Dose-Finding Study of the Multitargeted Tyrosine Kinase Inhibitor SU6668 in Patients with Advanced Malignancies

Bart C. Kuenen,\textsuperscript{1} Giuseppe Giaccone,\textsuperscript{1} Rita Ruijter,\textsuperscript{1} Astrid Kok,\textsuperscript{2} Casper Schalkwijk,\textsuperscript{2} Klaas Hoekman,\textsuperscript{1} and Herbert M. Pinedo\textsuperscript{1}

Abstract

**Purpose:** SU6668 is a tyrosine kinase inhibitor which targets platelet-derived growth factor receptor-\(\beta\), fibroblast growth factor receptor-1, vascular endothelial growth factor receptor-2, and KIT. We did a phase I study to define the maximum tolerated dose and to assess the pharmacokinetics of SU6668 administered orally thrice daily with food.

**Patients and Methods:** Patients with histologically proven, advanced, and progressive solid tumors were included at a starting dose level of 400 mg/m\(^2\) thrice daily. The early onset of dose-limiting toxicities (DLT) required dose reductions to 100 and 200 mg/m\(^2\) thrice daily. Pharmacokinetics was done on days 1, 28, and 56.

**Results:** Sixteen patients were included. Two of the first three patients developed DLTs, which consisted of grade 4 fatigue and grade 3 serositis-like pains. Six patients at dose level 100 mg/m\(^2\) thrice daily experienced no DLT. At dose level 200 mg/m\(^2\) thrice daily, two out of seven patients experienced DLTs consisting of grade 3 abdominal pain, grade 4 anorexia and grade 3 nausea/vomiting. Increasing doses resulted in a disproportional increase in area under the curve and \(C_{\text{max}}\) (peak plasma concentration). Both variables, however, decreased significantly on days 28 and 56 compared with day 1 (\(P<0.05\)). No objective responses were observed. Acute phase response, probably mediated by interleukin-6, was observed in serial blood samples.

**Conclusions:** The maximum tolerated dose of SU6668 given orally, thrice daily under fed conditions, is 100 mg/m\(^2\). Because of the low plasma levels reached at this dose level, the efficacy of SU6668 as a single agent is not to be expected.

Angiogenesis is essential for tumor growth and metastasis (1). The key regulator of the angiogenesis cascade is thought to be vascular endothelial growth factor (VEGF), which increases the permeability, proliferation, and migration of endothelial cells (2). However, inhibition of the VEGF receptor (VEGFR) pathway alone might be insufficient to substantially inhibit angiogenesis (3). The concerted process of angiogenesis is regulated by a large number of different factors, which have overlapping effects (redundancy). Tumor growth is the result of a complex interaction between tumor cells and their environment. This interaction is mediated by several factors, such as transcription factors (hypoxia-inducible factor 1 and 2), adhesion molecules, proteinases, and growth factors. The growth factors platelet-derived growth factor (PDGF) and basic fibroblast growth factor have both an autocrine and paracrine role in tumor growth and blood vessel formation by stimulating the proliferation and migration of tumor cells and of neighboring cells, such as stromal cells, endothelial cells, and their flanking pericytes.

VEGF, PDGF, and basic fibroblast growth factor exert their activity via receptors which belong to the family of receptor tyrosine kinases. Upon interaction with their respective ligand, receptor dimerization occurs with subsequent activation of the tyrosine kinase domain, resulting in activation of intracellular signaling pathways. Simultaneous blocking of the tyrosine kinase domains of the VEGFRs, PDGFRs, and fibroblast growth factor receptors (FGFR) may be an attractive anticancer therapy.

SU6668 ((Z)-3-[2,4-dimethyl-5-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid) is an orally available, small synthetic, lipophilic, highly protein-bound molecule that inhibits autophosphorylation of VEGFR-2, PDGFR-\(\beta\), FGFR-1, and KIT (4, 5). In biochemical assays, SU6668 inhibits VEGFR-2, PDGFR-\(\beta\), and FGFR-1 autophosphorylation competitively with \(IC_{50}\) values of 2.1, 0.008, and 1.2 \(\mu\)mol/L, respectively (6). In cellular assays, VEGF- and PDGFR-dependent signaling is inhibited at \(IC_{50}\) values of 0.5 and 1.0 \(\mu\)mol/L, respectively. In several human xenograft models, SU6668 induced regression of large, established tumors and resulted in significant decrease of microvessel density and mitotic index (6–8). Pharmacokinetic analysis of plasma samples of these animals showed that inhibition of VEGFR-2 phosphorylation in tumors was associated with sustained plasma concentrations of \(\geq 1 \mu\)g/mL (6).

**Authors’ Affiliations:** Departments of \textsuperscript{1}Medical Oncology and \textsuperscript{2}Clinical Chemistry, VU Medical Center, Amsterdam, the Netherlands

Received 12/1/04; revised 2/22/05; accepted 3/24/05.

Grant support: Partially supported by Sugen, Inc.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.


Requests for reprints: Giuseppe Giaccone, Department of Medical Oncology, VU Medical Center, De Boelelaan 1117, 1081 HV Amsterdam, the Netherlands. Phone: 31-20-444-4321; Fax: 31-20-444-4079; E-mail: G.Giaccone@vumc.nl.

\(d\)oi:10.1158/1078-0432.CCR-04-2466
Pharmacokinetic data from patients revealed that doses up to 2,000 mg/m² given orally once daily, in fasted conditions, were tolerated with minimal toxicity, but did not reach steady state trough levels (investigator’s brochure). Studies in dogs have shown that twice daily oral administration resulted in increased steady state trough levels, and a nearly 5-fold increase in oral bioavailability in fed compared with fasted animals. The primary objective of our phase I study was the definition of the maximum tolerated dose (MTD) and toxicity of SU6668 administered orally thrice daily with food. Secondary objectives were the assessment of pharmacokinetics and response rate.

**Patients and Methods**

**Eligibility criteria.** Entry in this open-label single center phase I study was restricted to patients aged ≥18 years with progressive advanced solid tumors who failed standard therapy. Karnofsky performance status had to be ≥60%. Evaluable or measurable disease was required. Patients with known brain metastases were excluded. Previous therapy for malignancy within 4 weeks prior to study drug administration was not allowed. Patients were required to have an absolute neutrophil count >1.5 × 10⁹/L, hemoglobin >6.0 mmol/L, platelet count ≥100 × 10⁹/L and a serum creatinine ≤150 mmol/L, or a creatinine clearance ≥40 mL/min. A total bilirubin >35 mmol/L or serum transaminases >3.0 × upper limit of normal was not allowed. A history of prior or concomitant malignant disease diagnosed within 5 years prior to study entry was not allowed, with the exception of in situ carcinoma of the cervix or basal cell carcinoma of the skin. Myocardial infarction, severe/unstable angina or coronary/peripheral artery bypass graft surgery within 6 months prior to study drug administration was not allowed. Patients with diabetes mellitus with clinical evidence of severe peripheral vascular disease or diabetic ulcers were also excluded. Manifestation of malabsorption or active inflammatory bowel disease was not allowed. Effective contraception by both male and female patients was required. The protocol was approved by the Institutional Ethics Committee and written informed consent was obtained before study entry.

**Drug administration.** The study drug was supplied as red-brown tablets containing 200 mg of SU6668. Each tablet also contained D-mannitol, carboxymethyl-cellulose calcium, magnesium stearate, hydroxypropylmethylcellulose 2208, polyethylene glycol 6000, and a ferric oxide film coating. The dose of SU6668 was based on body surface area in square meters, and was rounded to the appropriate 200 mg increment. SU6668 tablets were required to be ingested thrice daily – 8 hours apart within 1 hour after a meal minimally containing 20 g of fat. Patients with a break in therapy >3 weeks were withdrawn from study. Each period of 4 weeks was considered as one treatment cycle. Treatment could be continued to a maximum period of 1 year unless progressive disease or unacceptable toxicity occurred.

**Dose escalation.** The starting dose of SU6668 was 400 mg/m² thrice daily. Dose escalation would proceed by doubling the administered dose until grade 2 toxicity was observed occurring during the first cycle of therapy which could be considered as possibly or probably study drug-related. Once grade 2 study drug-related toxicity was observed, dose escalation would proceed with 40% dose increments until unacceptable toxicity was observed. Unacceptable toxicity was defined as grade 3 or greater toxicity, excluding nausea/vomiting and hematologic toxicity, or grade 4 hematologic toxicity or grade 4 nausea and vomiting refractory to antiemetic therapy, or a drug-related death.

**Pharmacokinetics.** On days 1, 28, and 56, blood samples for pharmacokinetics, patients received standard meals containing 20 g of fat at 7:30 a.m., 3:30 a.m., and 11:30 p.m. Samples were collected into lithium heparin tubes, placed on ice and centrifuged at 3,000 rpm at 4°C for 10 minutes. The upper layer was transferred with a glass pipette to a cryovial (1.5 mL), and stored at −70°C until further processing.

SU6668 plasma concentrations were determined using a validated high-pressure liquid chromatography assay with UV detection by MDS Pharma Services, Quebec Canada (9). Briefly, study samples (50 µL) and trilevel quality control samples were aliquoted in duplicate. β-Glucuronidase enzyme solution (10 µL) was added to one set and acetate buffer (10 µL) to the other set of study samples, quality controls and calibration curve specimens. Samples containing the β-glucuronidase enzyme were incubated at 45°C for 1 hour and the internal standard SU9905 was added to the study samples. The samples were vortexed, centrifuged and 30 µL injected onto a high-pressure liquid chromatography column (Zorbax Eclipse XDB C18 3.5 micron, 3.0 × 0.46 cm, flow rate 1.0 mL per minute). The UV-VIS detector was set at a wavelength of 440 nm. Under these conditions, retention time for SU6668 and the internal standard was 2.7 and 4.8 minutes, respectively, with a total run of 13 minutes. The PK variables Cₘₐₓ (peak plasma concentration), Tₘₐₓ (time of peak plasma concentration), AUC₀₋₁₂h (area under the plasma concentration-time curve from 0 to 16 hours), Vₖ/ₐ (volume of distribution), clearance/F and t₁/₂ (apparent terminal phase half-life) were calculated by non-compartmental analysis using WinNonLin, version 4.1 (Scientific Consulting Inc./Pharsight Corporation, Mountain View, CA).

**Toxicity evaluation.** Pretreatment evaluation was done within 7 days prior to initiation of therapy, and included a complete history and physical examination, urinalysis, 12-lead electrocardiogram, complete blood cell count, serum chemistry, and coagulation tests. A negative urine pregnancy test for all female patients at risk was demanded. Toxicity according to the National Cancer Institute-Common Toxicity Criteria (version 2.0) was monitored weekly for the first 8 weeks, every 2 weeks thereafter, and included a complete history, physical examination, serum chemistry, and hematology. Coagulation tests and electrocardiogram were done every 4 weeks. Any serious adverse event or unexpected event was reported within 24 hours, and a written report was submitted within one working day to Central Drug Safety Europe, Mannheim, Germany.

**Response evaluation.** Tumor staging according to the Response Evaluation Criteria in Solid Tumors was done by computed tomography or magnetic resonance imaging scans of all known tumor lesions within 2 weeks prior to the beginning of treatment (10). In case of multiple tumor sites, all measurable lesions were followed for the assessment of disease progression. Response evaluation was assessed every 8 weeks, or more frequently, if clinically indicated. In contrast to response criteria normally used in chemotherapy trials, two major differences were present. First, because an antitumor effect of SU6668 was not expected to occur rapidly, progressive disease during the first two treatment cycles was not an off-study indication provided that the patient’s clinical condition justified continuation of therapy. Second, the tumor assessment after the first 8 weeks of treatment was used as a new baseline for response evaluation.

**Additional laboratory variables.** Blood samples were drawn prior to the start of treatment, and during treatment on days 8, 15, and 28, and every 2 weeks thereafter, and in case of toxicity. Plasma was obtained from sodium citrate (9:1 vol/vol blood/citrate; final concentration, 0.32%) and centrifuged at 4,000 rpm at 4°C for 10 minutes. The separated plasma was then centrifuged a second time in an Eppendorf centrifuge at 14,000 rpm at 4°C for 3 minutes. After transfer to microtubes, these samples were stored at −80°C in 1 mL aliquots until further processing. Serum was obtained by centrifugation of blood at 4,000 rpm at 4°C for 10 minutes and stored at −80°C in 1 mL aliquots until further processing.

Human interleukin-6 (IL-6) was measured by sandwich enzyme immunoassay (Quantikine High Sensitivity, R&D Systems, Oxon, United Kingdom). Plasma von Willebrand factor antigen was measured.
by an ELISA, using rabbit anti-von Willebrand factor antigen as a
catching antibody and a peroxidase-conjugated rabbit anti-von Wille-
brand factor antigen as a detecting antibody (Dako, Copenhagen,
Denmark). Soluble E-selectin was assayed by ELISA (Diaclone,
Besancon, France) and human thrombin/antithrombin III complexes
by a sandwich enzyme immunoassay (Enzygnost TAT micro, Dade
Behring, Marburg, Germany). Human complement C3 was measured
with a rate nephelometric assay on an Array 360 System (Beckman
Instrument Inc., Brea, CA) with C3 antibody (Array C3 reagent,
Beckman Coulter, Inc., Galway, Ireland).

Statistics. Data are reported as means ± SD. Because a normal
distribution could not safely be assumed in the small groups, the data
were analyzed with a nonparametric method. The Wilcoxon signed ranks
test was used to assess significance of differences between days 1, 8, 15,
and 28. Differences were considered significant at the
P

value test.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>n = 16</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>median 52</td>
</tr>
<tr>
<td>range</td>
<td>33-68</td>
</tr>
<tr>
<td>Sex</td>
<td>male 9 (56)</td>
</tr>
<tr>
<td>female</td>
<td>7 (44)</td>
</tr>
<tr>
<td>Eastern Cooperative Oncology Group</td>
<td>0 (7 (44))</td>
</tr>
<tr>
<td>performance state</td>
<td>1 (9 (56))</td>
</tr>
<tr>
<td>Primary tumor</td>
<td>colorectal cancer 7 (56)</td>
</tr>
<tr>
<td></td>
<td>melanoma 2 (12)</td>
</tr>
<tr>
<td></td>
<td>sarcoma 2 (12)</td>
</tr>
<tr>
<td></td>
<td>ovarian cancer 1 (6)</td>
</tr>
<tr>
<td></td>
<td>non – small cell lung cancer 1 (6)</td>
</tr>
<tr>
<td></td>
<td>others 3 (19)</td>
</tr>
<tr>
<td>Prior treatment</td>
<td>surgery 15 (94)</td>
</tr>
<tr>
<td></td>
<td>chemotherapy 13 (81)</td>
</tr>
<tr>
<td></td>
<td>radiotherapy 7 (44)</td>
</tr>
<tr>
<td></td>
<td>experimental therapy 3 (19)</td>
</tr>
</tbody>
</table>
Three patients attained a confirmed stable disease, which disease in one. Twelve patients were evaluable for response. Development of severe toxicity in three, and to underlying response because they received less than one cycle, due to half-life of SU6668 is 2 to 4 hours comparing all doses, without to 12 L/h upon repeated dosing. The apparent terminal phase clearance was to 40 L at the start of treatment to 40 to 60 L by days 28 and 56. The first day of dosing and stabilized thereafter, increasing from 20

\[ C_{\text{max}} \text{ (µg/mL)} \]
\[ T_{\text{max}} \text{ (h)} \]
\[ \text{AUC}_{0-16\text{hrs}} \text{ (µg/mLh)} \]
\[ \text{Vz/F} \text{ (L/h)} \]
\[ \text{Clearance/F} \text{ (L/h)} \]
\[ t_{1/2} \text{ (h)} \]

SU6668 resulted in a disproportional increase of AUC and \( C_{\text{max}} \) over the dosing range tested. Although there were no differences in the administered dose per patient or the fat content of the meals between day 1 and days 28 and 56, both the AUC and \( C_{\text{max}} \) decreased upon repeated dosing. Comparing days 1, 28, and 56, AUC and \( C_{\text{max}} \) decreased significantly 50% (\( P = 0.002, 0.002, 0.007, \text{ and } 0.004, \) respectively). The volume of distribution seemed to increase with increasing doses on the first day of dosing and stabilized thereafter, increasing from 20 to 40 L at the start of treatment to 40 to 60 L by days 28 and 56. Clearance was ~6 to 9 L/h after the first dose, increasing to 11 to 12 L/h upon repeated dosing. The apparent terminal phase half-life of SU6668 is 2 to 4 hours comparing all doses, without significant change over time.

**Tumor response.** Four patients were not evaluable for response because they received less than one cycle, due to development of severe toxicity in three, and to underlying disease in one. Twelve patients were evaluable for response. Three patients attained a confirmed stable disease, which lasted 8 months in a patient with leiomyosarcoma and 6 months in a patient with melanoma. A patient with gastrointestinal autonomic nerve tumor who experienced stable disease discontinued treatment at her own request after 4 months of treatment. Unconfirmed stable disease was observed in a patient with parotid gland cancer, who discontinued treatment at his own request after 2 months of treatment. The plasma SU6668 levels of patients with stable disease were not different compared with those of the other patients. Nine patients developed progressive disease within 2 to 4 months of treatment, which in seven patients consisted of the occurrence of new tumor lesions, besides growth of the existing lesions.

**Laboratory variables.** No changes in kidney and liver function tests or hematology cell counts were observed. A significant increase of C-reactive protein within 4 weeks after the start of treatment with SU6668 was observed, which together with a significant decrease in albumin levels, indicated the occurrence of an acute phase response (Fig. 2A and B). This effect was probably mediated by IL-6, because a significant increase was observed after 1 week of treatment (Fig. 2C). The increase in platelets was probably also a consequence of this early IL-6 response (Fig. 2D). In addition, a significant increase of C3 levels occurred (Fig. 2E). von Willebrand factor levels also increased significantly, suggesting activation of endothelial cells (Fig. 2F). However, s-E-selectin remained unchanged throughout treatment (data not shown). No change was observed in the levels of thrombin/antithrombin complexes, indicating that no activation of the coagulation cascade occurred (data not shown).

**Discussion**

In this study, the MTD of SU6668 given thrice daily by oral administration under fed conditions was 100 mg/m². There seems to be a large difference with the nontoxic dose of 2,000 mg/m² once daily in fasted conditions. Due to the nearly 5-fold increase in oral bioavailability in fed compared with fasted conditions, the dose of 100 mg/m² thrice daily with food is probably at least equal to 1,500 mg/m² once daily in fasted conditions, but probably even more due to increased steady state trough levels. The plasma concentrations of SU6668 at the MTD in this study were in the range of 1 µg/mL, the level which in xenograft models was associated

**Table 2. Percentage of 15 patients experiencing SU6668 toxicity during all treatment cycles**

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>1/2 (%)</th>
<th>3/4 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anorexia</td>
<td>40</td>
<td>7</td>
</tr>
<tr>
<td>Nausea</td>
<td>73</td>
<td>27</td>
</tr>
<tr>
<td>Vomiting</td>
<td>53</td>
<td>—</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>33</td>
<td>7</td>
</tr>
<tr>
<td>Constipation</td>
<td>13</td>
<td>—</td>
</tr>
<tr>
<td>Change of taste</td>
<td>20</td>
<td>—</td>
</tr>
<tr>
<td>Weight loss</td>
<td>13</td>
<td>—</td>
</tr>
<tr>
<td>Fatigue</td>
<td>73</td>
<td>27</td>
</tr>
<tr>
<td>Flu-like complaints</td>
<td>47</td>
<td>—</td>
</tr>
<tr>
<td>Myalgia/arthralgia</td>
<td>27</td>
<td>—</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>33</td>
<td>20</td>
</tr>
<tr>
<td>Chest pain</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>20</td>
<td>—</td>
</tr>
<tr>
<td>Anemia</td>
<td>40</td>
<td>—</td>
</tr>
</tbody>
</table>

**Table 3. Pharmacokinetic parameters on days 1, 28, and 56 of SU6668 given thrice daily with food**

<table>
<thead>
<tr>
<th>Dose (mg/m²)</th>
<th>100</th>
<th>200</th>
<th>400</th>
<th>100</th>
<th>200</th>
<th>100</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (µg/mL)</td>
<td>4.7 ± 1.8</td>
<td>8.0 ± 2.0</td>
<td>11.4 ± 4.7</td>
<td>2.0 ± 1.7</td>
<td>4.0 ± 1.3</td>
<td>2.0 ± 0.5</td>
<td>4.1 ± 1.7</td>
</tr>
<tr>
<td>( T_{\text{max}} ) (h)</td>
<td>2.8 ± 2.2</td>
<td>3.6 ± 2.3</td>
<td>2.8 ± 0.6</td>
<td>4.9 ± 4.6</td>
<td>5.5 ± 4.6</td>
<td>8.6 ± 3.5</td>
<td>6.2 ± 6.0</td>
</tr>
<tr>
<td>( \text{AUC}_{0-16\text{hrs}} ) (µg/mLh)</td>
<td>31.0 ± 14.4</td>
<td>57.1 ± 11.6</td>
<td>64.7 ± 14.9</td>
<td>14.2 ± 3.6</td>
<td>28.2 ± 7.3</td>
<td>14.4 ± 5.0</td>
<td>27.8 ± 9.1</td>
</tr>
<tr>
<td>( \text{Vz/F} ) (L)</td>
<td>21.6 ± 11.4</td>
<td>25.4 ± 15.3</td>
<td>42.4 ± 27.5</td>
<td>40.0 ± 18.7</td>
<td>50.8 ± 19.9</td>
<td>63.2 ± 41.4</td>
<td>41.4 ± 14.5</td>
</tr>
<tr>
<td>Clearance/F (L/h)</td>
<td>6.1 ± 2.4</td>
<td>5.7 ± 1.1</td>
<td>9.1 ± 1.1</td>
<td>11.1 ± 2.5</td>
<td>11.8 ± 2.9</td>
<td>11.1 ± 4.4</td>
<td>12.5 ± 5.3</td>
</tr>
<tr>
<td>( t_{1/2} ) (h)</td>
<td>2.4 ± 0.7</td>
<td>3.0 ± 1.2</td>
<td>3.1 ± 1.7</td>
<td>2.5 ± 1.1</td>
<td>3.2 ± 1.6</td>
<td>4.3 ± 2.6</td>
<td>2.4 ± 0.6</td>
</tr>
</tbody>
</table>

**NOTE:** \( C_{\text{max}} \), peak plasma concentration; \( T_{\text{max}} \), time of peak plasma concentration; \( \text{AUC}_{0-16\text{hrs}} \), area under the plasma concentration-time curve; \( \text{Vz/F} \), volume of distribution; \( t_{1/2} \), apparent terminal phase half-life.
The occurrence of DLTs was correlated with higher plasma levels of SU6668 over time at all dose levels could be due to induction of metabolic enzymes. Preclinical studies with p.o. and i.v. administered [14C]-SU6668 showed evidence of a high presystemic clearance. Upon repeated i.v. and p.o. dosing a decreased AUC was observed, possibly as a result of change of metabolism of the parent drug (investigator’s brochure). However, PK of this study showed no significant change of the apparent terminal phase half-life of SU6668. The nature of the increase of the volume of distribution is unclear. An alternative explanation for the decrease in AUC and C_{max} over time could be a decreased absorption of the drug. Whether SU6668 is a substrate for hepatic and intestinal P450 enzymes is unknown, but could be a good explanation for the decreased bioavailability when SU6668 induces and is metabolized by P450 enzymes.

The occurrence of DLTs was correlated with higher plasma levels of SU6668 at dose levels 200 and 400 mg/m². These DLTs, which consisted of serositis-like pains, fatigue, and anorexia, were unexpected. Less severe grades of serositis-like pains were also frequently (53%) observed. Although other toxicities were mild, anorexia is problematic for an oral drug. No apparent antitumor activity was observed. The observed stable disease have to be judged in view of the permissive response criteria (allowing progressive disease during the first 8 weeks of treatment), and could furthermore be part of the natural history of these tumors.

The results of a clinical trial investigating 200 and 400 mg/m²/d SU6668, which were quite similar to our results, have recently been published (11). Although a limited number of patients was enrolled, a different dosing regimen was investigated and PK was done on days 1 and 22 (instead of day 28). PK was especially consistent with our data. Comparing days 1 and 22 an obvious decrease in C_{max} increase in the volume of distribution and increase of apparent oral clearance with an unchanged half-life were observed. Adverse events consisted of pain and abdominal pain which were mostly related to advanced disease. No responses and no serious adverse events related to SU6668 were observed, which was not expected regarding the dosing range studied.

The unexpected toxicities, the serositis-like pains and flu-like complaints, were intriguing. These observations in combination with an acute phase response mediated by IL-6, are indicative for the induction of an inflammatory reaction. Explaining these observations is difficult because SU6668 inhibits at least four different receptors.

The role of the VEGFRs, FGFRs, and PDGFRs and their ligands during adult life is not completely understood. During treatment with SU5416, a tyrosine kinase-inhibitor targeting the VEGFRs, we observed an increase of von Willebrand factor, s-E-selectin, and soluble tissue factor, indicating endothelial cell perturbation (12). Moreover, an increased incidence of thromboembolic events was observed when SU5416 was combined with cisplatin/gemcitabine (13). This indicates that VEGF also plays a role as maintenance and protection factor for endothelial cells. During treatment with SU6668, we observed an increase of von Willebrand factor levels, whereas the s-E-selectin levels remained normal, indicating that there was no extensive perturbation of endothelial cells or that von Willebrand factor was released by platelets. Neither thromboembolic events nor activation of coagulation variables were observed during treatment with SU6668. Of interest is that VEGF, together with basic fibroblast growth factor, synergistically enhanced endothelial cytoprotection via the induction of decay-accelerating factor (14). Decay-accelerating factor contributes to control of complement activation on the cell surface by preventing the formation and accelerating the decay of C3 and C5 convertase. It is conceivable that lower expression levels of decay-accelerating factor follow- ing inhibition of the VEGFR-2 and FGFR by SU6668 resulted in complement activation with subsequent chemotaxis of neutrophils. Decay-accelerating factor is not only expressed on endothelial cells, but also on a wide range of epithelial surfaces, such as pleural, pericardial, and synovial serosa (15). It is unknown how and which growth factors regulate the expression of decay-accelerating factor on these surfaces. The C3 levels in our patients increased during treatment, suggesting that no increased utilization of complement occurred. The increase of C3 levels is probably part of the acute phase response. Furthermore, basic fibroblast growth factor inhibits the

---

**Fig. 1.** Mean plasma concentration time profile following oral administration of SU6668 on (A) cycle 1 day 1, and (B) cycle 1 day 28 (unidirectional line = SD).
expression of adhesion molecules on endothelial cells, which can be blocked by SU6668 (16). Thus, inhibition of the FGFR-1 facilitates endothelial cell activation, and subsequent attachment and migration of monocytes. In conclusion, interfering in VEGF- and FGF-signaling at the same time may result in modification of the inflammatory process. PDGFR-β is markedly up-regulated in inflammatory tissue and experimental studies have shown that PDGF enhances the formation of granulation tissue (17–20). Furthermore, many different signaling properties and biological responses are induced by all isoforms of PDGF (21). These responses can be modified by other growth factors, such as IFN-γ and transforming growth factor-β (22). KIT and its ligand stem cell factor are known as inducers of mast cell proliferation and degranulation, and inducers of eosinophil activation and degranulation. They play an important role in inflammatory processes, especially in allergic diseases (23). Whether and how inflammatory processes are affected by inhibition of PDGFR-β and KIT remains to be investigated.

Fig. 2. Mean ± SD (A) C-reactive protein (CRP), (B) albumin, (C) interleukin-6 (IL-6), (D) platelets, (E) C3, and (F) von Willebrand factor (vWf) on days 1, 8, 15, and 28 of treatment with SU6668 (n = 14; bar, mean; unidirectional line, SD; *, significantly different compared with day 1).
SU6668 (100 mg/m²) given thrice daily with food resulted in plasma levels in the range which interferes with VEGFR phosphorylation in xenograft models. This dose was well tolerated, but will probably not induce tumor regressions when used as a single agent. The observation that inhibition of the PDGF receptor increases the efficacy of chemotherapy as a result of decreased interstitial fluid pressure indicates that it might be attractive to combine SU6668 with chemotherapy (24). However, we observed that SU6668, an antiangiogenic agent targeting multiple receptor pathways, as well as SU5416, an antiangiogenic agent targeting the VEGFR pathway, in combination with chemotherapy, induced unexpected toxicities (13). Therefore, close monitoring of patients receiving those experimental therapies is essential.

References
Dose-Finding Study of the Multitargeted Tyrosine Kinase Inhibitor SU6668 in Patients with Advanced Malignancies

Bart C. Kuenen, Giuseppe Giaccone, Rita Ruijter, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/11/17/6240

Cited articles
This article cites 20 articles, 12 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/11/17/6240.full#ref-list-1

Citing articles
This article has been cited by 6 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/11/17/6240.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.