vascular endothelial growth factor (VEGF) or its receptors (VEGFR). Rarefaction (a decrease in perfused microvessels) is suggested to play an important role in the development of this side-effect [1]. A reduction in capillary density has been demonstrated in patients treated with bevacizumab [2], sunitinib [3], and telatinib [4]. In the April issue of Annals of Oncology, Steeghoog et al. [5] have demonstrated that the reduction in capillary density induced by bevacizumab was reversible as measured over 3 months upon discontinuation of the drug. Preclinical data in the adult mouse showed rapid reversibility of capillary regression in normal organs within 2 weeks after cessation of VEGF inhibition [6]. Whether this is true in humans remains to be demonstrated. We here report rapid and full reversibility of microvessel perfusion upon discontinuation of sunitinib.

The tyrosine kinase inhibitor sunitinib targets VEGFR and is administered in a 4 weeks on 2 weeks off schedule. We have demonstrated earlier that the rise in blood pressure in patients with metastatic renal cell cancer (mRCC) during sunitinib was related with a decrease in capillary density [3]. We have also described that the blood pressure rise disappeared promptly during the drug holiday [7]. To investigate whether sunitinib-induced reduction in microvascular density is readily reversible within the 2 weeks off treatment, we measured 24 h ambulatory blood pressure and nailfold capillary density [5] at baseline, 14 days after the start of sunitinib 50 mg/day and 14 days after discontinuation of the drug in three consecutive patients with mRCC. As reported by us before [3], blood pressure increased, whereas the capillary density decreased. On day 42, 2 weeks after discontinuation of sunitinib, not only blood pressure but also capillary density fully recovered to baseline values in two of three patients (Figure 1). During the first treatment cycle, the third patient experienced objective sunitinib-induced grade 1–3 toxic effects. At the time of cycle 2, she still suffered from a series of grade 1 side-effects, which was reason to reduce the sunitinib dose to 37.5 mg/day. Apart from hypertension grade 2, cycle 2 was tolerated well. One week after discontinuation of sunitinib on the 2 weeks off period, antihypertensive medication could be stopped. Measurements of 24-h ambulatory blood pressure and nailfold capillary density were repeated on day 84 (2 weeks after discontinuation of sunitinib at 37.5 mg/day) and promptly showed recovery to baseline values (Figure 1).

Although the effects of VEGF/VEGFR inhibitors on blood pressure and microcirculation appear to be reversible, it is not known whether prolonged treatment with these drugs will cause permanent damage to the vascular system or even cause structural rarefaction and hypertension as a secondary phenomenon. Currently, these angiogenesis inhibitors are only administered in the palliative setting, but their prolonged administration in the curative setting is under investigation. Therefore, insight is required into potential long-lasting adverse effects of these drugs.
events in normal capillaries that are caused by chronic treatment with these drugs.

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None of the authors declare conflicts of interest.

references


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The development of targeted therapy has significantly improved the outcome of patients with metastatic renal cell cancer (mRCC). Among the approved targeted agents, sunitinib has achieved an important place in the treatment of this disease. In this thesis, several clinical and pharmacodynamic aspects regarding sunitinib treatment in mRCC patients are described. Chapter 1 provided an introduction on the treatment of mRCC with a particular focus on sunitinib. Thereafter, the contents of this thesis are divided in three parts.

Part I: Efficacy of sunitinib in renal cell cancer

In Chapter 2, the efficacy of sunitinib in primary renal cell cancer (RCC) tumors in seventeen patients who presented with primary metastatic disease was described. Although primary tumors are usually refractory to cytokine-based therapy, sunitinib induced a significant reduction in tumor volume with concomitant development of extensive tumor necrosis. As the drug was capable of inducing an important tumor response in primary tumors, this might result in improved surgical resection.

In Chapter 3A, the clinical impact of neoadjuvant sunitinib was explored on surgical management of primary tumors with surgery-limiting features which included complex primaries and/or bulky locoregional metastases. Although six out of ten surgery-limiting tumor sites showed a reduction in tumor size, the extent of downsizing by neoadjuvant sunitinib was limited and cytoreductive surgery was reconsidered in only three patients.

In addition, in Chapter 3B it was demonstrated that neoadjuvant sunitinib for resectable primary tumors can have a negative impact on surgical management. Two mRCC patients were described who developed a progressive caval vein thrombus during sunitinib treatment, consequently impeding the initially planned surgery.

Since localization of metastatic disease in the brain represents another RCC tumor location difficult to treat, the occurrence of brain metastases during sunitinib treatment was reported in Chapter 4. In a period of two years, nine out of 91 sunitinib-treated mRCC patients developed symptomatic brain metastases, which represented the first sign of progressive disease. Remarkably, six out of nine patients developed central nervous system (CNS) symptoms in the 2-week rest period. Lesions may have been masked by an anti-edema effect of sunitinib during the 4 weeks-on period of the treatment cycle. These findings suggest that sunitinib is inadequate for control of brain metastases and may temporarily suppress their existence. After radiotherapy or surgery for brain metastases, sunitinib could be safely continued and showed persisting efficacy in the extra-cerebral tumor sites. Therefore, new and isolated progression of RCC in CNS is no indication for permanent discontinuation of sunitinib.

In Chapter 5, it was examined whether genetic polymorphisms have predictive value for sunitinib efficacy in mRCC patients. To that end, a retrospective multicenter pharmaco-
genetic association study was performed in 136 clear cell mRCC patients treated with sunitinib. Thirty polymorphisms related to the pharmacokinetics and pharmacodynamics of the drug were investigated for a possible association with progression-free survival (PFS) and overall survival (OS). Apart from three clinical characteristics [Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic criteria, number of metastatic sites and age], genetic polymorphisms in three genes involved in the pharmacokinetics of sunitinib (CYP3A5, NR1I3 and ABCB1) were predictive factors for PFS. The results of this study warrant prospective validation and further clarification of the role of these genetic determinants in sunitinib exposure and efficacy.

Part II: Side-effects of sunitinib

In Chapter 6, the toxicity and efficacy of sunitinib were described in 82 mRCC patients included in a compassionate use programme. To this end, adverse events were graded according to Common Terminology Criteria for Adverse Events (CTCAE). The observed efficacy of sunitinib was comparable with that reported in previous phase III trials. However, the observed toxicity seemed to be more severe, as almost half of the group of patients needed a dose reduction because of treatment-related side-effects. Most important toxicities requiring a lower dose included stomatitis, fatigue, hand-foot syndrome and a combination of grade 1-2 side-effects. Severe toxicity, defined as dose reduction or permanent discontinuation, was highly related to low body surface area, high age and female gender. On the basis of these patient characteristics, a model could be developed to predict the probability of severe toxicity in patients treated with sunitinib.

In Chapter 7, the occurrence of severe cognitive disorders induced by sunitinib was reported. Three elderly patients with mRCC developed cognitive and behavioral changes while on sunitinib which were reversible upon discontinuation. Brain metastases were excluded and the neurological symptoms disappeared after discontinuation of the drug. All three patients had preexisting arteriosclerotic leukoencephalopathy which most likely has contributed to the development of these cognitive side-effects. Therefore, physicians should be aware of cognitive disorders in elderly patients who are treated with sunitinib. In case such cognitive disorders develop, brain metastases should be excluded and sunitinib should temporarily be discontinued. As described earlier, sunitinib-induced side-effects can be severe. Hence, tools are warranted to predict the toxicity of sunitinib in individual patients in order to select patients for alternative dosing.

In Chapter 8, genetic polymorphisms in the pharmacokinetic and pharmacodynamic pathways of sunitinib were identified that predispose for the development of sunitinib-induced side-effects. In 219 patients treated with sunitinib, several genetic variants were associated with the development of leucopenia, mucosal inflammation, hand-foot syn-
drome and any toxicity higher than grade 2. The identified genetic polymorphisms encoded for metabolizing enzymes, efflux transporters, and drug targets of sunitinib. Development of leucopenia was associated with genetic polymorphisms in $\textit{CYP1A1}$ 2455A/G, $\textit{FLT3}$ 738T/C and the $\textit{NR1I3}$ haplotype. In addition, mucosal inflammation and hand-foot syndrome were associated with genetic polymorphisms in $\textit{CYP1A1}$ 2455A/G and the $\textit{ABCB1}$ haplotype, respectively. Any toxicity higher than grade 2 prevalence was increased when the T allele of vascular endothelial growth factor receptor ($\textit{VEGFR}$)-2 1191C/T or a copy of TT in the $\textit{ABCG2}$ haplotype were present. Validation of the importance of specific genetic polymorphisms in the development of sunitinib-induced side-effects should be carried out in an independent patient population.

**Part III: Potential biomarkers**

In Chapter 9, new response criteria that incorporate the development of tumor necrosis were evaluated for early prediction of sunitinib efficacy. To that end, criteria defined by Choi et al were used in the evaluation of computed tomography (CT) scans in 55 sunitinib-treated mRCC patients. According to these criteria, a partial response (PR) was defined as a $\geq 10\%$ decrease in size or a $\geq 15\%$ decrease in attenuation. At first evaluation after a median period of 1.9 months, the Choi criteria were significantly better predictive for PFS and OS than the standard Response Evaluation Criteria In Solid Tumors (RECIST). However, the predictive value of the Choi criteria was similar to that of RECIST at later time points. Although the Choi criteria could be useful to early identify mRCC patients who benefit from sunitinib, these criteria were not able to early select patients without benefit from the drug. Therefore, the use of the Choi criteria will not change the management of sunitinib-treated mRCC patients.

In Chapter 10, the remarkable changes in hemoglobin levels during sunitinib treatment were reported. In 82 mRCC patients, a zig-zag pattern was observed in hemoglobin levels and erythrocyte numbers. During the 4 weeks-on treatment, a transient increase in hemoglobin and erythrocyte count occurred, which diminished rapidly during the 2-week rest period. Although the increase in erythrocyte numbers was accompanied by a rise in plasma erythropoietin, an erythropoietin-induced rise in erythrocytes is not expected to diminish rapidly within the 2 weeks of rest. On the basis of previous studies and our findings, it was hypothesized that the cyclic kinetics of hemoglobin and erythrocytes were not caused by an increase in erythropoiesis, but are likely the result of a temporary loss of intravascular fluid caused by inhibition of VEGFR-2 and subsequent reduction of nitric oxide.

In Chapter 11, the effects of sunitinib were measured on mature circulating endothelial cells (CEC) and hematopoietic progenitor cells (HPCs) in blood obtained from mRCC patients. Changes in circulating levels of CECs and HPCs may reflect sunitinib activity on
tumor neovasculature. In particular, the kinetics of specific populations of small VEGFR2-
expressing CECs [CD45\(^{-}\)/CD34\(^{\text{bright}}\)] and HPCs [CD45\(^{\text{dim}}\)/CD34\(^{\text{bright}}\)] were analyzed. These populations showed opposite kinetics; the CECs increased, whereas the HPCs decreased. This increase in CECs is likely the result of sunitinib activity in immature tumor vessels. In addition, an increased number of CECs after 14 days of sunitinib treatment was associated with a longer PFS when compared with patients with a decreased number of CECs.

To further investigate the effects of sunitinib on tumor endothelium, a study, reported in Chapter 12, was performed to measure changes in plasma proteins associated with activated tumor endothelium. To that end, plasma samples from sunitinib-treated mRCC patients were investigated for levels of the vascular endothelial growth factor (VEGF), soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular cell adhesion molecule-1 (sICAM-1), von Willebrand factor (vWF), circulating angiopoietin-2 (Ang-2) and soluble Tie-2 (sTie-2). This study showed that tumor burden was positively associated with baseline circulating Ang-2. Sunitinib induced a decrease in circulating Ang-2 and sTie-2 levels, whereas levels of sVCAM-1 and VEGF significantly increased. The decrease in circulating Ang-2 was positively associated with the percentage decrease in tumor burden after sunitinib treatment. Hence, the decline in circulating Ang-2 may represent a biomarker of sunitinib activity in RCC tumors.

Finally, in Chapter 13 the effects of sunitinib on the systemic vasculature were reported. Sunitinib treatment is associated with systemic hypertension, which may be caused by the development of functional rarefaction (a decrease in perfused microvessels) or structural rarefaction (a reduction in anatomic capillary density). In Chapter 13A, it was investigated whether sunitinib treatment leads to impairment of microvascular function and/or reduction of capillary density in the dorsal skin of the finger. In mRCC patients, sunitinib induced a rise in systolic and diastolic blood pressure, whereas the capillary density in the skin decreased. This decrease was associated with an increase in systolic and diastolic blood pressure. In Chapter 13B, it was demonstrated that these effects were reversible after discontinuation of sunitinib. Patients with a greater reduction in capillary density had a prolonged PFS. Therefore, reduction in skin capillary density might be a predictive marker of clinical outcome in sunitinib-treated mRCC patients.
Part I: Efficacy of sunitinib in renal cell cancer (RCC)

I.1 Drug of choice in the era of targeted therapy

The development of targeted therapy, including sunitinib, has proven to be effective in patients with metastatic renal cell cancer (mRCC; Figure 1) and has significantly improved their perspectives (Chapter 1). Nevertheless, it is not yet clear on how to decide on the optimal treatment regimen for an individual patient with mRCC (1).

Figure 1. An example of a mRCC patient who had a partial response (PR) during sunitinib treatment. Metastasis to the lip prior to sunitinib treatment (A) and after two weeks of sunitinib treatment (B). Computed tomography (CT) scans showing metastases (arrows) to lungs, pleura and subcutis before the start of sunitinib (C) and at fourteen weeks after initiation of sunitinib (D).

In mRCC, several factors should be taken into account for the appropriate choice of drug. First, it is important to determine whether the histology of the tumor mainly consists of the clear cell subtype. In case of dominant clear cell histology, patients are further stratified according to Memorial Sloan-Kettering Cancer Center (MSKCC) criteria, extent of disease, and presence of a primary tumor in the first-line setting. Based on the currently available data, sunitinib is justified as first-line standard of care for each patient with clear cell mRCC (2;3). Sunitinib has shown high efficacy in these patients even in the presence of poor risk factors (2). In case patients might not tolerate sunitinib,
pazopanib can be considered as choice (4). In addition, phase III data justify the combination of bevacizumab plus interferon-α as first-line treatment (5-8). However, it can be considered to restrict the latter combination to patients in the favorable risk group and limited disease in the lungs, since complete remission may occur for a prolonged period of time [Chapter 1; (9;10)]. Temsirolimus is recommended as first-line treatment in patients with poor risk factors (11). Besides the MSKCC-related criteria, other factors such as genetic polymorphisms (Chapter 5) and specific tumor features, may contribute to appropriate selection of targeted therapy, as optimal stratification of mRCC patients may further improve the efficacy of these drugs. It is also important to unravel mechanisms of primary (intrinsic) and secondary (acquired) resistance which is inevitable to occur in the course of the disease. Recently, lysosomal sequestration has been identified as a mechanism of sunitinib resistance (12).

1.2 Sunitinib treatment for localized disease

mRCC patients with potentially resectable primary tumors and/or a solitary metastasis are still candidates for nephrectomy and/or metastasectomy, as long-term survival has been reported in some patients (13). In the cytokine era, responses of the primary tumor were very rare (14) and patients presenting with primary metastatic disease and a resectable primary tumor usually underwent cytoreductive nephrectomy. This strategy was based on the results of two randomized phase III trials, which had shown that nephrectomy followed by interferon-α improved overall survival (OS) as compared with interferon-α alone (15;16). Currently, this treatment strategy is under debate since targeted therapy (17;18) can induce impressive responses in primary tumors (Chapter 2). Targeted therapy may even contribute to downsizing of primary tumors, thereby facilitating cytoreductive surgery [Chapter 3A; (19-21). However, mixed responses in the primary tumor and metastases may occur. It has been described that an initially resectable primary tumor may increase in size despite a treatment-induced response in the metastases (Chapter 3B). Therefore, early identification of progression of a resectable primary tumor is important to avoid a missed opportunity for cytoreductive nephrectomy. Patients with lung metastases only and a favorable prognostic score are most likely to benefit from cytoreductive surgery. The selection of mRCC patients for cytoreductive nephrectomy needs to be optimized. Recently, two randomized controlled trials have been initiated to determine the current role and optimal timing of removal of the primary tumor in patients with primary metastatic disease. Therefore, participation in a clinical trial is currently considered as the best management of mRCC patients presenting with a resectable primary and synchronous metastases.
**I.3 Second-line treatment**

After progression on a previous systemic treatment, several drugs can be considered as second-line therapy in mRCC patients. After failure of cytokine-based therapy, sunitinib, pazopanib, and sorafenib can be recommended (4;22;23). In addition, phase III data support second-line treatment with everolimus after failure of a receptor tyrosine kinase inhibitor (TKI) (11). In clinical practice however, another TKI is frequently given after failure of a TKI. For example, sunitinib-induced tumor response can still be observed after disease progression upon sorafenib treatment. However, the optimal treatment sequence of targeted agents after failure in first-line setting has not been defined yet. Therefore, clinical trials in the second-line and third-line setting are warranted in order to determine the optimal treatment strategy and drug sequence in individual patients with mRCC.

**I.4 Sunitinib for patients with non-clear cell histology**

Although efficacy of sunitinib is observed in patients with non-clear cell histology, evidence for use of sunitinib in this patient population is limited. In the majority of phase III trials on targeted drugs, mRCC patients with a predominant clear cell histology have been included. Temsirolimus has proven activity in patients with non-clear cell histology as observed in a subset analysis of the phase III trial (11). In addition, a few retrospective studies have reported minor efficacy of sunitinib and sorafenib in patients with non-clear cell histology [Chapter 6; (24;25)]. However, a recent phase II trial could not demonstrate significant efficacy of sunitinib in mRCC patients with non-clear cell histologies (26). In fact, there is no established effective therapy for patients with non-clear cell histology. Presently, treatment in a clinical trial is the preferred strategy for mRCC patients with non-clear cell histologies, because effective drugs need to be developed for this heterogeneous group (27). To that end, broad international collaboration is required, as non-clear histology accounts for a very small patient population.

**Part II: Side-effects of sunitinib**

**II.1 Prediction of side-effects**

In clinical practice, sunitinib treatment is associated with a wide range of grade 1-2 toxicities, frequently requiring dose reduction and/or discontinuation of treatment (Chapter 6). Therefore, patients should be monitored closely, in particular during the first weeks of sunitinib treatment. Early identification of patients who might develop severe toxicity may be helpful in order to reduce the standard dose of sunitinib prior to the start
of treatment. In Chapter 6, it was demonstrated that there was a significant correlation between severe sunitinib-related toxicity and patient characteristics including small body surface area, high age, and female gender. In addition, the data presented in Chapter 8 show that several genetic polymorphisms in the pharmacokinetic and pharmacodynamic pathways of sunitinib are associated with sunitinib-induced toxicity including leucopenia, mucosal inflammation, hand-foot syndrome, and any toxicity higher than grade 2. The high incidence of dose reduction and discontinuation of treatment, together with the findings in Chapters 6 and 8, suggest that the standard dosing schedule of sunitinib, which is 50 mg daily in a 4 weeks-on/2 weeks-off schedule, is not always optimal for unselected mRCC patients. Considering a relation between exposure of sunitinib and some toxicities (28), it is conceivable that patients with severe toxicity are overtreated, whereas patients who do not experience any toxicity may be undertreated. In that case, some side-effects such as hypertension may be an easy read-out of sufficiently reached plasma concentrations of sunitinib and its active metabolite SU12622. As an alternative, pharmacokinetic studies with intensive monitoring of plasma levels of sunitinib and its active metabolite SU12622 may be beneficial to further reveal the relationship between sunitinib exposure and sunitinib-induced toxicities. The need for pharmacokinetic studies is also supported by the findings in Chapter 5 in which genetic polymorphisms in three genes involved in the pharmacokinetics of sunitinib (CYP3A5, NR1I3 and ABCB1) were found to be associated with progression-free survival (PFS). In the future, these genetic variants may be useful to optimize dosing and scheduling of sunitinib in individual patients.

II.2 Palliation of side-effects
As sunitinib has proven efficacy in mRCC, optimal dosing, i.e. the highest tolerable dose, and adherence to sunitinib treatment are essential for palliation of disease-related symptoms and prolongation of survival. However, sunitinib frequently causes the accumulation of a series of grade 1 and 2 adverse events. As a result, sunitinib-induced toxicities can have a profound impact on daily life, thereby invalidating patients. For example, fatigue interferes excessively with daily life, mucositis and taste alteration require changes in food habits, hand-foot syndrome limits walking and the urgent pattern of diarrhea has a high risk of soiling. However, the evidence for adequate palliation of sunitinib-induced toxicities is rather limited, as these toxicities are completely different from those caused by traditional chemotherapy. More insight into the underlying mechanisms of sunitinib-induced side-effects may improve the palliation of these frequently observed symptoms. This is particularly important since sunitinib is considered palliative instead of curative for treatment of patients with mRCC.
II.2.1 Dose modifications
In general, sunitinib-induced toxicities will recover after dose reduction or temporarily discontinuation of treatment. When necessary, a 12.5 mg dose reduction is recommended from the regular dose of 50 mg (29). After discontinuation of sunitinib, recovery to acceptable levels of toxicity should occur within four weeks to allow sunitinib continuation. In the absence of hematological ≥ grade 3 or non-hematological ≥ grade 2 toxicities in the previous cycle, re-escalation to the previous dose level can be considered (29). However, dose reduction or interruption of sunitinib treatment should be prevented in order to achieve sufficient efficacy. If patients have symptoms of progressive disease (PD) during the rest period, continuous dosing of sunitinib at 37.5 mg per day is advised. Early detection and management reduces the severity of the side-effects, thereby improving quality of life and treatment adherence. To this end, frequent contact between patient and his/her treating physician is important, in particular during the first treatment cycle. In the paragraphs below, the current management of the most frequently reported toxicities associated with sunitinib treatment will be shortly discussed.

II.2.2 Fatigue
Fatigue is one of the most common side-effects associated with sunitinib treatment (2). Dose interruption or at least dose adjustment is usually required for grade 3 or 4 fatigue. Fatigue may be caused by other sunitinib-related toxicities, such as hypothyroidism (30) and anemia. Therefore, thyroid function and hemoglobin level should be monitored on a regular basis, as patients can be easily treated for these symptoms. Of note, onset of fatigue or its worsening could also indicate disease progression, as cancer-related fatigue is often perceived by mRCC patients.

II.2.3 Hypothyroidism
For all patients treated with sunitinib, it has been recommended that thyroid function tests are performed on days 1 and 28 of the first four cycles (31). Levels of the thyroid-stimulating hormone (TSH) should ideally be checked prior to start of sunitinib. By applying this intensive initial screening, patients at risk of developing sunitinib-induced thyroid dysfunction can be early detected following drug initiation. In addition, when patients do not have thyroid function test abnormalities within the first four cycles, monitoring of thyroid function tests can be subsequently performed less frequently, e.g. every three cycles, unless clinically indicated (31). Hormone replacement therapy should be initiated in patients with persistent TSH > 10 mIU/mL, even in the presence of normal T4 levels (31).
II.2.4 Hand-foot syndrome

Management of hand-foot syndrome can be initiated before symptoms develop. Complaints can be partly prevented by regular manicures and pedicures (32). Excessive pressure on bony prominences should be prevented by wearing appropriate footwear and by avoiding activities that place undue stress to the hands and feet. In addition, patients should be advised to avoid exposure of their hands and feet to hot water, as it is believed that hot water may exacerbate the symptoms (33). If hand-foot syndrome develops (Figure 2), moisturizing creams are recommended for relief.

![Figure 2](image)

**Figure 2.** Examples of grade 3 hand-foot syndrome in patients treated with sunitinib. (A) Painful skin changes of the hand consisting of blisters and peeling, interfering with activities of daily living (ADL). (B) Painful blister on the right foot of another patient, interfering with ADL.

In addition, keratolytics (e.g. urea 20-40% or salicylic acid 6%) can be given (33). In case of grade 2 hand-foot syndrome, topical analgesics such lidocaine 2% and pain killers may be given for pain control. Grade 3 hand-foot syndrome often requires interruption of treatment until hand-foot syndrome reaches grade 0 or 1. Thereafter, the treatment can be resumed at a reduced dose. Then, if toxicity does not occur, re-escalation of the dose can be attempted (33).

II.2.5 Oral mucositis and stomatitis

In clinical practice, the terms oral mucositis and stomatitis are often used interchangeable. Rigorous hygiene consisting of baking soda mouthwash or sodium chloride solutions may partly prevent oral mucositis and stomatitis (29). Treatment of ≥ grade 1 requires mixtures of mucosal coating agents and local anesthetics, whereas oral candidiasis requires oral nystatin or fluconazole. Of note, ketoconazole, which is a
selective inhibitor of CYP3A, is contraindicated as it may increase plasma concentrations of sunitinib. Development of grade 3 mucositis or stomatitis requires dose modification of sunitinib.

II.2.6 Gastrointestinal toxicity
Anorexia, nausea, vomiting and diarrhea are frequently reported side-effects of sunitinib (2). Symptomatic medications include megestrol acetate for anorexia, metoclopramide and ondansetron for nausea and/or vomiting, and loperamide for diarrhea (29;34). In case of nausea or vomiting, small amounts of food and drinks are advised. When patients have diarrhea, they are advised to avoid food that would aggravate this complaint (spicy fatty foods, caffeine) and to drink plenty of liquids for oral hydration (29).

II.2.7 Hypertension
The exact mechanism by which VEGF inhibitors induce hypertension has not yet been completely clarified. Previous studies have shown that VEGFR-2 is involved in the regulation of the vascular tone. Activation of VEGFR-2 via phosphoinositide-3 kinase (PI3K) and its downstream serine protein kinase Akt stimulates endothelium-derived nitric oxide synthase (eNOS), subsequently leading to the production of the potent vasodilator nitric oxide (NO) (Figure 3A). In addition, decreased NO bioavailability favors the production and/or activity of endothelin-1 (ET-1) through the loss of inhibitory effects of NO on ET-1 (35;36). Indeed, plasma ET-1 concentrations have been described to increase in subjects treated with sunitinib (37;38). Consequently, it could be hypothesized that inhibition of VEGFR-2 causes an imbalance between the vasodilator NO and the vasoconstrictor ET-1, favoring ET-1 and thereby resulting in the development of hypertension (Figure 3B). The recognition and management of hypertension by drugs affecting VEGF(R) signaling is important, as poorly controlled hypertension may lead to serious adverse events. Therefore, blood pressure should be monitored on a regular basis, in particularly in patients at high cardiovascular risk such as patients with pre-existent hypertension, diabetes mellitus and a history of cardiovascular disease. 24-hour ambulatory blood pressure measurements during the first treatment cycle can be used to early detect undiagnosed or uncontrolled hypertension (39). In line with the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC7) (40), it is currently recommended to apply behavioral and pharmacological interventions in order to achieve a blood pressure lower than 140/90 mmHg in patients treated with angiogenesis inhibitors (34;41). However, no clear recommendation can be made for anti-hypertensive drugs, as there is a lack of studies on treatment of this drug-induced hypertension (34;41). In fact, efficacy has
been observed for all types of anti-hypertensive drugs including diuretics, beta-blockers, angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor blockers. Therefore, drug selection should be based on the comorbidity, contraindications and drug interactions in an individual patient. As previously tested in a human colorectal tumor xenograft in nude rats, it has been demonstrated that anti-hypertensive treatment does not negatively affect the antitumor activity of the TKI cediranib (42). Recently, it has been reported that the development of hypertension during sunitinib treatment is associated with improved clinical outcome in mRCC, indicating that hypertension may be a biomarker of efficacy in sunitinib-treated mRCC patients (43-45).

**Figure 3.**
(A) Role of VEGFR-2 in regulation of the vascular tone.
(B) Inhibition of VEGFR-2 by sunitinib might result in a dysbalance between vasodilatation and vasoconstriction, leading to increased peripheral resistance and subsequent hypertension. R, receptor (extracellular domain); TK, tyrosine kinase; P, phosphorylation site (intracellular domain); PI-3K, phosphoinositide-3 kinase; eNOS, endothelium-derived nitric oxide synthase; NO, nitric oxide; ET-1, endothelin-1.
Part III: Potential biomarkers

III.1 Imaging of mRCC

As targeted therapy causes disease stabilization rather than substantial tumor regression, new response criteria and imaging techniques are being investigated for improved evaluation of the efficacy of these molecules in mRCC patients. Response Evaluation Criteria in Solid Tumors (RECIST) are usually applied to assess tumor response in mRCC and are able to identify patients with clinical benefit. However, RECIST is not able to early identify mRCC patients with a poor PFS during targeted therapy (Chapter 9). Incorporation of treatment-induced changes in tumor attenuation on contrast-enhanced computed tomography (CT) scans may have additional value to assess tumor response, but it is not clear whether inclusion of these changes in decision-making have significant impact on the current management of mRCC patients. Other imaging techniques including dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI), dynamic contrast-enhanced ultrasonography (DCE-US), and positron emission tomography (PET) are also under study for response evaluation of targeted therapy in mRCC patients (46).

Functional imaging, such as DCE-MRI and dynamic PET scans, is increasingly incorporated in the evaluation of anticancer drugs. These imaging techniques usually have a limited field of view, which may be a disadvantage. For example, the field of view of a dynamic PET scan is currently < 20 cm. Therefore, functional imaging can usually not be applied to evaluate the disease in the whole patient. However, the recent trend is towards a reduction of lesions to be measured, as RECIST version 1.1 has reduced the maximum number of target lesions from 10 to 5 lesions (47). In addition, Hillman et al (48) have reported that measurement of > two metastatic lesions when compared with two lesions did not alter the definitive response in solid tumors. However, functional imaging may fail to detect new lesions outside the field of view in case of progressive disease (PD), which is a potential limitation for routine use. In addition, functional imaging techniques may be significantly influenced by the hemodynamic changes that are caused by targeted agents, which hold especially for inhibitors of VEGF(R) signaling. As treatment with these drugs is associated with an increase in blood pressure (49), a decrease in heart rate (50), and a reduction in capillary density of the normal microcirculation (Chapter 13), these hemodynamic effects may affect the kinetic parameters that are obtained by functional imaging, such as PET and DCE-MRI. Therefore, methods need to be developed that correct for blood pressure and cardiac output in the kinetic modeling of functional imaging techniques.
As the PET technique is able to quantify tracer concentrations in absolute units, radiolabeling of targeted agents with short-lived positron emitting radionuclides, such as carbon-11 (\(^{11}\)C; half-life, 20.3 min) and fluorine-18 (\(^{18}\)F; half-life, 109.8 min), is an attractive approach to assess their uptake in tumor lesions. In dynamic PET studies, the optimal kinetic model of an injected tracer can be developed to quantify the tumor uptake of the radiolabeled drug. Currently, several targeted drugs have been radio-labeled as PET tracers and their number is still increasing (51). These specific PET tracers provide a unique opportunity for personalized treatment planning, since they may be able to predict tumor response before initiation of therapy. Of the approved targeted agents for treatment of mRCC, \([^{89}\)Zr\]bevacizumab and \([^{18}\)F\]sunitinib have been developed as PET tracers (52-54). Such PET studies using radiolabeled drugs may not only be used to explore a potential relationship between pretreatment uptake and response, but might also give insight into drug uptake during treatment and the development of tumor resistance.

Before implementation of new imaging tools and new response evaluation criteria to determine the efficacy of targeted therapies in the clinic, the feasibility, accuracy and reproducibility of the new methods should be determined. For the assessment of tumor response, the test-retest reproducibility of the method needs to be known in order to determine the minimal change in the variable that represents a true treatment effect. In particular, when relatively small changes are already considered as tumor response, the test-retest reproducibility needs to be high. In addition, there is a need for standardized protocols including timing of response evaluation, acquisition of imaging data and data analysis in order to optimally compare the results in individual patients at different time-points, but also to enable exchange of data among different institutes. With regard to the timing of response assessment, specific attention should be paid to sunitinib, as this TKI is usually administered in a schedule consisting of 4 weeks-on treatment followed by a 2-week rest period. Since temporarily discontinuation of sunitinib may result in flare-up mimicking PD (55), response measurements should not be performed during the rest period. As the new criteria and techniques have not yet been optimally validated, RECIST can still be considered as the preferred method to assess response to targeted therapy.

III.2 Circulating factors
Apart from imaging techniques, markers in blood may have promise as surrogate endpoints for sunitinib efficacy in mRCC patients. In comparison with imaging techniques, blood parameters are usually less expensive and less time-consuming. In Chapters 10, 11 and 12, it is demonstrated that changes in blood values including hemoglobin, circulating endothelial cells (CECs), hematopoietic progenitor cells (HPCs), and circulating...
proteins are partly reversible and related to the dosing schedule of sunitinib. The reversible pattern of these changes in blood values, however, suggests that the investigated markers rather reflect systemic exposure to sunitinib than a surrogate endpoint for tumor response. Among the investigated blood-based parameters in the present thesis, only CECs and circulating Ang-2 hold promise as surrogate markers, as changes in these blood parameters were associated with treatment outcome. An increased number of CECs after 14 days of sunitinib treatment was associated with a longer PFS in mRCC patients (Chapter 11), whereas the decrease in circulating Ang-2 was positively associated with the percentage decrease in tumor burden after sunitinib treatment (Chapter 12).

III.2.1 Circulating endothelial cells and progenitor cells

To date, the origin and phenotype of CECs and progenitor cells is not well defined. CECs are thought to be shed from mature blood vessels (56), whereas progenitor cells are considered to be bone marrow-derived cells, contributing to tumor angiogenesis (57). In sunitinib-treated mRCC patients, the increase in CECs may be caused by endothelial cell detachment and may reflect antivascular effects of sunitinib in tumors (Chapter 11). In a more recent study by Farace et al (58), baseline CD45\textsuperscript{dim}CD34\textsuperscript{+}VEGFR2\textsuperscript{+} progenitor cells were associated with PFS and OS in 55 mRCC patients treated with sunitinib or sorafenib. In that study, levels of CD45\textsuperscript{dim}CD34\textsuperscript{+}VEGFR2\textsuperscript{+} progenitor cells showed an increase on day 14 after start of treatment as compared with baseline levels, but this change was not significant (58). In addition, patients with stable or increased levels of CD45\textsuperscript{dim}CD34\textsuperscript{+}VEGFR2\textsuperscript{+} progenitor cells between baseline and day 14 had a lower risk of disease progression compared with patients whose CD45\textsuperscript{dim}CD34\textsuperscript{+}VEGFR2\textsuperscript{+} progenitor cell levels decreased over the same period (58). These results by Farace et al (58) are in contrast to the results described in Chapter 11, as a significant decrease in the level of CD45\textsuperscript{dim}/CD34\textsuperscript{bright}/VEGFR2\textsuperscript{+} HPCs was found in Chapter 11. This decrease in the level of CD45\textsuperscript{dim}/CD34\textsuperscript{bright}/VEGFR2\textsuperscript{+} HPCs may be caused by sunitinib-induced bone marrow suppression associated with FLT-3 inhibition of sunitinib (59). The discrepancy between the study by Farace et al (58) and our study may be explained by the fact that the CD45\textsuperscript{dim}/CD34\textsuperscript{bright}/VEGFR2\textsuperscript{+} HPCs in Chapter 11 are presented in absolute counts, whereas Farace et al presented the results as the percentage of VEGFR2\textsuperscript{+} cells among the circulating progenitor cells (CD45\textsuperscript{dim}/CD34\textsuperscript{+}). This difference in presentation of results on progenitor cells illustrates the lack of agreement on the methodology of studies investigating CECs and progenitor cells. Currently, identification and enumeration of CECs and progenitor cells remain difficult and are performed by non-standardized methods. As
agreement on the phenotypic differentiation of CECs and progenitor cells is still lacking, there is a need for consensus on the characterization of these cells.

III.2.2 Circulating Ang-2

The role of angiopoietin-2 (Ang-2) in tumor angiogenesis remains controversial and poorly defined. During sunitinib treatment, levels of circulating Ang-2 and sTie-2 decreased. The reduction in circulating Ang-2 was positively associated with the percentage decrease in tumor burden after sunitinib treatment (Chapter 12). Targeting Ang-2 may represent an additional beneficial effect of sunitinib in patients with mRCC. However, it is currently not known whether sunitinib directly targets Ang-2 or whether the decrease in circulating Ang-2 reflects an indirect effect of sunitinib on another target. As Ang-2 and von Willebrand factor (vWF) are both stored in the Weibel-Palade bodies of endothelial cells (EC) and plasma levels of vWF did not change in mRCC patients treated with sunitinib, it was suggested that sunitinib mostly reduced Ang-2 production from tumor cells (Chapter 12).

Presently, the interest in the Ang-2/Tie-2 pathway as an important factor for tumor angiogenesis is rapidly growing and drugs that selectively target the Ang-2/Tie-2 pathway have been developed (60). There is evidence that targeting the Ang-2/Tie-2 signaling pathway may inhibit the functions of Tie-2-expressing macrophages (TEM), which is a subset of tumor-associated macrophages having proangiogenic activity (61). Mazzieri et al (62) have shown that inhibition of Ang-2 regresses the tumor vasculature and inhibits progression of late-stage, metastatic MMTV-PyMT mammary carcinomas in a mouse tumor model. In that study, Ang-2 blockade did not inhibit recruitment of MRC1(+)TEMs, but inhibited their upregulation of Tie-2 and ability to restore angiogenesis in tumors. After a conditional knockdown of Tie-2 in hematopoietic-lineage cells, a lack of association was observed between TEMs and ECs in MMTV-PyMT tumors. Together, these findings suggest that Ang-2/Tie-2 signaling, or the upregulation of Tie-2 receptors on the surface of TEMs, is important to enable TEM interacting with angiogenic blood vessels [Figure 4, (61)]. This suggests that activated ECs in tumors release Ang-2, which in turn induces TEMs to interact with sprouting blood vessels through upregulation of Tie-2 on TEM as well as the formation of EC-TEM cell-to-cell contacts mediated by Ang-2 and Tie-2 (61). Although Ang-2 expression by tumor cells is not considered in the above described interactions between ECs and TEMs, it is conceivable that expression of Ang-2 by tumor cells, which has been observed for RCC tumor cells (63), can lead to interactions between tumor cells and TEMs. Since sunitinib treatment reduces levels of circulating Ang-2 and Tie-2 (Chapter 12), sunitinib may inhibit both ECs-TEMs and tumor cells-TEMs interactions. In addition, the inhibitory activity of sunitinib on bone marrow
may reduce the recruitment of TEMs and consequently their availability in tumors. As TEMs are known to have protumoral and proangiogenic activity that can counteract the efficacy of both anti-angiogenic and radiation therapy (61), the combined targeting of angiogenic ECs and proangiogenic TEMs by selective inhibition of the Ang-2/Tie-2-pathway is promising for the treatment of mRCC patients.

Figure 4. Modeling the effects of angiopoietin (Ang) inhibition on angiogenesis and TEMs in mouse mammary tumors. In mature blood vessels, Ang-1 is constitutively expressed by perivascular cells, such as pericytes and smooth muscle cells, and promotes EC quiescence by interacting with Tie-2 on ECs. In these conditions, ECs release little or no Ang-2 (not shown). (A) In growing tumors, ECs proliferate and form new blood vessels. This process is accompanied by the dissociation of pericytes from, and association of TEMs with, the sprouting ECs. At sites of sprouting angiogenesis (inset in A and B), activated ECs upregulate and release Ang-2 (which competes with Ang-1 for binding to Tie-2) in the peri-EC spaces, making it available for binding to Tie-2 on ECs and perivascular TEMs. The net outcome of the process, which occurs in the presence of VEGF and other proangiogenic factors, is stimulation of angiogenesis. (C) Ang-2 specific inhibition leads to regression of tumor blood vessels. This is accompanied by enhanced pericyte coverage of, and displacement of TEMs from, the remaining blood vessels. The former effect may be mediated, at least in part, by Ang-1, which outcompetes Ang-2 for binding to Tie-2. Note that TEMs express much lower Tie-2 levels in Ang-2 depleted tumors than in untreated tumors, suggesting that Ang-2 directly stimulates Tie-2 expression by TEMs.

TEM, Tie-2 expressing macrophages; Ang-1, angiopoietin-1; Ang-2, angiopoietin-1; EC, endothelial cell [reprinted with permission from reference (61)].
III.2.3 Capillary density and blood pressure

As described in Chapter 13, sunitinib treatment induced a decrease in capillary density in the dorsal finger of the skin, whereas blood pressure increased. These effects were related and probably reflect inhibition of VEGFR-2 signaling, leading to a decreased production of nitric oxide (NO) and subsequently peripheral vasoconstriction and hypertension (64). In this respect, polymorphisms in genes of the VEGFR-2/NO pathway may be useful to predict the development of hypertension in sunitinib-treated patients (65). As mentioned above (see paragraph II.2.7), retrospective studies have shown that the development of hypertension may be a simple surrogate marker for outcome in mRCC patients treated with sunitinib (43-45). However, it should be excluded that the observed relation between the development of hypertension and clinical outcome is not confounded by patient characteristics such preexistent hypertension and age. In addition, prospective studies with adequate blood pressure measurements are warranted to confirm the usefulness of hypertension as a surrogate marker of sunitinib efficacy. The mechanism of the relation between drug-induced hypertension and clinical outcome in patients treated with anti-angiogenic drugs has not yet been clarified. On the one hand, earlier studies have shown that the vasoconstrictor angiotensin II can enhance blood flow in hepatic tumors (66;67), suggesting increased drug delivery to tumors. On the other hand, a previous PET study using radioactive water and a radiolabeled anticancer drug ([11C]docetaxel) has shown that bevacizumab induces a rapid decrease in tumor perfusion and drug delivery to tumors in patients with non-small cell lung cancer (68). These findings indicate that more insight into the relation between drug-induced hypertension and clinical outcome is warranted, as it may also provide insight into the effects of anti-hypertensive medication on the efficacy of VEGF(R) inhibitors.

Conclusions

As multiple systemic treatment modalities arise, a further refinement is needed to identify mRCC patients who are likely to benefit from sunitinib treatment and patients who do not. Although the MSKCC criteria may be useful for risk stratification, more specific tools are required to select individual patients for a specific treatment. Since targeted drugs may fail in a number of patients and are associated with a wide range of toxicities, tools are needed for prediction of efficacy and drug-induced toxicity in order to personalize treatment planning in individual patients. To this end, prediction models, pharmacogenetic variables and radiolabeled drugs for PET may be useful to predict the efficacy and toxicity of targeted agents, including sunitinib, prior to the start of treatment. Furthermore, evaluation of tumor response needs to be improved in order to switch early from an ineffective drug to another one. Besides response evaluation by
tumor imaging, circulating biomarkers in blood may be promising surrogate markers for this purpose. To that end, extensive studies in larger numbers of cancer patients are required to validate markers for routine clinical use. In addition, it has become increasingly important to define the best sequence of drugs in order to maximize patient outcome. Finally, there is still a need for new active molecules since most mRCC patients will eventually acquire drug resistance and die from progressive disease. Therefore, participation in clinical trials should still be encouraged for patients with mRCC.
References


De ontwikkeling van medicamenten die beter gericht zijn tegen de kankercel, ook wel targetted therapie genoemd, heeft het perspectief van de patiënt met gemetastaseerd niercelcarcinoom significant verbeterd. Voor niercelcarcinoom is de vasculaire endotheliale groeifactor (VEGF) een belangrijk target voor therapie. Van de nieuwe medicamenten heeft sunitinib een belangrijke plaats verworven bij de behandeling van het niercelcarcinoom. Hoewel behandeling met sunitinib heeft geleid tot een betere overleving van patiënten met deze ziekte, was er enkele jaren geleden nog veel onduidelijk over de eigenschappen van dit middel. In dit proefschrift worden verscheidene klinische en farmacodynamische aspecten van sunitinib beschreven bij patiënten met gemetastaseerd niercelcarcinoom. Hoofdstuk 1 bevat een introductie tot de behandeling van gemetastaseerd niercelcarcinoom in het algemeen, met daarbij speciale aandacht voor sunitinib. Vervolgens is de inhoud van dit proefschrift opgedeeld in drie delen.

Deel I: Effectiviteit van sunitinib voor niercelcarcinoom
In Hoofdstuk 2 is de effectiviteit van sunitinib voor primaire tumoren van het niercelcarcinoom beschreven. Er werden zeventien patiënten met een primair gemetastaseerde ziekte behandeld met sunitinib. Hoewel primaire tumoren meestal refractair zijn voor een behandeling met cytokines, induceerde sunitinib zowel een significante verkleining in de tumorgrootte als een uitgebreide tumornecrose. Aangezien een klinisch indrukwekkende respons in primaire tumoren werd waargenomen, zou een voorbehandeling met sunitinib de chirurgische resectie van primaire tumoren sterk kunnen vereenvoudigen. Hoofdstuk 3A is gewijd aan de mogelijke waarde van een neoadjuvante behandeling met sunitinib voor inoperabele primaire tumoren. Tot deze inoperabele primaire tumoren werden complexe primaire tumoren en/of uitgebreide locoregionale metastasen gerekend. Hoewel zes van de tien inoperabele tumoren een afname in tumorgrootte toonden, kon slechts bij drie patiënten cytoreductieve chirurgie worden uitgevoerd. Verder werd duidelijk dat neoadjuvante behandeling met sunitinib een negatief effect kan hebben op mogelijk operabele primaire tumoren (Hoofdstuk 3B). Twee patiënten met gemetastaseerd niercelcarcinoom en een primaire tumor in situ toonden progressie van de ziekte tijdens neoadjuvante behandeling met sunitinib. Deze progressieve ziekte uitte zich in een trombus in de vena cava. Dit leidde ertoe dat de oorspronkelijk geplande operatie niet kon doorgaan. Aangezien metastasering naar de hersenen ook een moeilijk te behandelen tumorlocalisatie van niercelcarcinoom is, werd de incidentie van hersenmetastasen tijdens behandeling met sunitinib besproken in Hoofdstuk 4. Gedurende een periode van twee jaar kregen negen van 91 patiënten met gemetastaseerd niercelcarcinoom, die werden behandeld met sunitinib, symptomatiche hersenmetastasen. Deze hersenmetastasen waren het eerste teken van progressie van
de ziekte tijdens sunitinib. Het was opmerkelijk dat bij zes van de negen patiënten de symptomen ontstonden gedurende de tweewekelijkse rustperiode in het behandel-schema. Deze observatie doet vermoeden dat hersenmetastasen gemaskeerd kunnen worden door het anti-oedeem effect van sunitinib. Sunitinib lijkt dus onvoldoende effectief te zijn voor locale controle van hersenmetastasen, maar kan mogelijk wel tijdelijk de symptomen van hersenmetastasen onderdrukken. Na radioterapie of chirurgische behandeling van de hersenmetastasen kon de behandeling met sunitinib weer veilig worden voortgezet en bleek dit medicament nog langdurig effectief te zijn voor de behandeling van de extra-cerebrale tumorgebieden. Daarom is progressieve ziekte van het niercelcarcinoom in het centrale zenuwstelsel, mits deze progressie nieuw en geïsoleerd is, zeker geen reden om de behandeling met sunitinib definitief te stoppen.

Vervolgens is in Hoofdstuk 5 beschreven in hoeverre genetische polymorfismen de effectiviteit van sunitinib bij patiënten met gemetastaseerd niercelcarcinoom kunnen voorspellen. Daartoe werd een retrospectieve multicentrum studie verricht bij 136 patiënten met gemetastaseerd heldercellig niercelcarcinoom, die werden behandeld met sunitinib. Er werden dertig polymorfismen, die geassocieerd worden met de farmacokinetiek en de farmacodynamiek van sunitinib, onderzocht voor een mogelijke relatie met de progressievrije overleving en de totale overleving. Naast de drie klinische karakteristieken [Memorial Sloan-Kettering Cancer Center (MSKCC) prognostische criteria, aantal locaties met metastasen en leeftijd] bleken genetische polymorfismen in drie genen (CYP3A5, NR1I3 en ABCB1), die betrokken zijn bij de farmacokinetiek van sunitinib, voorspelling te zijn voor de progressievrije overleving. Toekomstige studies zijn nodig om deze genetische determinanten prospectief te valideren teneinde hun rol bij de blootstelling aan en de effectiviteit van sunitinib te verduidelijken.

Deel II: Bijwerkingen van sunitinib
In Hoofdstuk 6 zijn de toxiciteit en de effectiviteit van sunitinib beschreven bij 82 patiënten met gemetastaseerd niercelcarcinoom die werden behandeld in een 'compassionate use programme'. In dit ‘compassionate use programme’ waren de in- en exclusie criteria minder streng dan die in fase III studies. Op deze manier kon een beter beeld worden verkregen van het voordeel van sunitinib bij patiënten uit de dagelijkse oncologische praktijk. In deze studie bleek de effectiviteit van sunitinib vergelijkbaar met die in eerdere fase III studies. De geobserveerde toxiciteit, welke werd gegradeerd volgens de Common Terminology Criteria for Adverse Events (CTCAE), was echter veel ernstiger, aangezien bijna de helft van de patiënten een verlaging van de dosis nodig had om de ontstane bijwerkingen van sunitinib te verminderen. Stomatitis, vermoeidheid, hand-voet syndroom en een combinatie van verschillende graad 1-2 bijwerkingen waren

**Hoofdstuk 7** is gewijd aan het ontstaan van ernstige cognitieve symptomen tijdens behandeling met sunitinib. Drie bejaarde patiënten met gemetastaseerd niercelcarcinoom kregen cognitieve klachten en gedragsveranderingen tijdens het gebruik van sunitinib. Hersenmetastasen werden uitgesloten en na het staken van de behandeling verdwenen de cognitieve symptomen vanzelf. Alle drie patiënten had voorafgaande aan de behandeling met sunitinib reeds een arteriosclerotische leukoencefalopathie, hetgeen waarschijnlijk heeft bijgedragen aan het ontstaan van de cognitieve bijwerkingen. Daarom is het belangrijk dat artsen aandacht hebben voor cognitieve veranderingen in bejaarde patiënten aan wie sunitinib wordt voorgeschreven. Wanneer patiënten dergelijke symptomen ervaren, dienen hersenmetastasen te worden uitgesloten en dient de behandeling met sunitinib (tijdelijk) te worden gestaakt. Zoals eerder beschreven kunnen de bijwerkingen tijdens een behandeling met sunitinib ernstig zijn. Daarom is er een dringende behoefte aan hulpmiddelen die de toxiciteit van sunitinib bij individuele patiënten kunnen voorspellen, zodat de dosering zo nodig kan worden aangepast.

In **Hoofdstuk 8** is beschreven of specifieke genetische polymorfismen, die belangrijk zijn bij de farmacokinetiek en farmacodynamiek van sunitinib, kunnen worden geïdentificeerd. Deze genetische polymorfismen zouden patiënten gevoeliger kunnen maken voor de bijwerkingen van sunitinib. Bij 219 met sunitinib behandelde patiënten bleken verscheidene genetische polymorfismen aangewezen te kunnen worden, die geassocieerd waren met het ontstaan van leucopenie, mucositis, hand-voet syndroom en elke bijwerking ernstiger dan graad 2. Deze genetische polymorfismen codeerden voor metaboliserende enzymen, efflux transporters en specifieke targets van sunitinib. Zo was het ontstaan van leucopenie geassocieerd met genetische polymorfismen in CYP1A1 2455A/G, FLT3 738T/C en het NR1J3 haplotype. Het optreden van mucositis en het hand-voet syndroom was geassocieerd met genetische polymorfismen in respectievelijk CYP1A1 2455A/G en het ABCBI haplotype. De prevalentie van bijwerkingen die ernstiger waren dan graad 2 was verhoogd, wanneer het T allel van de vasculaire endotheliale groeifactorreceptor (VEGFR)-2 1191C/T of een kopie van TT in het ABCG2 haplotype aanwezig was. Het belang van deze specifieke genetische polymorfismen voor het ontstaan van bijwerkingen van sunitinib zal in een onafhankelijke patiëntenpopulatie moeten worden gevalideerd.
Deel III: Potentiële biomarkers

Om de effectiviteit van sunitinib te kunnen voorspellen werden nieuwe criteria voor het beoordelen van computertomografie (CT) scans geëvalueerd, zoals is beschreven in Hoofdstuk 9. Deze nieuwe criteria houden rekening met het ontstaan van tumornecrose en werden eerder door Choi en medewerkers ontwikkeld. In deze studie werden de criteria volgens Choi gebruikt voor de evaluatie van CT scans van 55 patiënten met gemetastaseerd niercelcarcinoom, die werden behandeld met sunitinib. Volgens Choi’s criteria is er sprake van een partiële remissie als de grootte van de tumor in één dimensie afneemt met tenminste 10% óf als de attenuatie van de tumor (uitgedrukt in Hounsfield Units) afneemt met tenminste 15%. Wanneer de grootte van de tumor toeneemt met tenminste 10% en er geen sprake is van een partiële respons op basis van de verandering in attenuatie, is er volgens Choi en medewerkers sprake van progressieve ziekte. Tijdens de eerste evaluatie na een mediane periode van 1,9 maanden hadden de Choi criteria voor de progressievrije overleving en voor de totale overleving een significant betere voorspellende waarde dan de gebruikelijke Response Evaluation Criteria In Solid Tumors (RECIST). Gedurende de verdere behandeling met sunitinib was de voorspellende waarde echter niet beter dan die van RECIST. De Choi criteria zouden kunnen worden toegepast om vroegtijdig patiënten met gemetastaseerd niercelcarcinoom te herkennen die baat hebben bij behandeling met sunitinib, maar ze zijn niet geschikt om patiënten die progressieve ziekte ontwikkelen vroegtijdig te identificeren. Daarom zal het gebruik van de respons criteria ontwikkeld door Choi en medewerkers geen invloed hebben op de beslissing of behandeling met sunitinib bij deze patiënten moet worden gestopt.

In Hoofdstuk 10 zijn de veranderingen in het hemoglobinegehalte tijdens de behandeling met sunitinib beschreven. Bij 82 patiënten met gemetastaseerd niercelcarcinoom werd een zig-zag patroon in het hemoglobinegehalte en het aantal erytrocyten waargenomen. Gedurende de vier weken behandeling ontstond een tijdelijke stijging in het hemoglobinegehalte en het erytrocytengetal welke verdween gedurende de rustperiode van twee weken. Hoewel de toename in het aantal erytrocyten gepaard ging met een stijging van het erytrocytenlacte in het plasma, was vanwege de prompte daling in de rustperiode een causaal verband minder waarschijnlijk. Gebaseerd op eerdere studies en onze bevindingen was een betere hypothese, dat de stijging in het hemoglobinegehalte meer het gevolg is van een tijdelijk verlies aan intravasculaire vloeistof door remming van VEGFR-2.

Hoofdstuk 11 is gewijd aan de effecten van sunitinib op rijke circulerende endotheel cellen (CECs) en hematopoietische stamcellen (HPCs) in het bloed van patiënten met gemetastaseerd niercelcarcinoom. De veranderingen in circulerende CECs en HPCs zouden de activiteit van sunitinib op de tumorvasculatuur kunnen weerspiegelen. In het
bijzonder werd de kinetiek geanalyiseerd van specifieke populaties CECs [CD45
neg)/CD34
bright) en HPCs [CD45
dim)/CD34
bright) die VEGFR-2 tot expressie brengen. Deze twee celpopulaties toonden een tegenovergestelde kinetiek: de CECs stegen terwijl de HPCs daalden. Deze stijging in CECs is waarschijnlijk het gevolg van de activiteit van sunitinib in onrijpe tumorvaten. Na veertien dagen behandeling met sunitinib hadden patiënten met een gestegen aantal CECs een langere progressievrije overleving dan patiënten met een gedaalde aantal CECs.

Om de effecten van sunitinib op het endotheel van tumoren verder te onderzoeken werd een studie, beschreven in Hoofdstuk 12, verricht om eiwitten in plasma te meten die geassocieerd zijn met geactiveerd tumorendotheel. Daartoe werd het plasma van patiënten met gemetastaseerd niercelcarcinoom, die werden behandeld met sunitinib, onderzocht op de hoeveelheid VEGF, soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular cell adhesion molecule-1 (sICAM-1), von Willebrand factor (vWF), circulerend angiopoietin-2 (Ang-2) en soluble Tie-2 (sTie-2). Voor het starten van de behandeling met sunitinib bleek de omvang van het ziekteproces positief geassocieerd te zijn met de hoeveelheid circulerend Ang-2. Behandeling met sunitinib leidde tot een daling van het circulerende Ang-2 en sTie-2, terwijl de hoeveelheden sVCAM-1 en VEGF juist significant toenamen. De daling in circulerend Ang-2 was positief geassocieerd met het totale percentage afname in alle tumorgebieden. Daarom zou de afname van circulerend Ang-2 een biomarker kunnen zijn voor de activiteit van sunitinib bij niercelcarcinoom.

Tenslotte werden in Hoofdstuk 13 de effecten van sunitinib op de systemische vasculatuur besproken. Behandeling met sunitinib is geassocieerd met het ontstaan van hypertensie, die toegeschreven wordt aan functionele rarefactie (een vermindering in geperfundeerde microvaatjes) of structurele rarefactie (een afname in de anatomische capillaire dichtheid). Bij patiënten met een gemetastaseerd niercelcarcinoom werd onderzocht of behandeling met sunitinib leidde tot een vermindering van de microvasculaire functie en/of afname van de capillaire dichtheid in de dorsale huid van de vinger (Hoofdstuk 13A). Bij deze patiënten werd een stijging van de systolische en diastolische bloeddruk waargenomen, die was geassocieerd met een afname van de capillaire dichtheid in de huid. In Hoofdstuk 13B werd beschreven dat deze effecten omkeerbaar zijn na het staken van de behandeling met sunitinib. Aangezien patiënten met een grotere afname in capillaire dichtheid tijdens het gebruik van sunitinib een langere progressievrije overleving hadden, zou deze waarneming voorspellend kunnen zijn voor een langere overleving.

In dit proefschrift zijn verscheidene klinische en farmacodynamische aspecten van de behandeling met sunitinib beschreven die belangrijk zijn voor patiënten met
gemetastaseerd niercelcarcinoom. De verschillende studies hebben meer inzicht verschaft in het vakkundig voorschrijven van sunitinib, het vermijden van ernstige bijwerkingen en het begrijpen van de effecten in het tumorproces en de vasculatuur. De gegevens bieden handvatten voor vervolgonderzoek om de behandeling voor patiënten met gemetastaseerd niercelcarcinoom verder te verbeteren.
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Curriculum vitae
Astrid Aplonia Maria van der Veldt was born on March 14th 1979 in Rotterdam, the Netherlands. After graduating from secondary school (gymnasium, Christelijk Lyceum Almelo) in 1997, she studied medicine at the University of Antwerp in Belgium. In 1999, she was allowed to study medicine in the Netherlands and she started her study at the VU University in Amsterdam. During her study, she participated in the 'VUmc Honours Programme'. In addition, she worked as a student assistant on the research project entitled ‘FDG PET in detecting recurrent cervical cancer’ at the Department of Nuclear Medicine & PET Research of the VU University Medical Center under supervision of dr. C.F.M. Molthoff and prof. dr. O.S. Hoekstra. In February 2006, she graduated from medical school with honors. From 2006 to 2007, she worked as a research fellow at the Department of Medical Oncology of the VU University Medical Center. In this period, she investigated the clinical and pharmacodynamic aspects of sunitinib for advanced renal cell cancer under supervision of prof. dr. E. Boven, prof. dr. J.B.A.G. Haanen, and dr. A.J.M. van den Eertwegh. For this research project, she also worked at the Department of Medical Oncology of The Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, the Netherlands. In 2007, she started her PhD project on $^{[11]}$C]docetaxel in lung cancer at the Department of Nuclear Medicine & PET Research of the VU University Medical Center under supervision of prof. dr. A.A. Lammertsma, prof. dr. E.F. Smit, dr. ir. M. Lubberink, and dr. N.H. Hendrikse. In this period, she continued her previous research on sunitinib for advanced renal cell cancer. For her research projects, she received several awards including the Merit Award of the American Society of Clinical Oncology and the Chris Meijer Award of the CCA/V-ICI. In January 2011, she started her specialization in Internal Medicine at the Department of Internal Medicine of the VU University Medical Center, headed by prof. dr. M.H.H. Kramer. The research projects performed at the Departments of Medical Oncology and Nuclear Medicine & PET Research have resulted in a double dissertation consisting of two different theses entitled ‘Sunitinib for advanced renal cell cancer – clinical and pharmacodynamic aspects’ and ‘Towards personalized treatment planning of chemotherapy: $^{[11]}$C]docetaxel PET studies in lung cancer patients’, respectively. In the future, Astrid hopes to combine patient care with scientific research.
PUBLICATIONS


