Serum C-Telopeptide Collagen Crosslinks and Plasma Soluble VEGFR2 as Pharmacodynamic Biomarkers in a Trial of Sequentially Administered Sunitinib and Cilengitide

Peter H. O’Donnell¹²³, Sanja Karovic¹, Theodore G. Karrison³⁴, Linda Janisch¹, Matthew R. Levine¹, Pamela J. Harris⁵, Blase N. Polite¹³, Ezra E.W. Cohen¹³, Gini F. Fleming¹²³, Mark J. Ratain¹²³, and Michael L. Maitland¹²³

Abstract

Purpose: Fit-for-purpose pharmacodynamic biomarkers could expedite development of combination antiangiogenic regimens. Plasma sVEGFR2 concentrations ([sVEGFR2]) mark sunitinib effects on the systemic vasculature. We hypothesized that cilengitide would impair microvasculature recovery during sunitinib withdrawal and could be detected through changes in [sVEGFR2].

Experimental Design: Advanced solid tumor patients received 50 mg sunitinib daily for 14 days. For the next 14 days, patients were randomized to arm A (cilengitide 2,000 mg administered intravenously twice weekly) or arm B (no treatment). The primary endpoint was change in [sVEGFR2] between days 14 and 28. A candidate pharmacodynamic biomarker of cilengitide inhibition of integrin αvβ3, serum c-telopeptide collagen crosslinks (CTx), was also measured.

Results: Of 21 patients, 14 (7 per arm) received all treatments without interruption and had all blood samples available for analysis. The mean change and SD of [sVEGFR2] for all sunitinib-treated patients was consistent with previous data. There was no significant difference in the mean change in [sVEGFR2] from days 14 to 28 between the arms [arm A: 2.8 ng/mL; 95% CI, 0.1–3.6 vs. arm B: 2.0 ng/mL; 95% CI, 0.7–3.4; P = 0.22, 2-sample t test]. Additional analyses suggested (i) prior bevacizumab therapy to be associated with unusually low baseline [sVEGFR2] and (ii) sunitinib causes measurable changes in CTx.

Conclusions: Cilengitide had no measurable effects on any circulating biomarkers. Sunitinib caused measurable declines in serum CTx. The properties of [sVEGFR2] and CTx observed in this study inform the design of future combination antiangiogenic therapy trials.

Introduction

Recently, combination cancer therapy regimens have improved the therapeutic index and outcomes for solid tumors (1–3). However, most efforts at developing new combination therapies with conventional strategies and clinical trial designs have not succeeded (4–7). Systematic, stepwise development on the basis of preliminary clinical studies and pharmacodynamic biomarker endpoints have been suggested to improve development of anticancer combination treatments (5, 7–9). In combination antiangiogenic therapy, there has been no reliable pharmacodynamic biomarker endpoint (10, 11), and few studies to evaluate dose and scheduling strategies in small sets of patients before performing larger phase II and III trials (4, 12).

The multi-kinase inhibitor sunitinib potently disrupts the VEGF signaling pathway (13). The drug is indicated as first-line therapy for renal cancer (14, 15) and pancreatic neuroendocrine tumors (16) and second-line therapy for gastrointestinal stromal tumors (17). The initial phase III trial dosing strategy entailed daily administration of 50 mg for 4 weeks, followed by a 2-week withdrawal. The 50-mg dose achieved a higher rate of tumor response than lower doses, but the 2-week withdrawal was necessary to make chronic administration tolerable (18).

Plasma concentration of soluble VEGF receptor-2 ([sVEGFR2]) is a pharmacodynamic biomarker for inhibitors of VEGF signaling. In patients with cancer treated with VEGFR2 kinase inhibitors, [sVEGFR2] routinely decreases on exposure to the drug and returns toward baseline measurements when treatment is discontinued, even for just 14 days (19–25). This has been recapitulated in mice where the decrease in [sVEGFR2] is dose-dependent and independent of the presence of tumors (26). Also in mice, the time course of these changes coincides with the regression and regrowth of microvessels and endothelial cells when a VEGFR2 inhibitor is administered and then withdrawn (27). The collective evidence suggests that during sunitinib “off” periods, surviving endothelial cells divide and migrate to restore microvessel integrity.
Translational Relevance

Drugs that target the transmembrane receptor protein VEGFR2 typically cause decreases in the circulating plasma protein sVEGFR2 in patients. This could make sVEGFR2 a useful tool in the future development of new combinations of angiogenesis inhibitor therapies. In this study, we demonstrated that measurements of sVEGFR2 performed as expected in a small group of patients when we treated them with the VEGFR2 inhibitor sunitinib. We also showed that adding a second drug with fewer side effects, cilengitide, had no effect on this marker. This implied that the second drug was not having its intended effect and that further development of this combination in this way is not warranted. In the future, similar use of circulating protein biomarkers should be a helpful way to more rapidly assess which drug combinations are showing evidence of having bigger effects on their intended targets without causing excessive treatment-related toxicities.

The αvβ3 integrin inhibitor cilengitide disrupts endothelial cell migration and has low systemic toxicity. A randomized dose ranging phase II trial showed cilengitide treatment to be associated with a longer progression-free survival than typically observed in advanced disease, but the low toxicity raised the question of whether the drug has been sufficiently dosed to have its intended effect routinely (30). A study of continuous infusion therapy was found similarly to have little significant toxicity but also no evidence of single-agent activity (29). A recently published phase III trial revealed no improvement in clinical outcomes when cilengitide was added to standard therapy in glioblastoma (30). One explanation is that this maximally administrable dose of cilengitide, 2,000 mg twice weekly does not have the intended pharmacodynamic activity in humans. We hypothesized that whether biologically active, the low toxicity of cilengitide would allow it to be readily combined with sunitinib. After sunitinib had been administered to maximize tumor response and tumor endothelial cell injury, cilengitide could prevent endothelial cell regrowth and migration during withdrawal from sunitinib therapy without the same magnitude of multi-kinase inhibition toxicity induced by continued high-dose sunitinib.

Beyond their role in endothelial cell/matrix interactions, αvβ3 integrins are the most abundant integrins on osteoclasts and mediate osteoclast adhesion to bone matrix (31–33). Multiple methods of blocking αvβ3 integrins have been shown to inhibit bone resorption (34–36). This led to human subject investigations of the αvβ3 integrin inhibitor L-000945704 as a potential osteoporosis therapeutic. Administration of this oral agent for 12 months showed dose-dependent effects on multiple markers of bone turnover in postmenopausal women, including serum C-telopeptide crosslinks, as quickly as 2 weeks after initial administration (37). Serum C-telopeptide crosslink assays are now well-standardized and available commercially to clinical diagnostic laboratories (38–40).

We hypothesized that biologic effect of the integrin inhibitor cilengitide at the maximally administrable dose would be demonstrated with measurable changes in these 2 probable valid pharmacodynamic biomarkers. The inhibition of αvβ3 integrin–dependent endothelial cell repopulation of the microvasculature would be indirectly detected through changes in [sVEGFR2] during sunitinib withdrawal. Untreated patients would show typical recovery of [sVEGFR2] toward baseline after 2 weeks of sunitinib withdrawal, whereas patients treated with cilengitide during the 2 weeks of sunitinib withdrawal should show lower [sVEGFR2] at the end of the interval. As another αvβ3 integrin inhibitor L-000945704 had already demonstrated reproducible effects on serum CTx marker, we expected cilengitide to cause measurable declines after 2 weeks of the maximally administrable dose, whereas patients not receiving cilengitide should demonstrate no measurable changes.

Materials and Methods

Study participants

We enrolled adults with advanced solid tumors that were refractory to standard therapy, for which no standard therapy existed or for whom sunitinib monotherapy would be appropriate. Patients had a Karnofsky performance status ≥ 70, normal organ and marrow function [as defined by leukocytes ≥ 3,000/μL, absolute neutrophil count ≥ 1,500/μL, platelets ≥ 100,000/μL, hemoglobin ≥ 9 g/dL, serum creatinine at or below the upper limit of institutional normal (1.4 mg/dL), AST/ALT < 2.5 times the institutional normal limit in the absence of liver metastases] and total bilirubin within normal institutional limits. Patients were excluded if they had surgery, radiotherapy, or chemotherapy within 4 weeks, had prior treatment with an antiangiogenic agent where the best response was progressive disease, a history of proved gastric or duodenal ulcer or clinically significant gastrointestinal blood loss in the 6 weeks prior to the start of treatment, a history of a central nervous system hemorrhage, a bone fracture in the past 12 months, a QTc ≥ 500 ms, or if they required use of a therapeutic dose of warfarin.

These eligibility criteria and the treatment plan were slightly modified after interim analysis. Patients enrolled under the original protocol are hereafter referred to as cohort 1. The protocol called for these patients to receive 28 days of sunitinib at 50 mg daily without interruption or dose reduction to be considered evaluable for the study biomarker-based primary endpoint. Many cohort 1 patients were unable to meet that criterion for evaluable; we also observed baseline [sVEGFR2] measures to be skewed by prior bevacizumab therapy. The protocol was therefore amended to exclude patients with recent prior VEGF signaling inhibitor therapy (bevacizumab, sorafenib, sunitinib, or investigational antiangiogenesis agents) and required 14 days of continuous sunitinib therapy at 50 mg daily for patients to be evaluable for the primary biomarker endpoint (the justification and further details are provided below). Patients enrolled after this amendment are referred to as cohort 2. This study was registered with ClinicalTrials.gov as NCT01122888.

Treatment

Cilengitide was supplied by the Cancer Therapy Evaluation Program of the Division of Cancer Treatment and Diagnosis (DCTD) of the National Cancer Institute (NCI) under a collaborative agreement with EMD Serono, Inc. The drug was administered intravenously twice weekly as a 1-hour infusion. Sunitinib was provided by DCTD under collaborative agreement with Pfizer and was administered orally.

Cohort 1 patients received sunitinib 50 mg daily for 28 days. They were randomized to study arms A and B, 1:1 by opening computer-generated random binary series, coded, profiled
envelopes at initiation of sunitinib therapy. Patients then received either cilengitide twice weekly during the ensuing 14 days off sunitinib (arm A) or no treatment for these 14 days (arm B). All subjects were then to resume sunitinib at the conclusion of the 14 days “off” period for 28 days at 50 mg daily followed by cilengitide for 14 days on all subsequent treatment cycles. Cohort 2 patients received sunitinib 50 mg daily for 14 days before the same randomized treatment arm assignment (A, 14 days twice weekly cilengitide infusion vs. B, no treatment). All subsequent cycles for cohort 2 patients entailed 14 days sunitinib followed by 14 days of cilengitide therapy.

Dose delays and adjustments for adverse events attributable to the protocol treatments were permitted. Patients not able to tolerate sunitinib at a dose of ≥25 mg/d, or cilengitide at ≥1,000 mg twice weekly, were removed from the study. Patients were evaluated for toxicity weekly during the first cycle of treatment and every 2 weeks thereafter with adverse event grading by the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Patients received full supportive care, including transfusions, antibiotics, antiemetics, antidiarrheal agents, antihypertensive management, etc., when appropriate.

**Pharmacodynamic measurements**

**Blood sampling.** Plasma samples were collected to determine [sVEGFR2] and serum for collagen C-telopeptide crosslinks (CTX) at baseline, conclusion of sunitinib therapy, at least weekly during the off-sunitinib interval, and prior to cycle 2 sunitinib administration (Fig. 1). For plasma, whole blood was collected in EDTA-containing tubes and placed on ice for 15 minutes. For serum, whole blood was collected in preservative-free tubes to clot at room temperature for 30 minutes. All tubes were centrifuged at ≥700 × g for 15 minutes at 4°C. The separated plasma and sera were transferred into at least 2 labeled polypropylene tubes, frozen at −70°C, and stored for analysis.

**sVEGFR2 measurements.** Freshly thawed plasma samples were assayed in triplicate according to the manufacturer’s protocol by colorimetric ELISA (R&D Systems) in the University of Chicago Comprehensive Cancer Center Core Immunologic Monitoring Laboratory. Performance according to manufacturer specifications [CV% for intraplate (7%) and interplate (10%) variance] was confirmed by distributing aliquots of the same serum sample from 2 volunteer control subjects to each plate. Measured values for all samples on a plate were then normalized by a plate factor derived from the ratio of the mean [sVEGFR2] for the 2 control subjects on that plate to the mean value across all plates. This methodology has yielded reproducible performance across assay instances and populations in studies of cancer patients and healthy volunteers alike (41, 42). For this assay, the typical range between minimum and maximum plasma [sVEGFR2] for an individual subject across multiple sampling time points without pharmacologic intervention is 1.3 ng/mL and the maximum range has been 1.5 ng/mL.

**Serum CTX.** Testing was performed by the University of Chicago Hospitals chemistry laboratory with the validated Roche Beta-CrossLaps assay (Roche Diagnostics; 07/2007), a 2-site immunometric (sandwich) electrochemiluminescence detection assay on the Roche Cobas 6000 e601 analyzer as used in prior studies of integrin inhibitors (37). The assay performed according to manufacturer’s specifications with typical interassay CV of <5% (39).

**Statistical analysis**

The primary endpoint of the study was the difference between arms A and B in the increase in [sVEGFR2] (ΔVEGFR2) over the 14-day interval from the end of the first 14 days of sunitinib administration to completion of the cilengitide (arm A) or no treatment (arm B) interval just prior to readministration of sunitinib. The null hypothesis was no difference in the change. The alternative hypothesis was that cilengitide causes a 50% reduction in ΔVEGFR2.

Our initial sample size estimates were based on measurements published by DePrimo and colleagues (20), and our unpublished data on the SD of absolute change in [sVEGFR2] in 62 patients who received sorafenib in a pilot study at the University of Chicago (43). See Supplementary Methods for the additional calculations and quantitative biomarker for study sample size.

For cohort 2, to detect a 50% reduction in the predicted ΔVEGFR2 (0.55 ng/mL) between cilengitide treatment (arm A) and no treatment (arm B) over the 14-day interval from the end of the first 14 days of sunitinib administration to completion of cilengitide treatment required 14 patients in each treatment arm. This was based on a one-tailed t test at α of 0.05 significance level to have 80% power. We assumed a SD of 0.57 ng/mL (half of 1.14 ng/mL SD from the sorafenib pilot study; ref. 43). Thus, for cohort 2, we planned to enroll 28 total evaluable patients randomized after an initial 2-week course of sunitinib. Prespecified interim analyses to test our quantitative assumptions on ΔVEGFR2 and SD were conducted after the first 31 subjects (of whom 23 were not evaluable) in cohort 1 were enrolled and again after the first 14 subjects in cohort 2 were evaluable for the primary endpoint.

**Results**

**Patients and tolerability**

The patients in cohort 1 did not tolerate continuous, full dose sunitinib for 28 days. Eight of the first 10 enrolled patients had to interrupt or reduce sunitinib dosing. For most of these patients, the need for interruption or reduction occurred after the first 14 days. To continue to pursue the primary biomarker endpoint with patients having uniform sunitinib exposure, we amended the protocol to treat patients daily for an initial 14 rather than 28
Table 1. Characteristics of 41 enrolled study subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>20 (49)</td>
</tr>
<tr>
<td>Men</td>
<td>21 (51)</td>
</tr>
<tr>
<td>Age, y median (range)</td>
<td>60 (31–81)</td>
</tr>
<tr>
<td><strong>Self-reported race/ethnicity</strong></td>
<td></td>
</tr>
<tr>
<td>Black non-Hispanic</td>
<td>4 (10)</td>
</tr>
<tr>
<td>White non-Hispanic</td>
<td>31 (75)</td>
</tr>
<tr>
<td>White Hispanic</td>
<td>4 (10)</td>
</tr>
<tr>
<td>East Asian</td>
<td>2 (5)</td>
</tr>
<tr>
<td><strong>Tumor type</strong></td>
<td></td>
</tr>
<tr>
<td>Adenoid cystic carcinoma</td>
<td>4 (10)</td>
</tr>
<tr>
<td>Primary brain tumor</td>
<td>4 (10)</td>
</tr>
<tr>
<td>Renal</td>
<td>3 (7)</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>3 (7)</td>
</tr>
<tr>
<td>Thyroid</td>
<td>3 (7)</td>
</tr>
<tr>
<td>Carcinosarcoma/Neuroendocrine</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Thymic carcinoma</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>1 (2)</td>
</tr>
</tbody>
</table>

days (Fig. 1). The amended schema commenced after the first 21 patients were enrolled. The patient characteristics for the 21 patients in cohort 1 and the 20 patients in cohort 2 are summarized in Table 1. Specific treatment-attributable adverse events and grades are summarized in Supplementary Table S1.

**[sVEGFR2] as a valid biomarker**

Of the 41 enrolled patients, 22 were evaluable for the primary analysis of [sVEGFR2]. Fourteen of the evaluable patients were from cohort 2; as described above, the majority of patients from cohort 1 (13 of 21) were not evaluable due to dose interruptions. For clarity of the biomarker analyses with a consistent schedule of sunitinib administration and serum collection (14 days of sunitinib, followed by no treatment or cilengitide over the subsequent 14 days), our primary analyses were restricted to the 14 evaluable cohort 2 patients.

The [sVEGFR2] measurements were within typical ranges for patients in both arms (Fig. 2). Sunitinib treatment caused similar magnitude decline in both study arms [arm A baseline mean [sVEGFR2]: 10.27 ng/mL, SD, 1.32; 14-day sunitinib [sVEGFR2]: 5.28 ng/mL, SD, 1.41; arm B baseline [sVEGFR2]: 9.18 ng/mL, SD, 2.07; 14-day sunitinib [sVEGFR2]: 5.77 ng/mL, SD = 1.66]. The similar distribution and variance of baseline and post-sunitinib [sVEGFR2] implies the study arms were adequately balanced for purposes of this analysis. These results recapitulate findings previously demonstrated for sunitinib and [sVEGFR2] (24), and the quantitative findings (the mean change in [sVEGFR2] and the SD) were consistent with our prestudy estimates, confirming use of [sVEGFR2] as an analytically valid pharmacodynamic biomarker for this drug.

**Cilengitide has no measurable effect on [sVEGFR2]**

Cilengitide did not affect the degree of [sVEGFR2] recovery during the sunitinib 2-week "off" period (Fig. 2). For arm A, the mean cohort [sVEGFR2] after 2-weeks-off sunitinib was 8.12 ng/mL (SD, 1.31) and for arm B, 7.82 ng/mL (SD, 1.59 ng/mL), not significantly different from each other. Expressed another way, the magnitude of rebound in [sVEGFR2] after the post-sunitinib nadir was 2.85 ng/mL (SD, 0.83 ng/mL) in Arm A versus 2.04 ng/mL (SD, 1.43 ng/mL) in arm B (P = 0.22 by 2-sample t test).

Unexpectedly, the cilengitide-treated arm A actually had in absolute and relative terms a greater recovery of [sVEGFR2] than the control arm B. We initially proposed to detect a 50% decrease in [sVEGFR2] recovery in these cilengitide-treated patients. We therefore performed a futility analysis to assess whether continuing this trial to enroll an additional 14 subjects could likely lead us to reject the initial null hypothesis. The conditional power, that is, the probability that the null hypothesis would be rejected after studying an additional 14 patients given the data observed thus far, was very low (<5%) and we therefore terminated the trial.

**Prior bevacizumab suppresses [sVEGFR2]**

In studies of previously untreated patients with cancer and larger populations without cancer, when multiple samples are run on the R&D Systems ELISA and reported population mean serum [sVEGFR2] is typically 9 to 10.7 ng/mL with SD approximately 1.5 ng/mL (20, 25, 41, 42, 44). However, the baseline [sVEGFR2] in cohort 1 patients was considerably lower than expected in such a small sample of patients. We inferred that our cohort 1 patient predisposition to low baseline [sVEGFR2]. After comparing various demographic and disease-related factors, a history of (even remote) bevacizumab treatment was most strongly associated with lower pre-sunitinib (baseline) [sVEGFR2] compared with other patients (Fig. 3). In patients previously treated with bevacizumab (n = 5), the mean baseline [sVEGFR2] was 7.53 ± 1.56 ng/mL, a full SD lower than the typical previously untreated patient or healthy subject population. For patients without a history of bevacizumab treatment (n = 15), the baseline [sVEGFR2] level was 9.72 ± 1.76 ng/mL, consistent with previously reported measurements for other populations. This difference was statistically significant (P = 0.03) and is consistent with bevacizumab having long-term effects of unclear significance on microvasculature. Regardless of the potential clinical significance, prior bevacizumab affected the reliability of [sVEGFR2] as a pharmacodynamic biomarker of sunitinib and cilengitide effect. Therefore, to achieve the goals of this investigation (testing the effects of sequential sunitinib and cilengitide on changes in [sVEGFR2]), we concluded it was appropriate to exclude patients with prior
Sunitinib effects on serum CTx

Serum CTx is a validated assay for bone turnover, used in clinical practice for osteoporosis and other bone metabolic disorders. In studies of a selective αβ3/αβ5 integrin small-molecule inhibitor, serum CTx measurements routinely declined after 2 weeks of therapy. We therefore expected serum CTx to be a likely useful pharmacodynamic biomarker for the selective integrin inhibitor cilengitide. The secondary endpoint of our study to describe the magnitude of change, time course, and interindividual variability of serum CTx declines was expected to serve as a positive control for sufficiency of cilengitide dosing. As a selective small-molecule integrin inhibitor had previously been shown to induce changes in serum CTx, we expected serum CTx would be unchanged by sunitinib exposure and provide evidence of cilengitide target engagement whether or not the additional antiangiogenic effects were detected with the recovery in [sVEGFR2]. Unexpectedly, sunitinib had significant effects on serum CTx (Fig. 4). For the 14 subjects in cohort 2, serum CTx declined from baseline serum concentrations of 414 ± 242 pg/mL to 293 ± 187 pg/mL after 2 weeks of sunitinib exposure. For 5 of the 14 patients, this constituted a decrease of more than 50%. Given this unexpected magnitude of change prior to any cilengitide exposure and the absence of prior data on the time course and variance in serum CTx with exposure to sunitinib, we abandoned further use of serum CTx as a pharmacodynamic biomarker specific to integrin inhibition.

Discussion

This randomized, controlled clinical investigation with a quantitative, serum pharmacodynamic biomarker endpoint provided sufficient evidence against “proof of concept” to discontinue our efforts to develop a sequential combination of sunitinib and cilengitide. We were able to make this decision based on the reproducible performance of the quantitative circulating peptide/pharmacodynamic biomarker [sVEGFR2] before sunitinib treatment, after sunitinib treatment and after withdrawal from sunitinib treatment. In the course of conducting the trial, we obtained initial evidence that bevacizumab might have prolonged effects on human endothelial cell function, and we unexpectedly detected significant effects of short term sunitinib exposure on the bone turnover marker serum C-telopeptide crosslinks.

Because we did not detect the expected pharmacodynamic biomarker effects of cilengitide on [sVEGFR2], a subsequent trial will not be conducted. This quantitative biomarker of sunitinib effect was predictable and reproducible. Future proof-of-concept and pharmacodynamic marker studies to select combination treatments with sunitinib or likely other VEGFR2 kinase inhibitors can be performed with a relatively small number of patients. At the time we began the study, cilengitide seemed a promising agent. One could speculate that the failure to detect effects of cilengitide on [sVEGFR2] was due to studying too few patients or too short a treatment course to demonstrate these pharmacodynamic effects. Currently, increasing the number of patients or prolonging the treatment interval would be clinically impractical. As many other alternative treatment strategies are in development and there is no evidence that cilengitide has the intended pharmacodynamic effects, we discontinued the trial.

The prior information on the distribution of [sVEGFR2] in other populations enabled us to discover that prolonged exposure to bevacizumab might have long-term effects to lower [sVEGFR2]. After measuring [sVEGFR2] in cohort 1, we recognized that the group mean was skewed significantly and these low values were most strongly associated with prior bevacizumab exposure. After the initial 3-week treatment interval with bevacizumab, [sVEGFR2] typically increases (47–49). Kopetz and colleagues (47) were the first to report a decrease in [sVEGFR2] at median time of 12 months after bevacizumab treatment in patients with colorectal cancer who had progressive disease. In a cohort of advanced solid tumor patients, we found evidence consistent with this being an effect of prolonged bevacizumab therapy. As circulating [sVEGFR2] is primarily derived from systemic endothelial cells, this observation is consistent with a hypothesis that bevacizumab might cause diminished function of the endothelium with long-term exposure. Consistent with our findings, Mourad
and colleagues (50) had previously demonstrated evidence of capillary rarefaction and endothelial dysfunction in a cohort of 18 patients who had received bevacizumab for 6 months. As numerous randomized trials of bevacizumab therapy with prolonged treatment have recently been completed and have included serial collection of blood samples for measurement of circulating peptide biomarkers, there will be opportunities in the near future to test further the hypothesis that prolonged bevacizumab exposure induces rarefaction and endothelial dysfunction that might be quantified with changes in concentrations of [sVEGFR2].

We also found the multi-kinase inhibitor sunitinib to cause decreases in bone turnover marked by serum CTx. This indicated that changes in serum CTx do not mark an αvβ3-specific integrin inhibitor effect. It also has implications for interpreting the biomarker effects of the multi-kinase inhibitor cabozantinib in patients with prostate cancer with bone metastases. The initial studies of cabozantinib suggested this agent might have pharmacologic effects distinct from other VEGFR2 kinase inhibitors such as sunitinib (51, 52). Multiple biomarkers and objective clinical observations were consistent with cabozantinib diminishing bone turnover associated with bone metastases: decreased narcotic use, changes on nuclear bone imaging, changes in alkaline phosphatase, and changes in serum CTx. Although in a small population, we found strikingly similar effects of sunitinib on serum CTx in this non–prostate cancer patient population. Similar to the reported changes among 66 patients with prostate cancer (52), we detected a mean decrease in serum CTx after just 14 days of sunitinib therapy of approximately 30%. In the initial report for cabozantinib, 45% of patients had a decrease of 50% or greater. In the sunitinib-treated patients, serum CTx decreased by at least 50% in 36% of patients. It is possible this is an effect of kinase inhibition on downstream integrin signaling or alternatively an indirect effect of these kinase inhibitors enhancing the clearance of serum CTx. Although we could not use serum CTx as a marker of cilengitide effect, the unexpected detection of clear decreases in this biomarker after sunitinib exposure suggest this is not an effect unique to cabozantinib.

Ordinarily, a small sample size study would be declared as a shortcoming. However, a goal for future clinical investigations in development of combination treatment strategies in oncology is to require fewer patients to determine whether a particular strategy is worth further investigation. Here, we demonstrated that a validated, quantitative pharmacodynamic biomarker and a mechanism-based hypothesis could be used to screen a treatment strategy with a small number of patients. We have demonstrated that [sVEGFR2] is a biomarker with reproducible performance and could be used to screen agents to complement sunitinib in a small group of patients. Agents impairing the recovery of [sVEGFR2] could then be considered for further study. In principle, this concept of screening combination therapy trials with quantitative biomarker endpoints might be extended to a larger set of anticancer agents where a serially evaluable, validated pharmacodynamic biomarker is available.

Disclosure of Potential Conflicts of Interest

M.J. Ratain is a consultant/advisory board member for EMD Serono. M.L. Maitland was a consultant/advisory board member for Amgen Inc., GlaxoSmithKline, and Pfizer. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: P.H. O’Donnell, T.G. Karrison, M.J. Ratain, M.L. Maitland

Development of methodology: P.H. O’Donnell, M.L. Maitland

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): P.H. O’Donnell, L. Janisch, M.R. Levine, B.N. Polite, E.W. Cohen, G.F. Fleming, M.J. Ratain, M.L. Maitland

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): P.H. O’Donnell, S. Karovic, T.G. Karrison, M.J. Ratain, M.L. Maitland

Writing, review, and/or revision of the manuscript: P.H. O’Donnell, S. Karovic, T.G. Karrison, P.J. Harris, B.N. Polite, E.W. Cohen, G.F. Fleming, M.J. Ratain, M.L. Maitland

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M.R. Levine, M.J. Ratain, M.L. Maitland

Study supervision: G.F. Fleming, M.J. Ratain, M.L. Maitland

Acknowledgments

Sunitinib and cilengitide were provided through the National Cancer Institute Investigational Drug Branch. The authors thank the patients and their families for their contributions to this study.

Grant Support

The clinical trial was sponsored by U01CA69852 (M.J. Ratain). This project was additionally supported by U.S. NIH grants R23CA124802 NCIC Career Development Award (M.L. Maitland), the University of Chicago Comprehensive Cancer Center (P30 CA14599) Clinical Pharmacology Core Laboratory, and U1MI CA186705. Additional support was provided by a Translational Research Professorship from the Conquer Cancer Foundation of the American Society of Clinical Oncology (P.H. O’Donnell, M.J. Ratain, and M.L. Maitland).

EMD Serono, Inc. provided funding to support the conduct of biomarker assays in the conduct of this trial.

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.

Received March 9, 2015; revised June 12, 2015; accepted June 29, 2015; published OnlineFirst July 21, 2015.

References


Ebos JM, Lee CR, Christensen KS, Mutsaers AJ, Kerbel RS. Multiple circulating proangiogenic factors induced by sunitinib malate are tumor-independent and correlate with antitumor efficacy. Proc Natl Acad Sci USA 2007;104:17093–74.


Administered Sunitinib and Cilengitide Sequentially

VEGFR2 as Pharmacodynamic Biomarkers in a Trial of Sequentially Administered Sunitinib and Cilengitide

Peter H. O'Donnell, Sanja Karovic, Theodore G. Karrison, et al.


Updated version
Access the most recent version of this article at:

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2015/07/21/1078-0432.CCR-15-0427.DC1

Cited articles
This article cites 52 articles, 26 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/21/22/5092.full.html#ref-list-1

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.