Abstract

Purpose: To determine the biological modulatory dose of SU5416, we employed a novel trial design, where “dose de-escalation” was based on demonstrable biological changes observed at the maximum tolerated dose. If such an effect was shown, dose de-escalation to a predefined dose level would occur to determine if the lower dose exhibited the same amount of pharmacodynamic effect as the higher dose.

Experimental Design: Ten patients with advanced solid tumors were enrolled at each dose level. One of the following pharmacodynamic effects was considered significant: (a) a 35% decrease in microvessel density in sequential tumor biopsies and (b) a 35% decrease in blood flow within tumors as assessed by dynamic contrast-enhanced magnetic resonance imaging. In addition, soluble E-selectin, soluble intercellular adhesion molecule, soluble vascular cell adhesion molecule, and plasma vascular endothelial growth factor were measured sequentially.

Results: Nineteen patients were enrolled. Sequential tumor biopsies in all evaluable patients showed an increase in microvessel density. Only one patient met the intended pharmacodynamic end point of >35% reduction in blood flow. There was a significant increase in both soluble E-selectin and soluble intercellular adhesion molecule levels pretreatment versus levels at the time of removal of patients from study (P = 0.04 and P = 0.0007, respectively). Levels of serum fibrinogen rose with therapy. There was a trend toward increase in plasma vascular endothelial growth factor levels.

Conclusion: SU5416 does not result in decreased blood flow in tumors or a decrease in microvessel density. This corresponds to the lack of clinical activity seen with this agent. Our clinical trial design termed dose de-escalation is a novel approach to determine the in vivo biological effects of targeted therapies in cancer patients.

The growth of tumors and subsequent development of metastases is dependent on the formation of new blood vessels or angiogenesis. The process of angiogenesis is tightly regulated by a series of proangiogenic and antiangiogenic factors. One of the most important proangiogenic factors is vascular endothelial growth factor (VEGF). VEGF overproduction has been identified as a major factor underlying pathologic angiogenesis in cancer (1). VEGF binds to high-affinity endothelial receptors. VEGF receptor tyrosine kinases are essential components of the endothelial cell signal transduction pathway. VEGF receptor-2/Flk-1/KDR is the target for the 3-substituted indolinone compound SU5416, where it binds to the ATP-binding pocket of the intracytoplasmic catalytic domain, inhibiting tyrosine kinase phosphorylation and downstream signaling (2).

SU5416 inhibits tumor growth in vivo in a dose-dependent manner, whereas it has no effect in vitro (3). SU5416 inhibits VEGF-induced mitogenesis of endothelial cells with an IC_{50} of 0.04 μmol/L. It has shown significant activity in tumor xenografts in nude mice (3, 4). SU5416 inhibited tumor metastases, microvessel formation, and cellular proliferation in a colon cancer xenograft model (4). In this experiment, an increase in tumor cell apoptotic rate was seen based on the terminal deoxynucleotidyl transferase–mediated nick-end labeling assay (4). Assessment of blood flow using an intravital multifluorescence videomicroscopy in a glioma xenograft model showed a significant reduction in blood flow by day 22 of treatment as well as a reduction in microvessel density (5).

Initial in vitro data suggested a biological half-life of >24 hours. A phase I dose escalation study has been completed with SU5416 on a twice-weekly schedule. Patients were treated with...
twice-weekly administration of SU5416 for 4 weeks followed by 2 weeks of rest (6, 7). Dose-limiting toxicity was achieved at 190 mg/m² and consisted of projectile vomiting and headache. The maximum tolerated dose (MTD) was determined to be 145 mg/m². There was evidence of clinical activity during this phase I trial.

The goal of the current study was to determine the biological effects of SU5416 at the tumor level during a phase I clinical trial. We employed a novel clinical trial design for this endeavor, where dose de-escalation was based on demonstrable biological changes observed at the MTD. The primary end point was to show a (a) 35% decline in tumor blood flow as determined by dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) or (b) 35% decrease in microvessel density on sequential tumor biopsies. The biological correlates and timing of anticipated drug effect seen in in vivo animal models were replicated in our phase I trial design. Indeed, Shaheen et al. (4) showed at least a 35% decrease in tumor microvessel density in a colon cancer xenograft model, and Vajkoczy et al. (5) showed at least a 35% decrease in blood flow and microvessel density by day 22.

Materials and Methods

Trial design

This phase I study has a novel design that we have referred to as “dose de-escalation design.” The rationale for this study design is based on the fact that the MTD of SU5416 had been previously determined. The trial was designed to show a pharmacodynamic effect at the MTD. If such an effect was shown, “dose de-escalation” to a predefined dose level would occur to determine if the lower dose exhibited the same amount of pharmacodynamic effect as the higher dose. The predetermined pharmacodynamic effects were based on preclinical data of SU5416. One of the following pharmacodynamic effects were considered significant for trial design: (a) a 35% decrease in microvessel density in sequential tumor biopsies and (b) a 35% decrease in blood flow within tumor as assessed by DCE-MRI. Ten patients were enrolled at the highest dose level (predetermined MTD). If 5 of 10 patients were seen to have the intended pharmacodynamic effect, dose de-escalation would continue until a decrease in pharmacodynamic effect was seen. The dose above the level in which a drop in pharmacodynamic effect is seen would be considered the biological modulatory dose (BMD) of SU5416.

Ten evaluable patients were to be enrolled at the first dose level (MTD) at 145 mg/m² given twice weekly. We required that at least five patients show the intended activity (i.e., that there be at least five biological responses) at the first dose level (the MTD) to continue this trial. If the intended effect is shown (i.e., at least five biological responses at the MTD), the trial would proceed to compare the MTD and the de-escalated dose (110 mg/m²) in terms of biological activity. The strategy is summarized in Table 1. The rationale is that if the lower dose seems similar in biological response rate to the MTD, it qualifies as a reasonable candidate for the BMD. Operationally, this means that the number of biological responses at the lower dose must not be less than the number of MTD responses minus 2 (e.g., if there are 9 of 10 responding at the MTD, there must be seven or more responding at the lower dose; at the minimum of five responses at the MTD, there must be three or more responding at the lower dose). Using a Fisher’s exact test (one sided), this rule represents a P > 0.20, showing lack of evidence that the two dose levels differed in terms of biological activity.

In the dose de-escalation trial, if the number of patients with the intended biological effect at the lower dose level is equal to (or exceeds) the number seen at the MTD, we could consider studying an even lower dose (a further de-escalation) in an additional group of 10 patients. Alternatively, if at the initial de-escalated dose level, biological activity is seen, but the number of biological responders is low (i.e., not within two of the number at the MTD), we would then dose escalate to an intermediate dose between the initial de-escalated dose and the MTD and study 10 additional patients at this intermediate dose. Of importance is that only two to three dose levels would be evaluated for such a design; therefore, the total number of patients would be 20 to 30.

Eligibility criteria

Patients with solid tumors not amenable to curative or otherwise effective surgical, radiation therapy, or chemotherapy treatment programs were eligible. Other eligibility included Eastern Cooperative Oncology Group performance status of 0, 1 or 2; life expectancy of at least 12 weeks; at least 4 weeks must have elapsed since prior large-field radiation therapy, and patients must have been off previous anticancer therapy for at least 3 weeks and recovered from all treatment-related toxicity; adequate hematologic variables (WBC >3,500/L, platelets >100,000/L, hemoglobin >9.0 g/dL); normal coagulation screen; and adequate renal and hepatic variables (creatinine <1.5 mg/dL, bilirubin <1.6 mg/dL, aspartate aminotransferase and alanine aminotransferase more than twice the normal). Patients must have at least two distinct lesions of metastatic or primary tumor >1 cm, which will permit biopsy of a single lesion for the laboratory correlative studies and another lesion for DCE-MRI tumor perfusion studies. It was important that two distinct lesions were identified so that correlative computed tomography/MRI–guided visceral tumor biopsy would not interfere with the correlative DCE-MRI tumor perfusion (blood flow) studies. All patients gave written informed consent.

<table>
<thead>
<tr>
<th>Table 1. Strategy for dose de-escalation trials to determine the BMD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. cases with the intended correlative laboratory change (10 patients per dose level)</strong></td>
</tr>
<tr>
<td><strong>First dose level (MTD)</strong></td>
</tr>
<tr>
<td>&lt;5</td>
</tr>
<tr>
<td>5-10</td>
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<td></td>
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</table>
Drug administration

SU5416 was given over 1 hour via a central venous access device. Treatment was given, without interruption, twice a week on Mondays and Thursdays. Cycles were repeated every 4 weeks. Premedications included diphenhydramine (25-50 mg) and ranitidine (50 mg i.v.). Dexamethasone was given at a dose of 10 mg i.v. for one dose before each dose of SU5416 or 10 mg orally 12 and 6 hours before each dose of SU5416.

Cell adhesion molecules and serum fibrinogen

Plasma samples collected from patients before SU5416 and at scheduled intervals according to treatment schema. Collisions correlated to day 0 (pretreatment), day 1 (cycle/day 1, C1D1), day 15 (C1D15), day 29 (C2D1), day 58 (C3D1), and day 140 (C5D1). Samples were analyzed for levels of soluble intercellular adhesion molecule-1 (ICAM-1), soluble vascular cell adhesion molecule-1 (VCAM-1), and soluble E-selectin using commercially available ELISA kits (R&D Systems, Minneapolis, MN). Samples and normals were assayed in triplicate. The limits of detection for each assay were 0.35 ng/mL for soluble ICAM-1, 2.0 ng/mL for soluble VCAM-1, and 0.1 ng/mL for soluble E-selectin. The mean intra-assay and interassay variability for the kits are 4.8% and 10.1%, respectively. Thirteen normal plasma samples considered as negative controls were collected and tested along with patient samples. Analysis of adhesion molecule data was done using GraphPad Prism version 3.00 statistical software (GraphPad Software, San Diego, CA). Two-tailed, paired t tests were done on intragroup comparison; P < 0.05 were considered significant.

Dynamic contrast-enhanced magnetic resonance imaging assessment of blood flow

Data acquisition. Patients were studied using a 1.5-T scanner (Siemens Magnetom Vision). Perfusion studies were obtained with a single slice Flash sequence (repetition time = 10, echo time = 4, flip angle = 30 degree, slice thickness = 10 mm, matrix = 128 × 256, field of view = 16 × 25 to 35 × 35 cm) repeated 128 times, once every 2.6 seconds. The first 10 images were obtained without contrast enhancement, after which an 10-second bolus injection of gadolinium-diethylenetriaminepentaacetic acid contrast agent (Magnevist, Berlex Laboratories, Montville, NJ) was administered i.v. Gadolinium is a paramagnetic agent that, proportional to its concentration, decreases both the longitudinal magnetization recovery time (T1) and the transverse magnetization decay time (T2) in tissue. In MRI, a shorter T1 increases signal intensity, whereas a shorter T2 decreases it. However, for the FLASH sequence described above, the signal decrease caused by T2* shortening is negligible (<2% signal change over the range of obtainable gadolinium-diethylenetriaminepentaacetic acid tissue concentrations at the study dosage). It can also be shown that over the same range of tissue concentration, the relative signal increase (postcontrast/baseline) has an essentially linear dependence on tissue signal intensity. This allows us to interpret proportional change in signal intensity over time as equivalent to proportional change in contrast agent tissue concentration. DCE-MRI was done twice on each patient: once to obtain a pretreatment baseline and once after two cycles of therapy (before cycle 3). The slice position and orientation between days within each patient was carefully matched to obtain data from the same region of interest in the tumor.

Post-processing and analysis. Image processing was done offline using a custom software package that permitted a trained rater to review all 128 images in a study and to interactively identify the tumor region of interest. Mean tumor signal intensity versus time curve for each subject was generated. These curves were then converted to proportional enhancement versus time curves by dividing by the precontrast mean signal intensity. Four measures were obtained from each set of curves. The uptake of contrast into the extracellular extravasation space from blood plasma is typically described by the two-compartment Kety equation (8). Using this model, mathematical simulations were done to assess the sensitivity of each of the variables to blood flow, variations in the arterial plasma concentration input function, and the extracellular extravasation space volume fraction. Three of the variables, gradient peak (Gpeak), enhancement (E), and time until gradient drops to 10% of Gpeak (tg10), were sensitive to flow; however, only Gpeak and E were approximately linear over the range of expected variation, and only Gpeak had the lowest variation with respect to changes in the extracellular extravasation space volume fraction (extracellular extravasation space volume — total tissue volume). All of the measures were sensitive to differences in the time-to-peak of the plasma input function, but only time-to-gradient peak (tgp) was essentially insensitive to changes in flow and therefore served as an index of the consistency of our bolus injections. Because relative signal change with respect to tissue concentration was different for each patient because of T1 differences between tumor types, pretreatment and posttreatment values of each variable were compared across patients nonparametrically using the Wilcoxon signed rank test, with two-tailed evaluation of significance levels.

Sequential tumor biopsies and microvessel density

Sequential tumor biopsies, using a core needle biopsy, were obtained before starting therapy and before cycle 3. Tumor biopsies were fixed in 10% buffered formalin and paraffin embedded. Three-micrometer tissue sections on a slide are deparaffinized by treatment with xylene followed by a 3-minute wash with absolute, 95%, and 70% ethanol. Sections were then treated by high-heat epitope enhancement for 10 minutes (70% of 850-W heat source) before antibody identification, to allow for antibody penetration and enhance antigen identification. Slides were then rinsed twice in a buffered saline (TBS) and then incubated for 10 minutes with 10% normal goat serum in 0.05 mol/L TBS to eliminate nonspecific background staining of the secondary antibody. Primary antibodies (CD34 and factor VIII) at the appropriate dilution were then applied to the sections, and the slides were placed in the humidity chamber overnight at room temperature. Slides were then rinsed, the biotinylated secondary antibody (Kirkegaard and Perry Laboratories, Gaithersburg, MD) was applied for 30 minutes, rinsed again, and incubated with horseradish peroxidase–tagged streptavidin antibody (Kirkegaard & Perry Laboratories) for 30 minutes. The samples were rinsed and incubated with freshly prepared 3,3'-diaminobenzidine (Sigma Chemical, St. Louis, MO) at a concentration of 0.75 mg/mL in Tris buffer containing 5 mL of 30% H2O2. Slides are observed for a brown reaction product and stopped by immersion in water. The slides are counterstained with hematoxylin, cleared, and mounted for microscopy. Immunoreactivity in the tissue biopsies was identified in small and medium-sized vessels under low power to identify areas with microvessel density. Angiogenic “hotspots.” Microvessel density per area (mean microvascular density/mm2) SD of three randomly selected hotspots were then determined under higher magnification (×400, 0.01885 mm2/field) as previously described (9–11). A single microvessel was considered as an endothelial cell or cell cluster with brown reaction product that is distinct from adjacent microvessels, tumor cells, or other tissue elements. Slides were independently read by three observers, and microvessel density count was averaged.

Results

Patients and clinical experience. A total of 19 patients were enrolled on this study. Patient characteristics include median age of 55 years (range, 33-76 years), 11 male and 8 female patients, and a median of two prior therapies. Pathologies enrolled include eight colon cancers, four breast cancers, three soft tissue sarcomas, and one of each of the following pathologies: esophageal cancer, adrenal carcinoma, carcinoid, and renal cell carcinoma. A total of 67 cycles were administered (range, 1-24) all at the initial 145 mg/m2 dose level. No
objective responses were seen. Four patients had stable disease (4 months, soft tissue sarcoma; 4 months, colon cancer; 6 months, soft tissue sarcoma; 24 months, renal cell). Toxicities are similar to other studies of this agent. Grade 1 and 2 headaches occurred during 40% of the cycles. Grade 1 and 2 elevations in transaminases occurred in 36% of the courses. Given the regular use of dexamethasone as premedication with this agent, frequent increases in blood glucose were seen. Grade 1 and 2 nausea and vomiting occurred in 26% and 25% of the courses, respectively. Grade 3 nausea was seen in 3% of the courses. Two cases of deep venous thrombosis were diagnosed during this study.

Microvessel density. From the 19 patients enrolled on this study, only five patients had tumor tissue evaluable in both the pretreatment biopsy and the biopsy before cycle 3. Sequential tumor biopsies in all five evaluable patients showed an increase in microvessel density over the 2-month period (Table 2). Figure 1 is a patient with recurrent breast cancer showing an increase in microvessel density count on pretreatment and posttreatment biopsy.

Dynamic contrast-enhanced magnetic resonance imaging assessment of tumor blood flow. A total of 11 of the 19 patients studied had paired DCE-MRI assessments of tumor blood flow suitable for analysis. A summary of the pretreatment and posttreatment $K_{ep}$ according to the Brix model is shown in Table 3. Only a single patient met the intended pharmacodynamic end point of >33% reduction in blood flow. $f_{gp}$ was not statistically significantly different between pretreatment and posttreatment session ($P = 0.3$).

Cell adhesion molecules. No differences were seen between pretreatment and C1D15 soluble E-selectin, soluble ICAM, or soluble VCAM levels. However, paired t test showed a significant increase in soluble E-selectin levels pretreatment ($56 \pm 22$) versus soluble E-selectin levels at the time of removal of patients from study ($63 \pm 19$, $P = 0.04$; Fig. 2). Similarly, an increase was seen in soluble ICAM levels between pretreatment levels ($343 \pm 146$) and end of study values ($432 \pm 150$, $P = 0.0007$). No such difference was seen for soluble VCAM levels ($514 \pm 259$ versus $486 \pm 207$, $P = 0.5$).

Vascular endothelial growth factor and basic fibroblast growth factor. There was a trend toward an increase in plasma VEGF levels from pretreatment ($41 \pm 61$) to C1D15 ($42 \pm 58$) to C2D1 (before SU5416 infusion, $57 \pm 86$). Comparison between pretreatment and C2D1 levels not did quite reach statistical significance ($P = 0.07$). Unlike VEGF levels, basic fibroblast growth factor remained relatively stable over the first cycle of treatment. Pretreatment levels were $2.5 \pm 1.4$ versus C1D15 $2.7 \pm 1.5$ versus C2D1 $2.8 \pm 1.6$.

Fibrinogen. Levels of serum fibrinogen rose with therapy (Fig. 3). Mean pretreatment fibrinogen was $487 \pm 109$ mg/dL.

Table 2. Changes in microvessel density in tumor tissue pretreatment and after two cycles of treatment with SU5416

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Biopsy site</th>
<th>Microvessel density, ×20</th>
<th>Microvessel density, ×40</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Breast cancer</td>
<td>Breast</td>
<td>Pretreatment ≥3</td>
<td>Posttreatment ≥3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Breast cancer</td>
<td>Breast</td>
<td>Pretreatment ≥1</td>
<td>Posttreatment ≥2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Soft tissue sarcoma</td>
<td>Upper extremity mass</td>
<td>Pretreatment ≥1</td>
<td>Posttreatment ≥2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Leiomyosarcoma</td>
<td>Liver</td>
<td>Pretreatment 0</td>
<td>Posttreatment ≥1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Colon</td>
<td>Abdominal mass</td>
<td>Pretreatment ≥1</td>
<td>Posttreatment ≥3</td>
</tr>
</tbody>
</table>

Fig. 1. Patient with breast cancer with pretreatment and pre-cycle 3 biopsy of breast mass showing increase in microvessel density.
This rose to 669 ± 160 mg/dL by C1D15. This difference was very highly significant \( P < 0.0001 \) (paired analysis). However, the levels then remained stable with day C1D28 levels at 657 ± 195 mg/dL. There was no difference between C1D15 and C1D28 levels, thus suggesting that the increase in fibrinogen was related to investigational drug rather than disease progression.

**Discussion**

This novel clinical trial design that we describe for the first time is based on the assumption that the traditional toxicity-based MTD has already been determined. In our dose de-escalation trial, the first dose level is actually the MTD. Subsequent dose levels are lower than the MTD. The goal is to identify the BMD. The number of patients per dose level is inevitably greater than a traditional phase I trial. We suggest that at least 10 patients should be enrolled per dose level. This is based on the assumption that different tumor types would be enrolled. The end point to be used for biological activity ("biological response") needs to be clearly defined based on preclinical models. For most biological end points, it would seem reasonable to require that at least five patients show the intended activity (i.e., that there be at least five biological responses) at the first dose level (the MTD) to continue the trial. This will allow the investigators to validate their laboratory correlative assays before continuing the trial. If the correlative laboratory assay does not show the anticipated change, one may conclude that either there are problems with the assay, the investigational agents presumed mechanism of action is incorrect, or the dose is inadequate (even at the MTD) to achieve the desired effect (i.e., missing the target). If the intended effect is shown (i.e., at least five biological responses at the MTD), the trial would proceed to compare the MTD and the de-escalated dose in terms of biological activity. The strategy is outlined in Table 2; admittedly, this is an ad hoc procedure, but one that seems to present a practical approach. The rationale is that if the lower dose seems similar in biological response rate to the MTD, it qualifies as a reasonable candidate for the BMD. Operationally, this means that the number of biological responses at the lower dose must not be less than the number of MTD responses minus 2 (e.g., if there are 9 of 10 responding at the MTD, there must be seven or more responding at the lower dose; at the minimum of five responses at the MTD, there must be three or more responding at the lower dose). Using a Fisher’s exact test (one sided), this rule represents a \( P > 0.20 \), showing lack of evidence that the two dose levels differed in terms of biological activity. With the small sample size, the confidence limits on the difference in biological activity will be relatively wide. Therefore, once this BMD is determined, consideration should be given to a randomized phase II trial comparing the BMD to MTD to determine if they differ in terms of clinical activity. If they do not differ, the recommended dose for further studies would be the BMD. In the dose de-escalation trial, if the number of patients with the intended biological effect at the lower dose level is equal to (or exceeds) the number seen at the MTD, one could consider studying an even lower dose (a further de-escalation) in an additional group of 10 patients. These further results would determine which de-escalated dose is advanced to a phase II comparison with the MTD. Alternatively, if at the initial de-escalated dose level, biological activity is seen, but the number of biological responders is low (i.e., not within two of

**Table 3.** Eleven evaluable patients for pretreatment and posttreatment DCE-MRI flow study with changes in \( K_{ep} \) (min \(^{-1}\)) according to Brix model

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pretreatment ( K_{ep} ) (min (^{-1}))</th>
<th>After two cycles ( K_{ep} ) (min (^{-1}))</th>
<th>% Change</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>1.58</td>
<td>0.889</td>
<td>44</td>
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<tr>
<td>2</td>
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<td>3</td>
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<td>1.29</td>
<td>1.13</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
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</tr>
<tr>
<td>6</td>
<td>0.79</td>
<td>0.91</td>
<td>15</td>
</tr>
<tr>
<td>7</td>
<td>0.89</td>
<td>1.09</td>
<td>22</td>
</tr>
<tr>
<td>8</td>
<td>0.90</td>
<td>1.25</td>
<td>39</td>
</tr>
<tr>
<td>9</td>
<td>1.09</td>
<td>2.18</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>0.945</td>
<td>1.973</td>
<td>109</td>
</tr>
<tr>
<td>11</td>
<td>2.21</td>
<td>5.35</td>
<td>143</td>
</tr>
<tr>
<td>Means</td>
<td>1.39</td>
<td>1.75</td>
<td>26</td>
</tr>
</tbody>
</table>

**NOTE:** Patients sorted based on % change.

Fig. 2. Measurements of E-selectin, VCAM, and ICAM in 19 patients. Pretreatment values and values at the time of study removal.
endothelial cells will be associated with the release of endothelial cell specific markers in plasma. The rationale for this hypothesis is supported by the detection of elevated levels of certain of these markers in thrombotic thrombocytopenic purpura (18, 19), a disorder characterized by endothelial cell apoptosis, as well as in hypercholesterolemia (20), another disorder characterized by endothelial cell dysfunction. Similarly, elevated levels of the endothelial cell–specific adhesion molecules E-selectin and VCAM-1 reflect early endothelial dysfunction in patients with glucose intolerance (21). Levels of VCAM-1 and E-selectin are also elevated in the plasma of patients receiving the antiangiogenic, vascular targeting agent CM-101 (22). Our trial showed no early change in any of these markers by C1D15. Comparison of pretreatment values to values obtained at the time patient was taken off study, however, showed a significant increase in E-selectin and soluble ICAM but not in soluble VCAM. It is, however, unclear if this reflects drug effect or just progressive disease. Another study of chemotherapy plus SU5416 showed a similar increase in soluble E-selectin (23).

Although no change in basic fibroblast growth factor was seen, an increase in plasma VEGF level was observed. Although levels of VEGF have correlated with advanced stage disease, poor prognosis, and chemotherapy refractoriness in many cancers (24), no data exists on changes of this marker during VEGF-directed therapies. This has not been evaluated with bevacizumab, a humanized monoclonal antibody targeting VEGF, as the use of antibodies makes plasma measurements useless. Therefore, our trial also constitutes the first trial demonstrating an increase in plasma VEGF level with VEGF receptor–targeted therapy and may constitute a sign of in vivo biological activity. When SU5416 has been combined with IFN-α in renal cell carcinoma, a decrease in plasma VEGF levels has been observed (25).

An increase in thrombotic rates has been reported in trials of SU5416 when combined with chemotherapy (26). In this study, the authors hypothesized that endothelial cells deprived of VEGF after exposure to SU5416 become activated and more susceptible to damage with chemotherapy. Our trial shows a significant increase in E-selectin and soluble ICAM—23]. Our trial showed no early change in any of these markers by C1D15. Comparison of pretreatment values to values obtained at the time patient was taken off study, however, showed a significant increase in E-selectin and soluble ICAM but not in soluble VCAM. It is, however, unclear if this reflects drug effect or just progressive disease. Another study of chemotherapy plus SU5416 showed a similar increase in soluble E-selectin (23).

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Although our hypothesis-driven study design dose de-escalation strategy to isolate a biological modulatory dose did not come to fruition, we believe this concept is valid. Perhaps the simplest explanation is that the investigational agent in this study is not clinically active. In addition, as experience with antiangiogenic agents in the clinic has evolved, the utility of biomarkers continues to be explored.

References

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**Fig. 3.** Increase in serum fibrinogen with SU5416 therapy.


Clinical Cancer Research

Novel Phase I Dose De-escalation Design Trial to Determine the Biological Modulatory Dose of the Antiangiogenic Agent SU5416

Afshin Dowlati, Kelly Robertson, Tomas Radivoyevitch, et al.


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