Understanding the molecular-based mechanism of action of the tyrosine kinase inhibitor: sunitinib

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Sunitinib is an orally available small-molecule multikinase inhibitor. This agent potently inhibits the vascular endothelial growth factor receptor, platelet-derived growth factor receptor, and c-Kit in addition to other kinases in biochemical and cell-based assays. In several relevant preclinical cancer models, sunitinib exerts significant antiangiogenesis and antitumor effects. In phase I studies, using intermittent dosing schedules, oral administration of doses up to 50 mg/day were reasonably well tolerated and resulted in plasma concentrations in the range of targeted levels needed for sustained kinase inhibition. Biomarker and functional imaging studies showed modulation of circulating markers of angiogenesis as well as a reduction in tumor metabolism. Sunitinib showed clinical activity in patients with renal cell cancer and in patients with imatinib-resistant gastrointestinal stromal tumors. Definitive randomized clinical trials showed significant clinical activity in these two indications leading to regulatory approval. In addition, this drug has showed activity in a variety of other tumor types such as breast, colon, and lung cancer and is being explored in combination with standard drugs in these diseases. The observation that biological and functional imaging effects are reduced during drug-free intervals has prompted the evaluation of protracted dosing schedules. A better understanding of mechanisms involved in resistance to sunitinib provides the rationale for combination strategies that hopefully will result in better clinical effect. Ongoing studies will elucidate the overall role of this drug in cancer treatment. *Anti-Cancer Drugs* **21 (suppl 1):S3–S11 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

This study reviews the role of angiogenesis in cancer and summarizes both the preclinical activity and the clinical development of sunitinib, including early clinical trials, phase I and pharmacological trials, and its late clinical development. It also contains a review of biomarker and functional imaging studies. It concludes with a review of the role of the combination of antiangiogenics and chemoradiotherapy regimens and future directions. Four case reports are also discussed.

Role of angiogenesis in cancer

More than three decades ago, Professor Folkman formulated the angiogenesis hypothesis. In brief, this hypothesis states that blood vessel formation is required for tumors to grow beyond a ~2 mm size [1]. The formation of blood vessels is also critical in the development of metastasis and results from the complex interplay among several cell types including the cancer cell, endothelial cell, pericytes, fibroblasts, and other components of the surrounding stroma. The therapeutic implication that directly follows this statement is that the inhibition of blood vessels formation could be a strategic target for drug development and cancer treatment. Studies conducted since then have shown the angiogenesis hypothesis to be correct, have provided important advances in our understanding of the mechanisms leading to abnormal blood vessel formation by cancer, and, perhaps more importantly, have brought to the clinical area agents that, by inhibiting this process, improve the outcome of cancer patients [2].

The formation of blood vessels is a highly regulated process in which proangiogenic factors operate in concert with antiangiogenesis factors. The list of these factors continues to grow with over 30 putative mediators known today. Two of the key mediators, both from a mechanistic and from a therapeutic perspective, are the vascular endothelial growth factor (VEGF) and the platelet-derived growth factor (PDGF). Binding of these ligands to their cognate receptors located in endothelial cells and pericytes results in the activation of the receptor tyrosine kinase activity (RTK) that will trigger the activation cascade of several intracytoplasmatic signal transduction mediators. This will lead to the proliferation of new endothelial cells and pericytes, recruitment of endothelial cell precursors, and the generation of capillaries. Given their significant role in cancer-mediated angiogenesis, these two receptors have become the focus of attention for therapeutic interventions. As with other ligand–receptor systems, treatment approaches have

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ranged from monoclonal antibodies directed against the ligand or the receptor and small molecule inhibitors of the RTK [3]. As angiogenesis is a complex phenomenon in which multiple receptors are involved, molecules that inhibit several of these receptors are likely to provide therapeutic advantages [4]. Thus, multitargeted agents such as sunitinib, sorafenib, axitinib, pazopanib, imatinib, etc., which block several of these kinases, may achieve a broader spectrum of antitumor effects.

The target: cancer angiogenesis

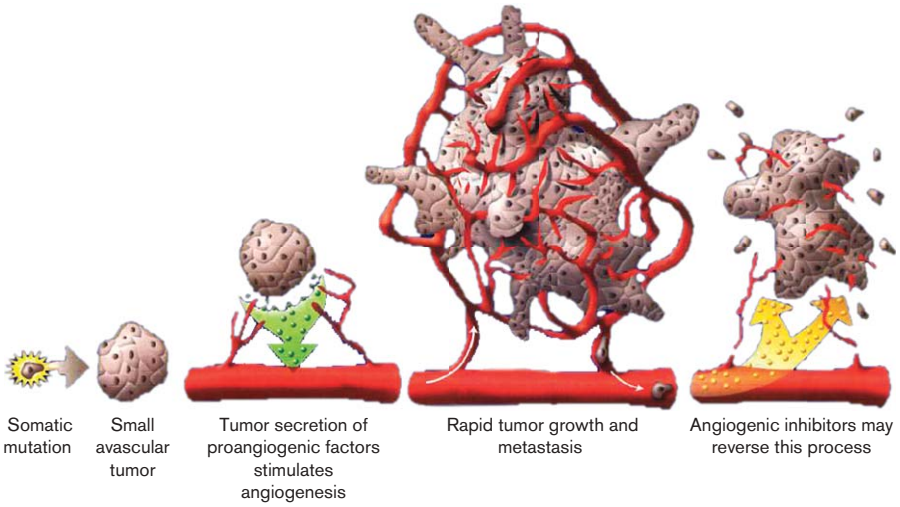
The ultimate effect of cancer angiogenesis is the recruitment and induced proliferation of endothelial cell precursors, endothelial cells, and pericytes to form new blood vessels. This process results from the complex interplay between cancer cells and supporting stroma such as fibroblasts, smooth muscle cells, and others. Angiogenesis is a complex process involving multiple steps including (a) the secretion of proteases by the endothelial cells that line existing blood vessels, and subsequent degradation of the basement membrane; (b) the migration of circulating endothelial cells through the membrane to form vessel sprouts; (c) the proliferation and differentiation of the endothelial cells to extend the sprouts, which then fuse to form loops; and (d) the secretion of growth factors by the endothelial cells, which attract supporting cells (e.g. pericytes and smooth muscle cells), and the formation of the basement membrane (Fig. 1) [3,5].

This process is initiated, at the molecular level, by an increment in the production of proangiogenesis growth factors and simultaneous loss of antiangiogenesis factors. The generation of proangiogenesis mediators is a consequence of tumor hypoxia, activation of oncogenes, inactivation of tumor suppressor genes, and by other growth factors and cytokines. One of the best characteristic mediators of angiogenesis is the hypoxia-inducible factor (HIF1), which, under conditions of hypoxia (a common phenomenon in ischemic tumors), increases the production of VEGF. HIF1 production is tightly regulated and is increased, for example, in tumors with the loss of the von–Hippel–Lindau (VHL) gene [5].

VEGF binds and activates the VEGF receptor (VEGFR) promoting endothelial cell migration, growth, and survival. VEGF also promotes vessel permeability and recruits endothelial cell precursors from the bone marrow to the tumoral site. Further, VEGF increases the survival of endothelial cells by activating prosurvival intracellular pathways such as the Akt signaling pathway. Endothelial cell survival is also promoted by other proteins, such as angiopoietin-1 (ANGP1), placental growth factor (PIGF), and endothelin 1 and 2 [6]. Importantly, these other molecules can act as rescue factors for endothelial cell survival in the context of VEGF inhibition which may lead to anti-VEGF treatment resistance.

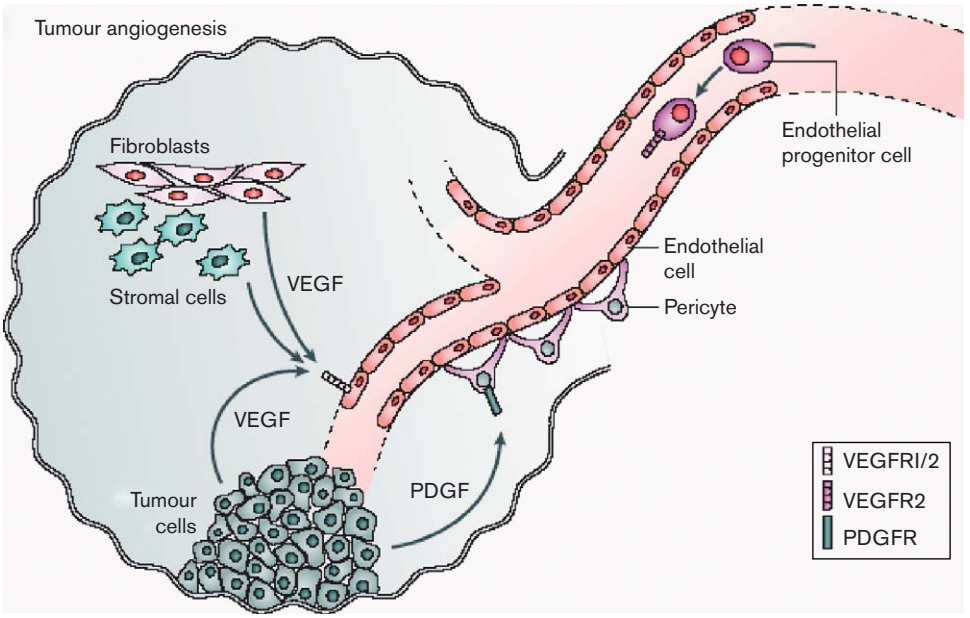
The VEGFR is a transmembrane RTK with structure and functionality similar to other receptors of this nature. The binding of the ligand to the extracellular domain of the receptor induces a process of receptor dimerization and activation of the intracellular TK, which, in turn, phosphorylates the receptor. The phosphorylated receptor acts as a docking domain for intracellular adaptors that lead to the activation of a cascade on intracellular signal transduction mediators, eventually leading to DNA synthesis, cell division, growth, proliferation, and migration [7,8]. As mentioned earlier, another important mediator of angiogenesis is the PDGF. There are four PDGF ligands that bind and activate PDGF receptor-α

Fig. 1



Cancer angiogenesis.

Fig. 2



Vascular endothelial (VEGF) and platelet-derived growth factor (PDGF) receptors implicated in angiogenesis. Adapted with permission from [3].

(PDGFR-α) and PDGFR-β. These last classes of receptors are important in vessel stability by recruiting pericytes to newly formed vessels that will support and stabilize them (Fig. 2).

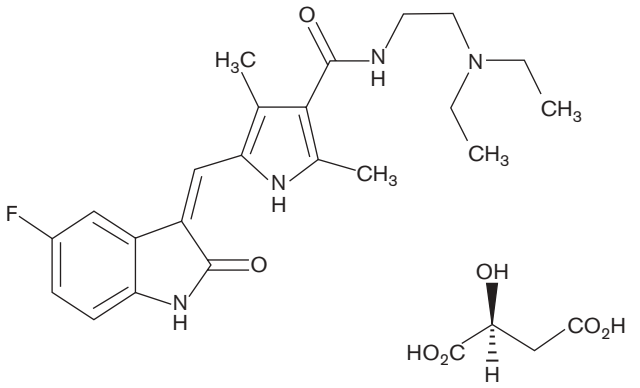
Tumor blood vessels are not physiologically stable but rather are unstable, tortuous and dilated vessels that have constantly been remodeled. These vessels are leaky and augment the intratumor pressure creating vast areas of hemorrhage. As a result, these vessels are not efficient in oxygen delivery and create areas of hypoxia within the tumor. As mentioned above, hypoxia is one of the key stimuli for HIF1 production, secretion of VEGF, and further angiogenesis [8,9].

Sunitinib also inhibits the stem-cell growth factor receptor (KIT), colony-stimulating factor (CSF)-1R, FMS-like tyrosine kinase-3 (FLT-3), and RET. c-KIT is expressed on hematopoietic progenitor cells, mast cells, germ cells, and the interstitial cells of Cajal. The receptor is activated by the stem cell factor and has been implicated in mastocytosis/mast cell leukemia, germ cell cancers, small-cell lung cancer, gastrointestinal stromal tumors (GISTs), acute myelogenous leukemia, neuroblastoma, melanoma, and ovarian and breast carcinoma [10]. CSF1R is expressed on monocytic progenitors [11]. The osteoclastogenic factor, macrophage CSF produced by tumor cells, binds to the CSF-1R receptors to stimulate osteoclasts and enhance the osteolytic activity of osteoclasts. CSF-1R expression has been reported in breast, ovarian, and endometrial cancers, and has been associated with poor prognosis and invasive potential. CSF-1R seems to be an attractive target for bone metastasis. FLT-3 is another TKR that, when mutated, may lead to the development of a particular type of leukemia, known as acute myeloid leukemia. Finally, sunitinib also targets mutant RET, which is involved in the multiple endocrine neoplasia types 2A and 2B autosomal-dominant syndromes, familial medullary thyroid carcinoma, and perhaps sporadic neuroendocrine tumors [12,13].

Preclinical review of sunitinib: activity in in-vitro and in-vivo models

Sunitinib (Sutent, Pfizer, New York, USA) is an oral indolin-2-one structural analog, which inhibits multiple RTK exerting potent antiangiogenesis and antitumor effects [14,15] (Fig. 3). The spectrum of kinases inhibited by sunitinib is listed in Table 1 and includes VEGFR1, 2, and 3, PDGFR-α and PDGFR-β, bFGF, c-Kit, FLT-3, RET, and CSF 1-R [3]. The agent exerted potent and specific inhibition of these targets in biochemical and cell-based assays [14,16]. In in-vitro studies, sunitinib inhibited VEGF-dependent proliferation and migration of human umbilical endothelial cells and disrupted capillary tube formation. In the in-vivo models of cancer angiogenesis, sunitinib decreased tumor microvessel density, blocked vascularization in the vascular-window tumor model, and decreased the metastatic potential of the Lewis lung cancer model. In in-vitro studies, the drug inhibited the proliferation of human cancer cells induced by VEGF, stem cell factors, and PDFG whilst inducing apoptosis in human umbilical endothelial cells at concentrations of 50 ng/ml and above.

Fig. 3



Chemical structure of sunitinib L-malate salt.

Table 1 Biological effects of sunitinib against target receptors

		Cellular IC_{50} (μmol/l)	
Receptor	Biochemical Ki (μmol/l)	Receptor phosphorylation	Proliferation
VEGFR1	0.002	ND	ND
VEGFR2	0.009	0.01	0.004
VEGFR3	0.017	ND	ND
PDGFR-β	0.008	0.01	0.039
PDGFR-α	ND	ND	0.069
KIT	0.004	0.001–0.01	0.002
FLT-3 (wild type)	ND	0.25	0.01–0.05
RET	ND	0.05	0.05
CSF 1-R	ND	0.05–0.1	ND

CSF1R, colony stimulating factor 1 receptor; FLT3, FMS-related tyrosine kinase; KIT, stem-cell growth factor receptor; PDGFR, platelet-derived growth factor receptor; VEGFR, vascular endothelial growth factor receptor.
Adapted with permission from [3].

Consistent with this finding, pharmacodynamic preclinical studies suggest that plasma concentrations in the range of 50–100 ng/ml inhibited target activation [14,16]. In in-vivo studies, sunitinib exerted significant antitumor activity in xenograft models from numerous tumor types including renal (786-0), colon (HT-29 and Colo205), breast, lung (NCI-H226), melanoma (WM-266-4), and epidermoid carcinoma (A431) with once daily dosing [17–20]. Antitumor activity was observed against well-established tumor types and resistance did not appear in these models. In addition, the effects were additive or synergistic with common chemotherapy agents such as docetaxel, fluorouracil, doxorubicin, and cisplatin [17,18,21,22]. Mechanistic studies showed that the supra-additive effects were because of the inhibition of compensatory prosurvival pathways.

The preclinical studies with sunitinib also explored biomarkers that could potentially be used in the subsequent clinical development of the agent. As angiogenesis was considered the principal target of the drug, the studies focused on dynamic contrast-enhanced magnetic resonance imaging to assess blood flow. A single dose of sunitinib resulted in a 42% inhibition of blood flow in the tumor rim and a 30% inhibition in the area under the concentration curve 24 h posttreatment. The observation was confirmed by histological studies showing a 30% inhibition in the mean vessel density inhibited and the vessel area in the sunitinib-treated group [23]. Other studies have evaluated gene expression profiling of treated tumors compared with controls. From these expression studies, cadherin 11 has emerged as a putative marker of sunitinib exposure [24].

Clinical review of sunitinib

Early clinical trials

On the basis of its tolerable toxicity and preclinical efficacy, clinical development of sunitinib was granted. Initial trials were conducted in healthy volunteers and the results showed that a single dose of the agent (up to 50 mg) was well tolerated without any clinically significant toxicities compared with placebo [25]. Absorption was slow, with a time of 8 h for peak plasma concentration (T_{max}). Neither the maximum concentration (C_{max}) nor the Area under the Curve (AUC) of sunitinib was affected by food. The estimated half-lives of sunitinib and SU012662 (the active *N*-desethyl metabolite) were 60 and 105 h, respectively. SU012662 has shown comparable biological activity and potency than sunitinib and is formed primarily by the cytochrome, P450-3A4 (CYP3A4), pathway in hepatic microsomes. Subsequent pharmacokinetic studies showed that inhibitors and inducers of the CYP3A4 affect the metabolism of sunitinib and should be avoided in patients treated with this compound.

Phase I trials and pharmacokinetic studies in cancer patients

Sunitinib was studied in cancer patients using various schedules, including a 2-week treatment followed by a 1-week rest period (schedule 2/1) or a 2-week rest period (schedule 2/2), or a 6-week cycle of treatment for 4 weeks followed by a 2-week rest period (schedule 4/2) [26,27]. These schedules explored both daily and every other day administration and incorporated planned restings periods suggested by the findings in preclinical models. Such animal models included dogs, rats, and monkeys and showed the development of necrotic suprarenal hemorrhages that led to severe hypoaldosteronism. This toxicity, combined with the induced neutropenia, led to the death of the animals. Findings have suggested that the adrenocortical axis is rarely affected in patients treated with sunitinib in the metastatic setting. In each of these schedules, doses of 75 mg per day or above resulted in dose-limiting toxicities of fatigue, asthenia, and thrombocytopenia, thereby establishing the recommended phase II dose of 50 mg on schedules 2/1, 2/2, or 4/2. Dose-dependent fatigue was the most commonly reported adverse event and was experienced by approximately

70% of the patients enrolled in phase I studies, regardless of tumor type. Patients complained of lethargy, asthenia, weakness, and malaise. The onset of fatigue generally occurred after 2 weeks of treatment and resolved during the 2-week rest period, further reinforcing the utility of a discontinuous dosing schedule. Thorough investigation for other causes of fatigue did not confirm any obvious etiologies. In several studies there was evidence that hypothyroidism could have been a causative and/or exacerbating factor of the fatigue, therefore suggesting that thyroid dysfunction should be considered in the differential diagnosis of fatigue during sunitinib therapy, and that thyroid hormone replacement in patients with confirmed hypothyroidism may improve sunitinib tolerance [28–30]. Other adverse events possibly related to sunitinib in 377 patients included nausea (56%), diarrhea (43%), skin discoloration (42%), anorexia (31%), dyspepsia (30.5%), and constipation (30%). The most common grade 3/4 toxicities occurring among 274 patients included fatigue (10.3%), neutropenia (8.2%), thrombocytopenia (7.2%), and asymptomatic lipase elevations (2.4%) [31].

In pharmacokinetic studies, peak plasma concentrations occurred 5 h after oral administration, and steady-state concentrations were reached by days 7 through to 10 and days 10 to 13 for sunitinib and SU012662, respectively. Sunitinib and its metabolite showed dose-proportional PK with the 50–75 mg/day doses and the half-lives were 40 and 80 h for sunitinib and SU012662, respectively. As expected, daily dosing for 28 days caused a 3.0 to 5.5-fold accumulation of sunitinib and a 7 to 15-fold accumulation of SU0112662, compared with day 1 [26]. Daily dosing with 50 mg was sufficient to produce target plasma concentrations above 50 ng/ml (required to inhibit the PDGFR and VEGFR RTKs in preclinical studies), whereas patients with dose-limiting toxicities exhibited total (sunitinib + SU0112662) through concentrations less than 100 ng/ml. Exposure to sunitinib was not affected by hepatic and renal function [32].

Asian patients showed a higher (26%) clearance of SU012662 relative to white patients. Females showed a decrease in clearance by 31 and 17%, and the apparent volume of distribution by 18 and 21% for sunitinib and SU012662, respectively. None of these factors, however, required a dose adjustment. The AUC of the total drug correlated with the incidence, but not the severity, of fatigue. Exposure to sunitinib correlated with an increase in diastolic blood pressure similar to the one observed with other antiangiogenesis agents.

Objective partial responses (PRs) were observed in several of the early studies in patients with thyroid cancer ($n = 1$), metastatic renal cell carcinoma ($n = 4$), neuroendocrine cancer ($n = 4$), GISTs ($n = 6$), sarcoma ($n = 1$), unknown primary adenocarcinoma ($n = 1$), non-small-cell lung cancer, and in melanoma at doses ranging from 50 to 75-mg on the 4/2 or 2/2 [26,27]. The activity noted in these studies formed the basis of the phase II/III clinical development program of sunitinib.

Late clinical development (renal cell cancer and gastrointestinal stromal tumors)

On the basis of the mechanism of action and clinical activity in early clinical trials, sunitinib has been developed for the treatment of patients with advanced renal cell cancer (RCC) and GISTs [33,34].

Renal cell cancer

RCCs are known to be highly vascular tumors with high expression of VEGF, VEGFR, PDGFR, and bFGF. The VHL gene is mutated in almost all hereditary RCC tumors and between 60 and 80% of the sporadic RCC. Frequent mutations in VHL results in the overexpression of HIF-1-α, which will release proangiogenic and protumoral factors. In two single-arm phase II trials conducted in cytokine-refractory RCC patients, sunitinib at a 50 mg dose on schedule 4/2 showed a 40 and 44% PR rate with a median time to progression of 8.7 and 8.3 months, respectively [35,36]. Toxicities in these trials were similar to those observed in early phase I studies. These results compared favorably with other clinically available angiogenesis inhibitors in this setting. Bevacizumab (10 mg/kg) resulted in a longer time to progression of 4.8 versus 2.5 months when compared with placebo ($P < 0.001$) and a 10% response rate, but no survival benefit was shown over placebo [37]. Axitinib (AG-013736), an oral inhibitor of VEGFR1, VEGFR2, and PDGFR-β RTK activity which is similar to sunitinib, has shown a 45% response rate and a median time to progression of 15.7 months in 52 patients with advanced RCC [38]. Finally, sorafenib, an inhibitor of Raf kinase, VEGFR2, VEGFR3, FLT3, PDGFR, and KIT showed a higher progression-free survival of 6 months compared with 3 months in the placebo group. In contrast to sunitinib, PRs were, however, rare. Furthermore, sunitinib showed significant activity even on a subpopulation of RCC patients previously treated with bevacizumab. The overall response rate of sunitinib in 61 patients with bevacizumab-resistant RCC was 23.0%, with a median progression-free survival of 30.4 weeks [39].

Subsequently, a phase III randomized trial compared interferon-α with sunitinib (50 mg/day 4/2 schedule) in 750 patients with previously untreated, metastatic RCC. The primary endpoint was progression-free survival and secondary endpoints included the objective response rate, overall survival, patient-reported outcomes, and safety. The median progression-free survival was significantly longer in the sunitinib group (11 months) than in the interferon-α group (5 months), corresponding to a hazard ratio (HR) of 0.42 (95% confidence interval, 0.32–0.54; $P < 0.001$). Sunitinib was also associated with a higher objective response rate than interferon-α

(31 vs. 6%, $P < 0.001$). The proportion of patients with grade 3 or 4 treatment-related fatigue was significantly higher in the group treated with interferon-α, whereas diarrhea was more frequent in the sunitinib group ($P < 0.05$) [40]. A recently reported update of this trial showed a median overall survival of 26.4 months for patients treated with sunitinib compared with 21.8 months for those receiving interferon-α (HR = 0.821; $P = 0.0510$) [41].

Patients in the sunitinib group also reported a significantly better quality of life than did patients in the interferon-α group for all the scales that were used ($P < 0.001$) [42].

Gastrointestinal stromal tumors

The discovery and clinical development of imatinib dramatically changed the outcome of patients with GIST and validated c-kit as a target in this disease. From a molecular perspective, approximately 85% of GISTs harbor KIT mutations in exons 9, 11, or 13 that are sensitive to imatinib, whereas only 5% have mutations in PDGFR-β. However, despite the high response rate and durability of responses to imatinib, 20% of GIST patients show primary resistance to imatinib, whereas secondary resistance develops after about 1 year and is characterized by secondary mutations in the KIT or PDGFR-β kinases, gene amplification, or loss of target kinase expression [43,44].

These data prompted the evaluation of sunitinib in patients with GIST who experienced disease progression or were refractory or intolerant to imatinib. On a 2/2 schedule, at the maximum tolerated dose of 50 mg, 61% of patients achieved disease regression or stable disease lasting more than 4 months. A subsequent more detailed dosing study was performed assessing 25, 50, and 75 mg daily on schedules 4/2, 2/2, or 2/1 in a similar GIST population. In the 97 patients enrolled, seven (7%) had PRs and remained on therapy for 9–18 cycles; 26 patients (27%) had stable disease for 6 months or longer, whereas the overall time to tumor progression was 34 weeks. These promising results of sunitinib in imatinib-refractory (or intolerant) GISTs were confirmed in a double-blind placebo-controlled, randomized phase III trial of sunitinib (50 mg once daily on schedule 4/2). Three hundred and twelve patients were randomized in a 2 : 1 ratio to receive sunitinib ($n = 207$) or placebo ($n = 105$); the trial was unblinded early when a planned interim analysis showed a significantly longer time to tumor progression with sunitinib. Median time to tumor progression was 27.3 weeks in patients receiving sunitinib and 6.4 weeks in those on placebo (HR = 0.33; $P < 0.0001$). Therapy was reasonably well tolerated; the most common treatment-related adverse events were fatigue, diarrhea, skin discoloration, and nausea. These results led to the approval of sunitinib for the treatment of patients with imatinib-refractory (or intolerant) GISTs [45,46].

Dosing schedules of sunitinib in renal cell cancer and gastrointestinal stromal tumors

Fractionated schedules were based on human PK studies as well as concerns regarding drug and toxicities accumulation with protracted dosing schedules. PK studies did not, however, corroborate significant drug accumulation. Furthermore, biological effects noted in plasma biomarkers and functional imaging studies suggested loss of activity in drug-free intervals. These observations led to the examination of the continuous dosing schedule. A continuous oral daily sunitinib (37.5 mg/day) has been tested in patients with advanced, cytokine-refractory RCC, and GIST [47,48]. In 69 patients, for whom data are available, the median time on sunitinib was 11.6 weeks (range, 2 to 32 weeks) at a median daily dose of 37.5 mg. Thirty-five patients tolerated 37.5 mg for up to 32 weeks, whereas another 17 patients (each) were dose escalated, or required dose reduction, to 50 and 25 mg/day, respectively. The most common toxicities leading to transient or sustained dose reduction were mucositis, thrombocytopenia, nausea/vomiting, hand–foot syndrome, asthenia/fatigue, diarrhea, and neutropenia. Of the 40 patients assessable for efficacy, tumor regressions were reported in 10%. A similarly designed phase II study was conducted in GIST patients. The results showed a median time on sunitinib of 16.8 weeks (range, 5.2–29.6 weeks) at a median daily dose of 37.5 mg/day. In this study, only one patient was escalated to 50 mg/day and two patients were reduced to 25 mg/day, in response to fatigue and hand–foot syndrome. The spectrum of toxicities was similar to those described above and clinical data thus far indicate that the continuous dosing schedule results in comparable clinical benefit in this population.

Biomarker and functional imaging studies

As with other targeted agents, the clinical development of sunitinib has been accompanied with correlative studies. Broadly, these studies aimed to show the pharmacodynamic effects and the mechanism of action of the drug in humans and to define biomarkers that may assist in the selection of patients more likely to benefit from treatment with the drug. Studies have ranged from analysis of molecular markers in the plasma of patients to functional imaging.

Assessment of circulating VEGF in the plasma of patients treated in a phase I trial showed a more than 3-fold increase in plasma VEGF concentrations within 4 weeks of therapy in 65% of the patients, believed possibly to be because of local tumor hypoxia resulting from angiogenesis blockage [49,50]. The phase II studies in RCC measured circulating soluble VEGFR2, VEGF-A, and PIGF (which is a specific ligand of VEGFR1) [39]. As expected in this advanced-cancer population, circulating soluble VEGFR2 levels were highly elevated at baseline. Interestingly, the levels declined by the end of the

4-week dosing period, followed by a gradual increase before cycle 2. In addition, near the end of the dosing period increases of VEGF-A levels and PIGF were observed. This observation, together with the absence of drug accumulation, prompted the assessment of continuous dosing schedules, which could theoretically prevent the increases observed in VEGFR2, VEGF-A, and PIGF during rest periods, perhaps also resulting in better antitumor efficacy.

Similarly, in breast cancer, the analysis of circulating biomarkers showed more than 3-fold increases in VEGF relative to baseline, and decreases in soluble sVEGFR2 levels by 30% in at least 88% of the patients treated with sunitinib in this study. VEGF and sVEGFR2 levels returned to baseline after 2 weeks of treatment. Furthermore, soluble KIT decreased by more than 50% by the end of cycle 2 and correlated with time to progression ($P < 0.001$) and survival ($P < 0.03$). On the basis of these early results, circulating VEGF, sVEGFR2, and soluble KIT may be potential pharmacodynamic markers of sunitinib activity in metastatic breast cancer and should be evaluated further [51].

Tissue and blood-based markers were also assessed in the studies conducted in imatinib-refractory GIST. Sunitinib resulted in the inhibition of activated PDGFR-β in paired tumor biopsies of responding patients versus patients resistant to treatment. Responders also exhibited a 3.6 to 6-fold increase in endothelial and tumor cell apoptosis compared with patients with progressive disease ($P < 0.05$). In addition, the drug induced a statistically significant increase in mature circulating endothelial cells responding patients. Other observations included increases in plasma VEGF that correlated with drug exposure, and conversely, decreases in soluble plasma KIT concentrations in some patients. Potentially more important was that imatinib-refractory GIST patients with KIT exon 9 mutations seemed to derive the greatest benefit (11 of 14 patients with PR or SD; 79%), compared to those patients with KIT exon 11 mutations (8 of 24 patients with PR or SD; 33%). A recent study update confirmed that the PR rate for primary KIT exon 9 mutations was significantly higher (37 vs. 5%; $P < 0.003$) than that for KIT exon 11 mutations, and this was associated with a trend toward improved overall survival [52]. Patients with secondary KIT mutations in exons 13 or 14 also showed a higher clinical benefit rate (65 vs. 9%) than those patients with secondary KIT mutations in exons 17 or 18. Moreover, both progression-free survival and overall survival correlated primary GIST genotype. Indeed, progression-free survival was better in those patients with primary exon 9 mutations (19.4 weeks; $P = 0.0005$) or with a wild-type genotype (19.0 weeks; $P = 0.0356$) than for those with KIT exon 11 mutations (5.1 weeks; $P = 0.724$) The same pattern was observed with overall survival (26.9 vs. 30.5 vs. 12.3 weeks) [53]. On the basis of these results, it seems that KIT sequencing holds the great promise for predicting clinical benefit. [18F] Fluorodeoxyglucose positron emission tomography (FDG-PET) was used in the early studies to determine whether sunitinib treatment would result in a decrease in tumor metabolism. Treatment with 50 mg daily of sunitinib for 4 weeks resulted in a more than 20% reduction in FDG standard uptake value (SUV) in 13 of 31 patients with solid tumors. Importantly, tumor regrowth and increased PET metabolism were evident during the off-therapy period between cycles. Owing to the previously observed correlation of FDG-PET responses with imatinib in GIST patients, PET was further examined as an early indicator of clinical benefit after sunitinib therapy in this patient population. In one study, patients with good PET response after 7 days of treatment derived better clinical benefit at 6 months. However, increased FDG uptake was noted in more than 80% of patients during the initial rest period that led to the exploration of continuous dosing in phase II studies in patients with GIST tumors [53]. A more direct assessment of the biological effects of sunitinib was conducted with oxygen-15 $(O_{15})-H_2O$ PET, which allows imaging of tumor blood flow. In this pilot study of 55 patients, sunitinib treatment produced changes in plasma VEGFR and soluble VEGFR2 levels that seemed to be associated with decreases in blood flow and a decrease in the O15–H_2O SUV in the tumor. Interestingly, these changes also correlated with a decrease in FDG-SUV on parallel standard FDG-PET imaging.

Studies of sunitinib in other tumor types (apart from renal cell cancer and gastrointestinal stromal tumors)

In addition to the studies discussed above, sunitinib has been tested as a single agent in several phase II studies, which are summarized in Table 2. These trials used a

Table 2 Summary of antitumor activity of sunitinib in other tumor types (excluding RCC and GIST)

Tumor type	Dose/schedule	No patients	Primary endpoint	Outcome (%)	References
NSCLC	50 mg/day on 4/2	63	ORR	11.1	[51]
MBC	50 mg/day on 4/2	64	ORR	11	[54]
CRC	50 mg/day on 4/2	84	ORR	1.1	[55]
Neuroendocrine	50 mg/day on 4/2	41 carcinoid	ORR	16.7	[56]
		66 pancreatic islet cell tumors		2.4	

CRC, colorectal carcinoma; GIST, gastrointestinal stromal tumor; MBC, metastatic breast cancer; NSCLC, non-small cell lung cancer; ORR, overall response rate; schedule 4/2, 6-week cycle of treatment for 4 weeks followed by 2-week rest period; RCC, renal cell cancer.

50 mg/day dose on a 4 weeks/on–2 weeks/off schedule. In breast cancer, the single agent, sunitinib, resulted in an 11% response rate. The agent is currently being evaluated in several phase III studies, either alone or in combination with chemotherapy versus standard to care, in several subgroups of breast cancer patients [51]. Similarly, an 11% response rate was noted in patients with non-small-cell lung cancer who had failed the standard of care. This level of activity compares favorably with other targeted agents, such as erlotinib, that are being tested in this setting [54]. A phase III study of sunitinib in combination with erlotinib versus erlotinib is currently ongoing. A proof-of-concept phase II trial, with sunitinib given as a single agent in 82 metastatic colorectal cancer patients, who had previously failed standard therapy, showed a median overall survival of 7.1 months in the earlier bevacizumab cohort and 10.2 months in the bevacizumab-naive cohort. The safety profile was similar to other studies with sunitinib in other tumor types [57]. A phase III study in first-line setting of sunitinib in combination with 5-fluorouracil–irinotecan chemotherapy has been completed. It is expected that these and other planned studies will provide a full evaluation of the potential of sunitinib as an anticancer drug. Clinical data on neuroendocrine and prostate cancer patients treated with sunitinib after progression to standard approaches are also available [55].

Role of the combination of antiangiogenics and chemotherapy regimens

An important area in the clinical development of sunitinib is to explore the combination of the drug with other compounds including conventional cytotoxic agents and other targeted compounds. In general, these studies have shown that sunitinib can be combined with a variety of drugs at relevant doses and with manageable toxicity. Combinations included interferon, capecitabine, docetaxel, fluorouracil–oxaliplatin, erlotinib, gemcitabine, and bevacizumab to name the most matured ones [58–63]. The strategy includes combinations of sunitinib with cytotoxic agents that may be more effective against vascularized tumor rims and with other targeted agents that are able to modulate the mechanism of resistance to sunitinib. These combinations form the basis for subsequent phase III studies.

Future directions

Future directions for the use of sunitinib should include individualized treatment strategies to suit both patient and tumor characteristics. Another important issue is the research of new strategies to overcome drug resistance. These will include the association of sunitinib in multimodality treatments, combining its administration with both other targeted therapies and also other chemotherapy schemas. The development of new biomarkers will also allow the optimization of its use [3].

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References

1 Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971; **285**:1182–1186.
2 Kumaran G, Clamp AR, Jayson GC. Angiogenesis as a therapeutic target in cancer. *Clin Med* 2008; **8**:455-458.
3 Faivre S, Demetri G, Sargent W, Raymond E. Molecular basis for sunitinib efficacy and future clinical development. *Nat Rev Drug Discov* 2007; **6**:734–745.
4 Le TC, Faivre S, Raymond E. New developments in multitargeted therapy for patients with solid tumours. *Cancer Treat Rev* 2008; **34**:37–48.
5 Poon RT, Fan ST, Wong J. Clinical implications of circulating angiogenic factors in cancer patients. *J Clin Oncol* 2001; **19**:1207–1225.
6 Semenza GL. Vasculogenesis, angiogenesis, and arteriogenesis: mechanisms of blood vessel formation and remodeling. *J Cell Biochem* 2007; **102**:840–847.
7 Adini A, Kornaga T, Firoozbakht F, Benjamin LE. Placental growth factor is a survival factor for tumor endothelial cells and macrophages. *Cancer Res* 2002; **62**:2749–2752.
8 Hicklin DJ, Ellis LM. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. *J Clin Oncol* 2005; **23**:1011–1027.
9 Ellis LM, Hicklin DJ. VEGF-targeted therapy: mechanisms of anti-tumour activity. *Nat Rev Cancer* 2008; **8**:579–591.
10 Duda DG, Jain RK, Willett CG. Antiangiogenics: the potential role of integrating this novel treatment modality with chemoradiation for solid cancers. *J Clin Oncol* 2007; **25**:4033–4042.
11 Heinrich MC, Blanke CD, Druker BJ, Corless CL. Inhibition of KIT tyrosine kinase activity: a novel molecular approach to the treatment of KIT-positive malignancies. *J Clin Oncol* 2002; **20**:1692–1703.
12 Kacinski BM. CSF-1 and its receptor in ovarian, endometrial and breast cancer. *Ann Med* 1995; **27**:79–85.
13 Skinner MA, Safford SD, Freemerman AJ. RET tyrosine kinase and medullary thyroid cells are unaffected by clinical doses of STI571. *Anticancer Res* 2003; **23**:3601–3606.
14 Kim DW, Jo YS, Jung HS, Chung HK, Song JH, Park KC, *et al.* An orally administered multitarget tyrosine kinase inhibitor, SU11248, is a novel potent inhibitor of thyroid oncogenic RET/papillary thyroid cancer kinases. *J Clin Endocrinol Metab* 2006; **91**:4070–4076.
15 Mendel DB, Laird AD, Xin X, Louie SG, Christensen JG, Li G, *et al. In vivo* antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. *Clin Cancer Res* 2003; **9**:327–337.
16 Atkins M, Jones CA, Kirkpatrick P. Sunitinib maleate. *Nat Rev Drug Discov* 2006; **5**:279–280.
17 Osusky KL, Hallahan DE, Fu A, Ye F, Shyr Y, Geng L. The receptor tyrosine kinase inhibitor SU11248 impedes endothelial cell migration, tubule formation, and blood vessel formation *in vivo*, but has little effect on existing tumor vessels. *Angiogenesis* 2004; **7**:225–233.
18 Abrams TJ, Lee LB, Murray LJ, Pryer NK, Cherrington JM. SU11248 inhibits KIT and platelet-derived growth factor receptor beta in preclinical models of human small cell lung cancer. *Mol Cancer Ther* 2003; **2**:471–478.
19 Abrams TJ, Murray LJ, Pesenti E, Holway VW, Colombo T, Lee LB, *et al.* Preclinical evaluation of the tyrosine kinase inhibitor SU11248 as a single agent and in combination with "standard of care" therapeutic agents for the treatment of breast cancer. *Mol Cancer Ther* 2003; **2**:1011–1021.
20 Christensen JG. A preclinical review of sunitinib, a multitargeted receptor tyrosine kinase inhibitor with anti-angiogenic and antitumour activities. *Ann Oncol* 2007; **18**:3–10.
21 de Bouard S, Herlin P, Christensen JG, Lemoisson E, Gauduchon P, Raymond E, Guillamo J-S. Antiangiogenic and anti-invasive effects of sunitinib on experimental human glioblastoma. *Neuro Oncol* 2007; **9**:412–423.
22 Cumashi A, Tinari N, Rossi C, Lattanzio R, Natoli C, Piantelli M, Iacobelli S. Sunitinib malate (SU-11248) alone or in combination with low-dose

docetaxel inhibits the growth of DU-145 prostate cancer xenografts. *Cancer Lett* 2008; **270**:229–233.
23 Cuneo KC, Geng L, Fu A, Orton D, Hallahan DE, Chakravarthy AB. SU11248 (sunitinib) sensitizes pancreatic cancer to the cytotoxic effects of ionizing radiation. *Int J Radiat Oncol Biol Phys* 2008; **71**:873–879.
24 Marzola P, Degrassi A, Calderan L, Farace P, Nicolato E, Crescimanno C, *et al*. Early antiangiogenic activity of SU11248 evaluated in vivo by dynamic contrast-enhanced magnetic resonance imaging in an experimental model of colon carcinoma. *Clin Cancer Res* 2005; **11**:5827–5832.
25 Morimoto AM, Tan N, West K, McArthur G, Toner GC, Manning WC, *et al*. Gene expression profiling of human colon xenograft tumors following treatment with SU11248, a multitargeted tyrosine kinase inhibitor. *Oncogene* 2004; **23**:1618–1626.
26 Bello CL, Sherman L, Zhou J, Verkh L, Smeraglia J, Mount J, Klamerus KJ. Effect of food on the pharmacokinetics of sunitinib malate (SU11248), a multi-targeted receptor tyrosine kinase inhibitor: results from a phase I study in healthy subjects. *Anticancer Drugs* 2006; **17**:353–358.
27 Faivre S, Delbaldo C, Vera K, Robert C, Lozahic S, Lassau N, *et al*. Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. *J Clin Oncol* 2006; **24**:25–35.
28 Britten CD, Kabbinavar F, Hecht JR, Bello CL, Li J, Baum C, Slamon D. A phase I and pharmacokinetic study of sunitinib administered daily for 2 weeks, followed by a 1-week off period. *Cancer Chemother Pharmacol* 2008; **61**:515–524.
29 Wong E, Rosen LS, Mulay M, VanVugt A, Dinolfo M, Tomoda C, *et al*. Sunitinib induces hypothyroidism in advanced cancer patients and may inhibit thyroid peroxidase activity. *Thyroid* 2007; **17**:351–355.
30 Desai J, Yassa L, Marqusee E, George S, Frates MC, Chen MH, *et al*. Hypothyroidism after sunitinib treatment for patients with gastrointestinal stromal tumors. *Ann Intern Med* 2006; **145**:660–664.
31 Wolter P, Dumez H, Schoffski P. Sunitinib and hypothyroidism. *N Engl J Med* 2007; **356**:1580–1581.
32 Tsai KY, Yang CH, Kuo TT, Hong HS, Chang JWC. Hand–foot syndrome and seborrheic dermatitis-like rash induced by sunitinib in a patient with advanced renal cell carcinoma. *J Clin Oncol* 2006; **24**:5786–5788.
33 Khosravan R, Toh M, La FJ, Ni G, Bello C. Sunitinib pharmacokinetic (PK) and safety data in subjects with renal impairment and on hemodialysis. *J Clin Oncol* 2008; **26**:2578.
34 Goodman VL, Rock EP, Dagher R, Ramchandani RP, Abraham S, Gobbura JVS, *et al*. Approval summary: sunitinib for the treatment of imatinib refractory or intolerant gastrointestinal stromal tumors and advanced renal cell carcinoma. *Clin Cancer Res* 2007; **13**:1367–1373.
35 Rock EP, Goodman V, Jiang JX, Mahjoob K, Verbois SL, Morse D, *et al*. Food and drug administration drug approval summary: sunitinib malate for the treatment of gastrointestinal stromal tumor and advanced renal cell carcinoma. *Oncologist* 2007; **12**:107–113.
36 Motzer RJ, Michaelson MD, Redman BG, Hudes GR, Wilding G, Figlin RA, *et al*. Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. *J Clin Oncol* 2006; **24**:16–24.
37 Stein MN, Flaherty KT. CCR drug updates: sorafenib and sunitinib in renal cell carcinoma. *Clin Cancer Res* 2007; **13**:3765–3770.
38 Yang JC, Haworth L, Sherry RM, Hwu P, Schwartzentruber DJ, Topalian SL, *et al*. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. *N Engl J Med* 2003; **349**:427–434.
39 Rixe O, Bukowski RM, Michaelson MD, Wilding G, Hudes GR, Bolte O, *et al*. Axitinib treatment in patients with cytokine-refractory metastatic renal-cell cancer: a phase II study. *Lancet Oncol* 2007; **8**:975–984.
40 Rini BI, Michaelson MD, Rosenberg JE, Bukowski RM, Sosman JA, Stadler WM, *et al*. Antitumor activity and biomarker analysis of sunitinib in patients with bevacizumab-refractory metastatic renal cell carcinoma. *J Clin Oncol* 2008; **26**:3743–3748.
41 Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, *et al*. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. *N Engl J Med* 2007; **356**:115–124.
42 Figlin RA, Hutson TE, Tmczak P, Michaelson MD, Bukowski RM, Négrier S, *et al*. Overall survival with sunitinib versus interferon (IFN)-alpha as first-line treatment of metastatic renal cell carcinoma (mRCC). *J Clin Oncol* 2008; **26**:5024.
43 Cella D, Li JZ, Cappelleri JC, Bushmakin A, Charbonneau C, Kim ST, *et al*. Quality of life in patients with metastatic renal cell carcinoma treated with sunitinib or interferon-alpha: results from a phase III randomized trial. *J Clin Oncol* 2008; **26**:3763–3769.
44 Van Glabbeke M, Verweij J, Casali PG, Cesne AL, Hohenberger P, Ray-Coquard I, *et al*. Initial and late resistance to imatinib in advanced gastrointestinal stromal tumors are predicted by different prognostic factors: a European Organisation for Research and Treatment of Cancer-Italian Sarcoma Group-Australasian Gastrointestinal Trials Group study. *J Clin Oncol* 2005; **23**:5795–5804.
45 Sleijfer S, Wiemer E, Seynaeve C, Verweij J. Improved insight into resistance mechanisms to imatinib in gastrointestinal stromal tumors: a basis for novel approaches and individualization of treatment. *Oncologist* 2007; **12**:719–726.
46 Prenen H, Cools J, Mentens N, Folens C, Sciot R, Schöffski P, *et al*. Efficacy of the kinase inhibitor SU11248 against gastrointestinal stromal tumor mutants refractory to imatinib mesylate. *Clin Cancer Res* 2006; **12**:2622–2627.
47 Demetri GD, van Oosterom AT, Garrett CR, Blackstein ME, Shah MH, Verweij J, *et al*. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. *Lancet* 2006; **368**:1329–1338.
48 De Mulder PH, Roigas J, Gillessen S, Srinivas S, Pisa P, Vogelzang N, *et al*. A phase II study of sunitinib administered in a continuous daily regimen in patients with cytokine-refractory metastatic renal cell carcinoma (mRCC). *J Clin Oncol* 2006; **223**:4529.
49 George S, Casali PG, Blay J, Cesne AL, Tyler AR, Quigley MT, *et al*. Phase II study of sunitinib administered in a continuous daily dosing regimen in patients with advanced GIST. *J Clin Oncol* 2006; **24**:527s. abstract 9532.
50 DePrimo SE, Bello C. Surrogate biomarkers in evaluating response to anti-angiogenic agents: focus on sunitinib. *Ann Oncol* 2007; **18** (**Suppl 10**):11–19.
51 Deprimo SE, Bello CL, Smeraglia J, Baum CM, Spinella D, Rini BI, *et al*. Circulating protein biomarkers of pharmacodynamic activity of sunitinib in patients with metastatic renal cell carcinoma: modulation of VEGF and VEGF-related proteins. *J Transl Med* 2007; **5**:32.
52 Burstein HJ, Elias AD, Rugo HS, Cobleigh MA, Wolff AC, Eisenberg PD, *et al*. Phase II study of sunitinib malate, an oral multitargeted tyrosine kinase inhibitor, in patients with metastatic breast cancer previously treated with an anthracycline and a taxane. *J Clin Oncol* 2008; **26**:1810–1816.
53 Heinrich MC, Maki RG, Corless CL, Antonescu CR, Harlow A, Griffith D, *et al*. Primary and secondary kinase genotypes correlate with the biological and clinical activity of sunitinib in imatinib-resistant gastrointestinal stromal tumor. *J Clin Oncol* 2008; **26**:5352–5359.
54 Prior JO, Montemurro M, Orcurto MV, Michielin O, Luthi F, Benhattar J, *et al*. Early prediction of response to sunitinib after imatinib failure by ^{18}f-fluorodeoxyglucose positron emission tomography in patients with gastrointestinal stromal tumor. *J Clin Oncol* 2009; **20**:439–445.
55 Saltz LB, Rosen LS, Marshall JL, Belt RJ, Hurwitz HI, Eckhardt SG, *et al*. Phase II trial of sunitinib in patients with metastatic colorectal cancer after failure of standard therapy. *J Clin Oncol* 2007; **20**:4793–4799.
56 Kulke MH, Lenz HJ, Meropol NJ, Posey J, Ryan DP, Picus J, *et al*. Activity of sunitinib in patients with advanced neuroendocrine tumors. *J Clin Oncol* 2008; **26**:3403–3410.
57 Socinski MA, Novello S, Brahmer JR, Rosell R, Sanchez JM, Belani CP, *et al*. Multicenter, phase II trial of sunitinib in previously treated, advanced non-small-cell lung cancer. *J Clin Oncol* 2008; **26**:650–656.
58 George DJ, Liu G, Wilding G, Hutson TE, Chen I, Maneval EC, *et al*. Sunitinib in combination with docetaxel and prednisone in patients with metastatic hormone-refractory prostate cancer (mHRPC)-preliminary results. *J Clin Oncol* 2008; **26**:5131.
59 Kondagunta GV, Hudes GR, Figlin R, Wilding G, Hariharan S, Kempin SN, *et al*. Sunitinib malate (SU) plus interferon (IFN) in first line metastatic renal cell cancer (mRCC): results of a dose-finding study. *J Clin Oncol* 2007;**25**:5101.
60 Sweeney C, Verschraegen C, Chiorean G, Lee F, Jones S, Tye L, *et al*. A phase I dose escalation study of sunitinib plus capecitabine in patients with advanced solid tumors. *J Clin Oncol* 2007;**25**:3592.
61 Patel PH, Kondagunta GV, Redman BG, Hudes GR, Kim ST, Chen I, Motzer RJ. Phase I/II study of sunitinib malate in combination with gefitinib in patients with metastatic renal cell carcinoma (mRCC). *J Clin Oncol* 2007; **25**:5097.
62 Traynor AM, Sandler AB, Sandler JH, Schiller J, Ilagan WL, Vermeulen G, *et al*. Phase I dose-escalation and pharmacokinetic study of sunitinib plus docetaxel in patients with advanced solid tumors. *J Clin Oncol* 2008; **26**:3564.
63 Leong S, Eckhardt SG, Chan E, Chow LQ, Vermeulen W, Camridge DR, et al. Phase I study of sunitinib in combination with modified FOLFOX 6 (mFOLFOX6) chemotherapy. 2007 Gastrointestinal Cancers Symposium Abstract book, Abstract 285.