A Phase II Study of Razoxane, an Antiangiogenic Topoisomerase II Inhibitor, in Renal Cell Cancer with Assessment of Potential Surrogate Markers of Angiogenesis


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ABSTRACT
Renal cell carcinoma (RCC) is an angiogenic tumor resistant to standard cytotoxic chemotherapeutic agents. Although often responsive to immunomodulatory agents including interleukin 2 and IFN-α, the overall results in randomized Phase III studies are disappointing with only modest improvements in overall survival. This Phase II study evaluated the efficacy and tolerability of razoxane, an antiangiogenic topoisomerase II inhibitor, in 40 patients (32 men, 8 women; age: range, 31–76 years; median, 58 years) with inoperable RCC. Twenty patients received razoxane 125 mg p.o., twice a day for 5 days each week for 8 weeks (one cycle). It was repeated in patients with stable disease (StD), but was discontinued after 16 weeks if there was no evidence of an objective response. Because minimal toxicity was seen, subsequent patients (n = 20) were treated until progressive disease (PD) was documented. Of 38 evaluable patients, 11 (29%) had StD for a minimum of 4 months, and the remainder had PD. Median overall survival was 7.3 months. Duration of survival was significantly better in patients with StD compared with those with PD (P = 0.003).

The effect of treatment on six potential surrogate serum/plasma (vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), urokinase plasminogen activator soluble receptor (upAsr), E-selectin, vascular cell adhesion molecule-1 (VCAM-1) and von Willebrand’s factor (vWF)) markers of angiogenesis was evaluated before and after 1 cycle of treatment. Pretreatment serum VEGF and E-selectin levels above the median value were associated with a poor prognosis. Serum VCAM-1 levels and urinary VEGF levels rose significantly after one cycle in patients with PD but not in those with StD. Serum VEGF, bFGF, VCAM-1 and vWF, plasma upAsr and urinary bFGF levels were significantly higher in PD patients compared with StD patients before and/or after 1 cycle of treatment. In conclusion, razoxane is an antiangiogenic agent that has minimal toxicity and that requires further evaluation in combination with other active agents in the treatment of RCC. Surrogate serum and urinary markers of angiogenesis may have a role in predicting disease response and overall survival in RCC.

INTRODUCTION
Approximately 25% of patients with RCC present with metastatic disease. Although the subsequent progress of the disease is highly variable, the overall prognosis is poor with a 5-year survival in reported studies of 0–18%. RCC is highly resistant to conventional cytotoxic chemotherapy. An extensive review of 39 drugs used to treat RCC revealed all to have response rates ≤9%. Radiotherapy may be used in the palliation of symptoms but likewise is associated with a poor response rate (1–6).

Razoxane (ICRF 159; (±)-1,2-di(3,5-dioxopiperazin-1-yl) propane) belongs to the family of bis-dioxopiperazines, developed in the 1960s as derivatives of the chelating agent EDTA. Early in its development it was shown to have potent antiangiogenic activity, with vessels at the periphery of tumors reverting from an abnormal tumor-related vasculature to a normal phenotype (7–10). The precise molecular mechanism of this antiangiogenic action is not known. More recently razoxane has been shown to be a noncleavable inhibitor of topoisomerase II (11). Razoxane inhibits metastatic spread of Lewis lung 3LL, hamster lymphoma ML, and murine squamous carcinoma G
cells in experimental animals and causes a marked increase in the sensitivity of tumors to radiation (reviewed in Refs. 12 and 13). Initial Phase I and II clinical trials demonstrated modest antitumor activity in advanced colorectal and head and neck carcinomas, lymphomas, lymphosarcomas, and acute leukemias. In combination with radiotherapy, razoxane has activity against liver metastases from colorectal, inoperable nonmetastatic rectal, bladder, vulval, and lung carcinomas; soft tissue and osteosarcomas; and central nervous system tumors including malignant glioma and astrocytoma (reviewed in Refs. 12 and 13).

Razoxane is absorbed from the gastrointestinal tract in a schedule-dependent manner. Absorption is poor with large single doses but is satisfactory with small divided doses (14). The plasma half-life in humans is 3.5 h. It is well tolerated with few side effects seen in early clinical trials. However, with high-dose razoxane, neutropenia and thrombocytopenia are seen (15) because of the topoisomerase activity and inhibition of cell proliferation. Hematological toxicity was rarely seen with prolonged low-dose schedules and razoxane was well tolerated when evaluated in the adjuvant treatment of colorectal carcinoma at a dose of 125 mg b.d. p.o. for 5 days every week given for up to 2 years, (16, 17). As such, oral razoxane is an inexpensive antiangiogenic and antiproliferative agent with few side effects.

Angiogenesis, the process of new microvessel formation, is necessary for a tumor to grow beyond 1–2 mm in diameter. Neovascularization also plays an important role in the metastatic spread of malignant disease (18). New vessel formation, with accentuation of capillary vessels, is a feature of RCC (19). High microvessel counts in RCC are associated with a poor prognosis (20, 21). IFN-α and IL-2 are antiangiogenic. This observation may in part explain their antitumor activity in RCC (22, 23). Therefore, investigation of inexpensive, established agents with antiangiogenic properties requires evaluation in the management of RCC. Angiogenic growth factors and their receptors, proteases and protease receptors, and endothelial cell adhesion molecules play important roles in the pathogenesis of malignant tumors. These factors include VEGF (24), bFGF (25, 26), uPA/sr (27), E-selectin, VCAM-1 (28), and vWF (29). Elevated levels of these proteins may be detected in the blood and urine of patients with malignant disease (28–33).

The purpose of this study was to evaluate the efficacy of prolonged low-dose oral razoxane in the treatment of metastatic RCC. Antiangiogenic agents may not produce regression of established blood vessels; therefore, we considered StD to be a relevant end point. Treatment with antiangiogenic drugs may be required over a protracted period of time; therefore, we wanted to assess whether it was possible to detect antiangiogenic effects early in the course of treatment. We hypothesized that potential surrogate plasma/serum and urine markers of angiogenesis would be influenced by therapy and would predict subsequent disease status.

PATIENTS AND METHODS

Eligibility Criteria—Single-Center Open-Label Phase II Study. Patient inclusion criteria included: cytologically and/or histologically proven metastatic RCC; age, ≥18 years; WHO performance status, ≤2; expected survival, ≥3 months; hemoglobin, ≥10 g/dl; white blood count, ≥3 × 10⁹/liter; absolute neutrophil count, ≥2 × 10⁹/liter; platelet count, ≥100 × 10⁹/liter; bilirubin, ≤2 × normal; AST/ALT, ≤3 × normal (unless because of metastases, in which AST/ALT ≤5 × normal is accepted); and creatinine within the normal range for our institution (70–150 μmol/liter, Oxford Radcliffe Hospital). Patients with previous or intermittent malignancies at other sites, with the exception of adequately treated cone-biopsied carcinoma of the cervix and basal or squamous cell carcinoma of the skin were excluded from the study. Other exclusion criteria were intensive chemotherapy or radiotherapy <3 weeks before inclusion, pregnancy, or women likely to become pregnant during the trial. Patients of childbearing age had to take adequate contraceptive precautions during the trial and for 4 weeks after completing treatment. The study was approved by the Central Oxford Research Ethics committee and conducted according to the recommendations of the Declaration of Helsinki and the Association of British Pharmaceutical Industry guidelines for good clinical practice. Informed written consent was obtained from each patient prior to entry into the study.

Patient Evaluation. In the 3 weeks prior to commencing treatment, each patient had the following assessments: physical examination with clinical evaluation of all of the sites of disease; a full blood count and renal, liver, and bone biochemistry screen; and establishment of a measurable lesion(s) using additional investigations as clinically indicated (plain X-rays, ultrasound, computed tomographic imaging, magnetic resonance imaging). On the day of prescription of razoxane, the patient had a further clinical evaluation. A full blood count was checked every 2 weeks while the patient was on study. Each patient was reexamined every 4 weeks and at each time had a biochemistry profile.

Treatment Schedule. In the first 20 patients recruited to the study, 125 mg razoxane was administered b.d. p.o. for 5 days a week for a cycle duration of 8 weeks. After formal disease assessment, the cycle was repeated in patients with either StD or responding disease and discontinued after 16 weeks (two cycles) if there was no evidence of objective disease response. Because the agent was well tolerated in these patients, subsequent patients were treated until PD was documented.

Toxicities and Dose Modifications. Toxicity was graded according to the Cancer and Leukemia Group B expanded CTC. Treatment delay of 1 week’s duration was considered for any ≥ grade 2 toxicity apart from anemia, alopecia, and nausea and vomiting controlled with antiemetics. If the grade 2 toxicity persisted for >1 week or if ≥ grade 3 neutropenia occurred, the treatment was reduced by 30% with razoxane 125 mg once a day on days 1, 3, and 5 and 125 mg b.d. on days 2 and 4 of the 5-day schedule. Razoxane treatment was discontinued if a further dose reduction was indicated, if grade 4 toxicity (CTC scale) was experienced at the reduced dose level, or if a life-threatening event occurred that was deemed directly related to razoxane therapy.

Response Assessment. The assessable lesion/lesions were clearly measurable according to CRC recommendations. A lesion was considered measurable if it measured ≥1 cm in diameter on computed tomographic or magnetic resonance imaging, or at least 2 cm on plain X-ray or ultrasound, in each of two perpendicular dimensions. The established measurable le-
tection(s) were evaluated after each 8-week cycle to assess response. A CR was defined as clearance of all of the measurable or evaluable disease for ≥8 weeks. A PR was defined as a reduction in the sum of the product of all of the bidimensional measurements of the lesions evaluated at baseline by ≥50% for ≥8 weeks. Disease was considered stable if the lesions measured at baseline reduced in size by <50% or increased by <25% after two cycles of treatment. PD was defined as an increase of ≥25% in the sum of the product of the bidimensionally measurable disease sites, or the appearance of new metastases <16 weeks (two cycles) after commencing razoxane.

**Surrogate Blood and Urine Markers of Angiogenesis.** Prior to treatment, two 10-ml blood samples for serum and plasma, and a urine sample, were obtained to analyze potential surrogate markers of angiogenesis. The blood sample was chilled on ice, centrifuged at 2000 rpm for 10 min at 4°C and stored in 1.5-ml aliquots at −70°C until analysis. The urine samples were either brought in by the patient or taken fresh on the morning of treatment and were frozen at −70°C until analysis. Additional samples were obtained after 4 and 8 weeks (1 cycle) of treatment.

 Serum E-selectin, VCAM-1, and serum and urine bFGF and VEGF were measured using ELISA kits supplied by R&D systems (Abingdon, United Kingdom). The serum vWF and plasma uPAsr levels were analyzed by ELISA using in-house methods as described previously (31, 34). The normal range (or mean ± SD) for each assay was as previously published or determined as indicated: serum VEGF, 62–707 pg/ml (mean, 220 pg/ml)

<table>
<thead>
<tr>
<th></th>
<th>Serum VEGF</th>
<th>Urine VEGF</th>
<th>Serum bFGF</th>
<th>Urine bFGF</th>
<th>Serum E-selectin</th>
<th>Urine E-selectin</th>
<th>Plasma uPAsr</th>
<th>Urine uPAsr</th>
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</thead>
<tbody>
<tr>
<td>Mean</td>
<td>62–707 pg/ml</td>
<td>73–144 ng/g creatinine</td>
<td>0–4.4 pg/ml</td>
<td>0.6–8.1 ng/g creatinine</td>
<td>220 pg/ml</td>
<td>0.83–1.7 ng/ml</td>
<td>252 ± 173 ng/ml</td>
<td>48.37 ± 19.6 ng/ml</td>
</tr>
<tr>
<td>Median</td>
<td>62–707 pg/ml</td>
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<td>252 ± 173 ng/ml</td>
<td>48.37 ± 19.6 ng/ml</td>
</tr>
<tr>
<td>Range</td>
<td>525 pg/ml</td>
<td>620–1440 ng/g creatinine</td>
<td>0–4.4 pg/ml</td>
<td>0.6–8.1 ng/g creatinine</td>
<td>220 pg/ml</td>
<td>0.83–1.7 ng/ml</td>
<td>150–173 ng/ml</td>
<td>48.37–19.6 ng/ml</td>
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</table>

Statistical Methods. The Wilcoxon rank-sum test was used to analyze differences in surrogate markers before and after one cycle of chemotherapy within the StD and PD groups. The Mann-Whitney test was used to test for differences in the markers between patients with StD and PD. Fisher’s exact test was used for testing relationships between categorical variables and Student’s t test to compare pretreatment angiogenic marker levels with control values as appropriate. The analysis was performed using the Stata statistical software, release 5.0 package (Stata Corp., College Station, TX).

**RESULTS**

**Patients.** Between August 1994 and February 1997, 40 patients with inoperable RCC were recruited to the study. Patient characteristics are summarized in Table 1. Only five patients had received previous treatment, and of these, only one had received cytokine therapy using IL-2.

**Response and Survival.** Thirty-eight of the 40 patients were evaluable for response. Response was classified at 16 weeks, those progressing before that time being included in the PD group. Although no objective tumor responses were seen, 11 patients had StD for ≥16 weeks’ duration. All of these patients were either newly diagnosed (n = 4) or had evidence of objective tumor progression in the 6 months prior to starting treatment (n = 7). The median survival for all of the 40 patients included in the study was 7.3 months. In evaluable patients, the median survival for those with StD was 399 days and for the 27 patients with PD, 127 days (P = 0.0026). The TTP was 12 weeks.

Patients were characterized as having a good, moderate, or poor prognosis based on the criteria of Jones et al. This involves the application of a simple index based on the presence or absence of each of the following three risk factors: performance status ≥1 (rather than 0); time from diagnosis <2 years; and more than one site of metastases. Good-prognosis patients have ≥1 risk factor; moderate-prognosis patients, any two risk factors; and poor-prognosis patients, all three risk factors. Of the 40 patients studied, 9 had good prognostic features, 18 had moderate, and 13 had poor. Although the good prognostic group had a better overall survival, this was not statistically signifi-
Table 2  Number of patients developing grade 1, 2, 3, and 4 toxicity based on Cancer and Leukemia Group B common toxicity criteria (total number of patients evaluated = 40)

<table>
<thead>
<tr>
<th>Toxicity (n = 40)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<td>Nausea/Vomiting</td>
<td>17</td>
<td>6</td>
<td>1</td>
<td>0</td>
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<tr>
<td>Fatigue</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Neutopenia</td>
<td>4</td>
<td>7</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Epistaxis</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Taste</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Hypercalcemia</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mucositis</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>CNS*</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Allergic reaction</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

* CNS, central nervous system.

Of the 38 evaluable patients, no association was found between the prognostic groups and the development of PD. Three of 9, 5 of 18, and 3 of 11 patients had StD ≥16 weeks in the good, moderate, and poor prognostic groups, respectively. Elevated pretreatment neutrophil counts (>8.25 × 10⁹/liter; P = 0.016), lactate dehydrogenase levels (>170 IU/liter; P = 0.016), and platelet counts (>450 × 10⁹/liter; P = 0.015), above the median for the patient group, were associated with a poor prognosis as reported previously (4, 35). An unresected primary tumor (P = 0.08), the presence of bone metastases (P = 1) or extrapulmonary metastases (P = 1), or a hemoglobin <10 g/dl (P = 0.366) lacked prognostic significance.

Toxicity and Dose Modifications. Toxicity is summarized in Table 2. Grade 3 toxicity was rare and included neutropenia (three patients), nausea and vomiting (one patient), and fatigue and diarrhea (one patient each). One patient had a delayed hypersensitivity reaction to razoxane characterized by the development of an urticarial rash and arthritis, whereas another developed cerebellar symptoms after three cycles of treatment. In both cases, the symptoms resolved on withdrawal of the drug. Treatment was delayed in 10 patients because of neutropenia (8 patients), continuing vomiting (1 patient), and mucositis (1 patient). The dose was reduced by 30% in four patients on one occasion only, because of grade 3 or persistent grade 2 neutropenia.

Surrogate Blood and Urine Markers of Angiogenesis. The results for the plasma/serum and urinary surrogate markers of angiogenesis are summarized in Fig. 1 and Table 3. Serum VEGF levels were analyzed in 35 patients before treatment. Elevated levels above the normal range were seen in 12 (34%). Pretreatment levels were significantly higher in patients who subsequently developed PD compared with those with StD (P = 0.042). No change in overall values, either for the whole group or for those with StD or PD, was seen during the first cycle of treatment. Patients with pretreatment serum levels above the median for the group had a worse prognosis (P = 0.023). A significant correlation was seen between pretreatment serum VEGF levels and platelet counts (r = 0.69; correlation coefficient, 0.0001; correlation coefficient, r, 0.69). Urinary VEGF levels were analyzed in 34 patients and were elevated in 10 prior to treatment (30%). A significant rise in urinary VEGF levels was seen in patients who developed PD (P = 0.006) but not in those with StD (P = 0.09).

Serum bFGF was elevated in 15 (43%) of 35 patients analyzed prior to razoxane. Although there was no significant difference between bFGF levels in StD versus PD patients pretreatment, a trend toward a reduction in levels in StD patients and an increase in levels in PD patients resulted in a significant difference in the values between the groups after treatment (P = 0.006). Urinary bFGF levels were elevated in only 1 of 34 patients pretreatment. Pretreatment levels were higher in patients who developed PD as compared with those with StD, although this difference was not maintained after treatment.

uPAsr levels were elevated in patients (mean ± SD, 1.95 ± 1.4 μg/liter) compared with a normal control population (0.72 μg/liter; P = 0.0001) with 12 (37%) of 32 having elevated levels pretreatment. There was a trend for levels to increase with razoxane therapy in the PD group and to fall in the StD group. Levels were significantly higher in the PD as compared with the StD group before (P = 0.04) and after (P = 0.028) 1 cycle of treatment.

Pretreatment serum VCAM-1 levels were significantly higher in patients compared with our control population (P = 0.0001). Levels were significantly higher in patients with PD (P = 0.039) being higher than those in patients with StD after treatment (P = 0.031). Although no significant differences were found in E-selectin levels between patients and the normal range (P = 0.22) or in the StD and PD groups before or after treatment, E-selectin levels above the median were associated with a worse outcome (P = 0.022). Pretreatment vWF levels were elevated in 6 (17%) of 35 patients. No significant difference was seen between vWF levels in patients and our controls (P = 0.112). Although no significant change in levels was seen on treatment, values in patients with PD were significantly higher than those with StD, both before (P = 0.026) and after (P = 0.024) one cycle of razoxane.

DISCUSSION

Targeting of angiogenesis represents a new approach to the management of solid tumors, particularly those that are poorly responsive to chemotherapy. In this study, we evaluated razoxane, an antiangiogenic topoisomerase II inhibitor (7–17) in the treatment of RCC, a highly angiogenic tumor (19) resistant to standard cytotoxic regimens (1). We observed disease stabilization in 11 (29%) of 38 patients evaluable for response with a median survival of 399 days for this group. All of these patients were either newly diagnosed or had documented PD within the 6 months prior to razoxane. On the basis of known risk-factor stratification, there were no significant differences between the patients with StD as compared with those with PD, whose median survival was 127 days (3). This suggests that in this study, razoxane may be inhibiting tumor growth in patients with StD but not causing tumor regression. This must be treated with caution based on the sample size and the highly variable growth rates seen in RCC.

This raises the issue as to how we should assess the effectiveness of antiangiogenic therapy. The drugs being considered for this role are, in general, cytostatic rather than cytotoxic to endothelial cells, inhibiting their proliferation and/or elongation (36). It seems reasonable to suppose that tumor...
Surrogate blood and urine markers of angiogenesis. Pre- and posttreatment levels of serum (A) and urinary (B) VEGF, serum (C) and urinary (D) bFGF, plasma uPAsr (E), serum VCAM-1 (F), serum E-selectin (G) and serum vWF (H), were measured in patients with RCC as described in “Patients and Methods.” Individual patient values (Y axis) are shown divided into StD and PD groups before and after razoxane (X axis). Statistical analyses for the changes in each marker are indicated, and these results are summarized in Table 3.
Razoxane in RCC

Phase II study of low-dose continuous thalidomide had RCC; of antiangiogenic drugs. Eighteen of 66 patients recruited to a RCC is similar to those seen in recent studies of other potential tumor response evaluation. If StD is considered a valid end prolongation of survival, would be realistic end points in terms stabilization or reduced tumor-growth kinetics, with a resultant prolongation of survival, would be realistic end points in terms of tumor response evaluation. If StD is considered a valid end point, then the response rate with razoxane in the treatment of RCC is similar to those seen in recent studies of other potential antiangiogenic drugs. Eighteen of 66 patients recruited to a Phase II study of low-dose continuous thalidomide had RCC; of these, 3 had a PR and another 3 had StD, giving a 33% response rate (CR + PR + StD; Ref. 37). Likewise in a Phase I dose-escalating study of IL-12 (38) and a Phase II study of TNP-470 (39), 8 (29%) of 28 and 7 (21%) of 33 patients responded to treatment.

These results are comparable with those from three randomized trials with IL-2 and IFN. In the study comparing IL-2, IFN-α, and IL-2 plus IFN-α, the event-free survival of all of the 3 arms was ≥10 weeks, whereas the response rate (CR + PR + StD) in evaluable patients was 33, 40, and 48%, respectively, at 10 weeks and 12, 19, and 20% at 25 weeks (5). In the trial comparing IFN-α and medroxyprogesterone acetate the progression-free survival of each group was 4 and 3 months, respectively; however, the overall response rates were difficult to determine (6). Finally, in the study comparing IFN-γ with placebo, TTP for both arms was 1.9 months, with a best response at 8 weeks of 37 and 36%, respectively (40). By comparison, 47, 29, and 13% of the patients in our study had StD at 8, 16, and 24 weeks, respectively, whereas TTP was 12 weeks. These findings suggest that StD is an important end point for assessment of antiangiogenic agents and that, in this Phase II study, razoxane had activity that was comparable with more established treatments. Additional randomized trials with antiangiogenic agents, including razoxane, are warranted.

The evaluation of surrogate serum, plasma, and urinary markers of endothelial function or angiogenesis may have a role to play in assessing the efficacy of novel antiangiogenic drugs (36). Pretreatment serum VEGF and E-selectin levels above the median value were directly associated with a worse prognosis, as previously reported in other studies (41, 42). A significant rise in urinary VEGF levels was seen in patients with PD (Table 3), but this rise was not significant when compared with the StD patients (Fig. 1B). No correlation was seen between serum and urinary VEGF levels, or between urinary levels and the presence of the primary tumor. This is in keeping with previous work in bladder cancer (32).

Plasma uPAsr levels were higher in patients with RCC than in a normal control population. uPAsr was significantly elevated in patients who developed PD on razoxane compared with those with StD both before treatment and after 8 weeks and, therefore, is not serially predictive of which patients will respond. Similarly vWF, urine bFGF, and serum VEGF were significantly different pretreatment in patients who developed PD compared with those with StD but this did not alter after treatment and, thus, cannot be used as a marker of response to razoxane therapy.

However, serum VCAM-1 and serum bFGF showed no difference between patient groups before treatment; but, after therapy with razoxane, there was a significant difference between patients with PD compared with those with StD. This was attributable to a rise in VCAM-1 in patients with PD and a small fall in patients with StD (Fig. 1F) and to a rise in bFGF in patients with PD (Fig. 1C). These results underscore those of previous studies showing VCAM-1 to be elevated in patients with solid tumors, including breast, renal, colorectal, gastric, and ovarian cancer and malignant melanoma, and its association with disease progression and a poor prognosis (28, 35, 43–45). The significant rise in serum VCAM-1, urinary VEGF, and serum bFGF levels in patients with PD indicates that these angiogenic growth factors should be further evaluated as potential surrogate markers of tumor response in RCC and in malignant diseases in general. Although these results have to be interpreted with caution, markers of endothelial cell proliferation and protease activation may be useful in suggesting possible molecular mechanisms of action for razoxane on endothelial cells. This requires further investigation in the preclinical setting. Further clinical evaluation of the antiangiogenic effect of razoxane could be provided by looking at tissue staining for surrogate markers and by using noninvasive radiology (e.g., positron emission tomography scans).

The prognostic significance of the absolute neutrophil count, platelet counts, and lactate dehydrogenase levels seen in this study is well established for malignant diseases including RCC (4, 46). A significant correlation was found between pretreatment platelet counts and serum VEGF levels. Platelets

<table>
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<tr>
<th>Marker</th>
<th>Elevated levels Pre vs. Post</th>
<th>Pre PD &gt; StD (Mann-Whitney, P)</th>
<th>Post PD &gt; StD (Mann-Whitney, P)</th>
<th>StD Pre vs. Post (Wilcoxon rank-sum, P)</th>
<th>PD Pre vs. Post (Wilcoxon rank-sum, P)</th>
<th>Survival, ≥ median vs. median (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum VEGF</td>
<td>12/35 (34%)</td>
<td>0.042b</td>
<td>0.088</td>
<td>0.464</td>
<td>0.738</td>
<td>0.023b</td>
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<tr>
<td>Urinary VEGF</td>
<td>10/34 (30%)</td>
<td>0.233</td>
<td>0.313</td>
<td>0.09</td>
<td>0.006b</td>
<td>0.790</td>
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<tr>
<td>Serum bFGF</td>
<td>15/35 (43%)</td>
<td>0.814</td>
<td>0.0057b</td>
<td>0.114</td>
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<td>Urinary bFGF</td>
<td>1/34 (3%)</td>
<td>0.035c</td>
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<td>12/32 (37%)</td>
<td>0.040b</td>
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<td>0.066</td>
<td>0.211</td>
<td>0.288</td>
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<tr>
<td>Serum VCAM-1</td>
<td>7/35 (20%)</td>
<td>0.170</td>
<td>0.031b</td>
<td>0.906</td>
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<td>0.333</td>
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<tr>
<td>Serum E-selectin</td>
<td>1/19 (5%)</td>
<td>0.729</td>
<td>0.729</td>
<td>0.223</td>
<td>0.624</td>
<td>0.022b</td>
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<tr>
<td>Serum vWF</td>
<td>6/35 (17%)</td>
<td>0.026e</td>
<td>0.024e</td>
<td>0.406</td>
<td>0.948</td>
<td>0.442</td>
</tr>
</tbody>
</table>

*P* Pre, pretreatment; Post, posttreatment; pts, patients.

* Significant difference.

* Significant increase.
transport and, after activation, release VEGF. Intratumoral platelet activation may result in the release of high local concentrations of the angiogenic growth factor and induce tumor angiogenesis. Therefore, serum VEGF may be an important marker for VEGF that could be delivered to tumors by platelets (47). In our study, elevated serum VEGF was associated with a worse prognosis. VEGF can induce the release of vWF from endothelial cells (48). We found no correlation between VEGF and vWF levels in our patients, or between vWF and platelet counts.

Razoxane, at the dose schedule used in this study, was well tolerated. Grade 3 toxicities were rare and included nausea and vomiting in one patient, diarrhea in one, neutropenia in three, and fatigue in another. As such, the side-effect profile was in keeping with that expected for razoxane (12) apart from two patients: one who developed cerebellar symptoms and the other, a delayed hypersensitivity reaction (Table 2). Some concern has been expressed that prolonged treatment with razoxane may predispose to acute promyelocytic leukemia (APL) and acute myeloid leukemia. Whereas in many of these cases patients received a variety of drugs prior to razoxane therapy, APL has developed in chemotherapy-naïve patients (13, 49). Although this does not necessarily imply cause and effect, it may be important if razoxane is used for prolonged therapy.

Antiangiogenic agents, including razoxane, have been shown to enhance the antitumor activity of cytotoxic drugs (18). The combination of two or more antiangiogenic agents with different modes of action may further enhance this effect, e.g., TNP-470, an inhibitor of endothelial cell proliferation, and minocycline, an inhibitor of gelatinase activity, combined with cyclophosphamide (50). The combination of razoxane with agents with antiangiogenic/immunomodulatory activity such as the cytokines—IFN-α, IL-2, and IL-12—and thalidomide would appear to be particularly attractive in the setting of RCC.

In conclusion, razoxane is a well-tolerated, antiangiogenic topoisomerase II inhibitor that may have a role to play in the management of solid, cytotoxic chemotherapy-resistant, tumors. Serum VCAM-1, urinary VEGF, and serum bFGF were identified as the most promising potential markers of response to antiangiogenic therapy. Because of the favorable toxicity profile, razoxane should be considered for combination studies with other antiangiogenic, biological, and cytotoxic agents in the treatment of RCC, both in the experimental setting and in clinical studies. Surrogate markers to assess the antiangiogenic activity of any drug in the treatment of solid tumors require further evaluation.

REFERENCES


A Phase II Study of Razoxane, an Antiangiogenic Topoisomerase II Inhibitor, in Renal Cell Cancer with Assessment of Potential Surrogate Markers of Angiogenesis
