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Serum C-telopeptide collagen crosslinks and plasma soluble VEGFR2 as pharmacodynamic biomarkers in a trial of sequentially administered sunitinib and cilengitide

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Abstract

Background—Fit-for-purpose pharmacodynamic biomarkers could expedite development of combination anti-angiogenic 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].

Methods—Advanced solid tumor patients received sunitinib 50 mg daily for 14 days. For the next 14 days, patients were randomized to Arm A (cilengitide 2000 mg administered intravenously twice weekly (BIW)), or Arm B (no treatment). The primary endpoint was change in [sVEGFR2] between Day 14 and Day 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/arm) received all treatments without interruption and had all blood samples available for analysis. The mean change and standard deviation of [sVEGFR2] for all sunitinib-treated patients was consistent with previous data. There was no significant difference in the mean change in [sVEGFR2] from Day 14 to Day 28 between the arms (Arm A: 2.8 ng/mL

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[95% CI 2.1, 3.6] vs. Arm B: 2.0 ng/mL [95% CI 0.72, 3.4] $P = 0.22$, two sample t test). Additional analyses suggested: 1) prior bevacizumab therapy to be associated with unusually low baseline [sVEGFR2], and 2) 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 anti-angiogenic therapy trials.

Keywords

pharmacodynamic biomarkers; C-telopeptide crosslinks; sunitinib; cilengitide; integrin inhibitors; VEGFR2

INTRODUCTION

Recently, combination cancer therapy regimens have improved the therapeutic index and outcomes for solid tumors (1–3). But most efforts at developing new combination therapies with conventional strategies and clinical trial designs have not succeeded (4–7). Systematic, stepwise development based on preliminary clinical studies and pharmacodynamic biomarker endpoints have been suggested to improved development of anticancer combination treatments (5, 7–9). In combination anti-angiogenic 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 phase III trials(4, 12).

The multi-kinase inhibitor sunitinib potently disrupts the vascular endothelial growth factor (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 vascular endothelial growth factor receptor-2 ([sVEGFR2]) is a pharmacodynamic biomarker for inhibitors of VEGF signaling. In cancer patients 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.

The α_Vß3 integrin inhibitor cilengitide disrupts endothelial cell migration and has low systemic toxicity. A randomized dose ranging phase 2 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 (28). 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 3 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, 2000 mg twice weekly does not have the intended pharmacodynamic activity in humans. We hypothesized that if 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 re-growth 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, a_Vß3 integrins are the most abundant integrins on osteoclasts and mediate osteoclast adhesion to bone matrix (31–33). Multiple methods of blocking a_Vß3 integrins have been shown to inhibit bone resorption (34–36). This led to human subject investigations of the a_Vß3 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 post-menopausal 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 biological 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 a_Vß3 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 while patients treated with cilengitide during the 2 weeks of sunitinib withdrawal should show lower [sVEGFR2] at the end of the interval. As another a_Vß3 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, while patients not receiving cilengitide should demonstrate no measurable changes.

MATERIAL 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 anti-angiogenic 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 $QT_C \geq 500$ msec, or if they required use of a therapeutic dose of warfarin.

These eligibility criteria and 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 50mg daily without interruption or dose-reduction in order to be considered evaluable for the study biomarker-based primary endpoint. Many cohort 1 patients were unable to meet that criterion for evaluability; 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 anti-angiogenesis 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.

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 one-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, pre-filled 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 per day, or cilengitide at ≥1000 mg twice weekly, were removed from the study. Patients were evaluated for toxicity weekly during the first cycle of treatment and every two 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 ≥ 700g for 15 minutes at 4°C. The separated plasma and sera were transferred into at least two 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, Minneapolis, MN) in the University of Chicago Comprehensive Cancer Center Core Immunologic Monitoring Laboratory. Performance according to manufacturer specifications (CV% for intra-plate (7%) and inter-plate (10%) variance) was confirmed by distributing aliquots of the same serum sample from two 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 two 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, Indianapolis, IN 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 inter-assay CV of < 5% (39).

Statistical analysis

The primary endpoint of the study was the difference between Arm A and Arm 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 re-administration 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,(20) and our unpublished data on the standard deviation of absolute change in [sVEGFR2] in 62 patients who received sorafenib in a pilot study at the University of Chicago(43). See Supplemental Methods for the initial 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 the alpha = 0.05 significance level to have 80% power. We assumed a standard deviation of 0.57 ng/mL (half of 1.14 ng/mL standard deviation from the sorafenib pilot study (43)). Thus, for Cohort 2 we planned to enroll 28 total evaluable patients randomized after an initial two-week course of sunitinib. Pre-specified interim analyses to test our quantitative assumptions on ΔVEGFR2 and standard deviation 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 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 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, standard deviation = 1.32, 14-days-sunitinib [sVEGFR2] 5.28 ng/mL, stand. dev. = 1.14; Arm B baseline [sVEGFR2] 9.18 ng/mL, stand. dev. = 2.07, 14-days sunitinib [sVEGFR2] 5.77 ng/mL, stand. dev. = 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 standard deviation) were consistent with our pre-study estimates, confirming utility 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 (std. dev.1.31) and for Arm B, 7.82 ng/mL (std. dev. 1.59), not significantly different from each other. Expressed another way, the magnitude of rebound in [sVEGFR2] after the post-sunitinib nadir was 2.85 (std. dev. 0.83) ng/mL in Arm A, versus 2.04 (std. dev. 1.43) ng/mL in Arm B (P=0.22 by two 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, i.e., the probability that the null hypothesis would be rejected after studying an additional 14 patients given the data observed thus far, was very low (less than 5%) and we therefore terminated the trial.

Prior bevacizumab suppresses [sVEGFR2]

In studies of previously untreated cancer patients and larger populations without cancer when multiple samples are run on the R&D Systems ELISA and reported, population mean serum [sVEGFR2] is typically 9–10.7 ng/mL with standard deviation 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 1patient population, prior to enrollment in this trial, had some unusual 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 to 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 standard deviation 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]) weconcluded it was appropriate to exclude patients with prior bevacizumab exposure from enrollment. This exclusion resulted in two small randomized study arms to have baseline and post-sunitinib therapy [sVEGFR2] measurements consistent with our predictions. In this setting, we concluded that [sVEGFR2] serves as a fit-for-purpose pharmacodynamic biomarker(45, 46).

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 a_Vß3/a_Vß5 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 anti-angiogenic 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 two 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.

Since 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, et al (47) were the first to report a decrease in [sVEGFR2] at median time of 12 months after bevacizumab treatment in colorectal patients 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, et al. (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 a_Vß3-specific integrin inhibitor effect. It also has implications for interpreting the biomarker effects of the multi-kinase inhibitor cabozantinib in prostate cancer patients 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 prostate cancer patients (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 employ 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Baselga J, Campone M, Piccart M, Burris HA 3rd, Rugo HS, Sahmoud T, et al. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. The New England Journal of Medicine. 2012; 366:520–9. [PubMed: 22149876]
2. Baselga J, Cortes J, Kim SB, Im SA, Hegg R, Im YH, et al. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. The New England Journal of Medicine. 2012; 366:109–19. [PubMed: 22149875]
3. Robert C, Karaszewska B, Schachter J, Rutkowski P, Mackiewicz A, Stroiakovski D, et al. Improved Overall Survival in Melanoma with Combined Dabrafenib and Trametinib. The New England Journal of Medicine. 2014
4. Azad NS, Posadas EM, Kwitkowski VE, Steinberg SM, Jain L, Annunziata CM, et al. Combination targeted therapy with sorafenib and bevacizumab results in enhanced toxicity and antitumor activity. J Clin Oncol. 2008; 26:3709–14. [PubMed: 18669456]
5. Hamberg P, Verweij J. Phase I drug combination trial design: walking the tightrope. J Clin Oncol. 2009; 27:4441–3. [PubMed: 19704054]
6. Maitland ML, Hudoba C, Snider KL, Ratain MJ. Analysis of the yield of phase II combination therapy trials in medical oncology. Clin Cancer Res. 2010; 16:5296–302. [PubMed: 20837695]
7. Verweij J, Disis ML, Cannistra SA. Phase I studies of drug combinations. J Clin Oncol. 2010; 28:4545–6. [PubMed: 20855831]
8. Yap TA, Sandhu SK, Workman P, de Bono JS. Envisioning the future of early anticancer drug development. Nature Reviews Cancer. 2010; 10:514–23.
9. Paller CJ, Bradbury PA, Ivy SP, Seymour L, LoRusso PM, Baker L, et al. Design of phase I combination trials: recommendations of the Clinical Trial Design Task Force of the NCI Investigational Drug Steering Committee. Clin Cancer Res. 2014; 20:4210–7. [PubMed: 25125258]
10. Jayson GC, Hicklin DJ, Ellis LM. Antiangiogenic therapy–evolving view based on clinical trial results. Nature Reviews Clinical Oncology. 2012; 9:297–303.
11. Maru D, Venook AP, Ellis LM. Predictive biomarkers for bevacizumab: are we there yet? Clin Cancer Res. 2013; 19:2824–7. [PubMed: 23549876]
12. Moreno Garcia V, Basu B, Molife LR, Kaye SB. Combining antiangiogenics to overcome resistance: rationale and clinical experience. Clin Cancer Res. 2012; 18:3750–61. [PubMed: 22547772]
13. Mendel DB, Laird AD, Xin X, Louie SG, Christensen JG, Li G, et al. In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and

platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. Clin Cancer Res. 2003; 9:327–37. [PubMed: 12538485]

14. Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, et al. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. The New England Journal of Medicine. 2007; 356:115–24. [PubMed: 17215529]
15. Motzer RJ, Rini BI, Bukowski RM, Curti BD, George DJ, Hudes GR, et al. Sunitinib in patients with metastatic renal cell carcinoma. JAMA. 2006; 295:2516–24. [PubMed: 16757724]
16. Raymond E, Dahan L, Raoul JL, Bang YJ, Borbath I, Lombard-Bohas C, et al. Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. The New England Journal of Medicine. 2011; 364:501–13. [PubMed: 21306237]
17. Demetri GD, van Oosterom AT, Garrett CR, Blackstein ME, Shah MH, Verweij J, et al. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. Lancet. 2006; 368:1329–38. [PubMed: 17046465]
18. Faivre S, Delbaldo C, Vera K, Robert C, Lozahic S, Lassau N, et al. Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. J Clin Oncol. 2006; 24:25–35. [PubMed: 16314617]
19. Bass MB, Sherman SI, Schlumberger MJ, Davis MT, Kivman L, Khoo HM, et al. Biomarkers as predictors of response to treatment with motesanib in patients with progressive advanced thyroid cancer. The Journal of Clinical Endocrinology and Metabolism. 2010; 95:5018–27. [PubMed: 20739388]
20. Deprimo SE, Bello CL, Smeraglia J, Baum CM, Spinella D, Rini BI, et al. Circulating protein biomarkers of pharmacodynamic activity of sunitinib in patients with metastatic renal cell carcinoma: modulation of VEGF and VEGF-related proteins. Journal of Translational Medicine. 2007; 5:32. [PubMed: 17605814]
21. Deprimo SE, Huang X, Blackstein ME, Garrett CR, Harmon CS, Schoffski P, et al. Circulating levels of soluble KIT serve as a biomarker for clinical outcome in gastrointestinal stromal tumor patients receiving sunitinib following imatinib failure. Clin Cancer Res. 2009; 15:5869–77. [PubMed: 19737953]
22. Nikolinakos PG, Altorki N, Yankelevitz D, Tran HT, Yan S, Rajagopalan D, et al. Plasma cytokine and angiogenic factor profiling identifies markers associated with tumor shrinkage in early-stage non-small cell lung cancer patients treated with pazopanib. Cancer Research. 2010; 70:2171–9. [PubMed: 20215520]
23. Norden-Zfoni A, Desai J, Manola J, Beaudry P, Force J, Maki R, et al. Blood-based biomarkers of SU11248 activity and clinical outcome in patients with metastatic imatinib-resistant gastrointestinal stromal tumor. Clin Cancer Res. 2007; 13:2643–50. [PubMed: 17473195]
24. Motzer RJ, Michaelson MD, Redman BG, Hudes GR, Wilding G, Figlin RA, et al. Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. J Clin Oncol. 2006; 24:16–24. [PubMed: 16330672]
25. Cohen EE, Rosen LS, Vokes EE, Kies MS, Forastiere AA, Worden FP, et al. Axitinib is an active treatment for all histologic subtypes of advanced thyroid cancer: results from a phase II study. J Clin Oncol. 2008; 26:4708–13. [PubMed: 18541897]
26. Ebos JM, Lee CR, Christensen JG, Mutsaers AJ, Kerbel RS. Multiple circulating proangiogenic factors induced by sunitinib malate are tumor-independent and correlate with antitumor efficacy. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104:17069–74. [PubMed: 17942672]
27. Baffert F, Le T, Sennino B, Thurston G, Kuo CJ, Hu-Lowe D, et al. Cellular changes in normal blood capillaries undergoing regression after inhibition of VEGF signaling. American Journal of Physiology. 2006; 290:H547–59. [PubMed: 16172161]
28. Reardon DA, Fink KL, Mikkelsen T, Cloughesy TF, O'Neill A, Plotkin S, et al. Randomized phase II study of cilengitide, an integrin-targeting arginine-glycine-aspartic acid peptide, in recurrent glioblastoma multiforme. J Clin Oncol. 2008; 26:5610–7. [PubMed: 18981465]

29. O'Donnell PH, Undevia SD, Stadler WM, Karrison TM, Nicholas MK, Janisch L, et al. A phase I study of continuous infusion cilengitide in patients with solid tumors. Investigational New Drugs. 2012; 30:604–10. [PubMed: 20839028]
30. Stupp R, Hegi ME, Gorlia T, Erridge SC, Perry J, Hong YK, et al. Cilengitide combined with standard treatment for patients with newly diagnosed glioblastoma with methylated MGMT promoter (CENTRIC EORTC 26071–22072 study): a multicentre, randomised, open-label, phase 3 trial. The Lancet Oncology. 2014; 15:1100–8. [PubMed: 25163906]
31. Duong LT, Rodan GA. The role of integrins in osteoclast function. Journal of Bone and Mineral Metabolism. 1999; 17:1–6. [PubMed: 10084394]
32. Rodan SB, Rodan GA. Integrin function in osteoclasts. The Journal of Endocrinology. 1997; 154(Suppl):S47–56. [PubMed: 9379137]
33. Teitelbaum SL. Bone resorption by osteoclasts. Science. 2000; 289:1504–8. [PubMed: 10968780]
34. Brashear KM, Hunt CA, Kucer BT, Duggan ME, Hartman GD, Rodan GA, et al. Non-peptide alpha(v)beta(3) antagonists. Part 5: identification of potent RGD mimetics incorporating 2-aryl beta-amino acids as aspartic acid replacements. Bioorganic & Medicinal Chemistry Letters. 2002; 12:3483–6. [PubMed: 12419389]
35. Breslin MJ, Duggan ME, Halczenko W, Fernandez-Metzler C, Hunt CA, Leu CT, et al. Non-peptide alphavbeta3 antagonists. Part 6: design and synthesis of alphavbeta3 antagonists containing a pyridone or pyrazinone central scaffold. Bioorganic & Medicinal Chemistry Letters. 2003; 13:1809–12. [PubMed: 12729670]
36. Hutchinson JH, Halczenko W, Brashear KM, Breslin MJ, Coleman PJ, Duong le T, et al. Nonpeptide alphavbeta3 antagonists. 8. In vitro and in vivo evaluation of a potent alphavbeta3 antagonist for the prevention and treatment of osteoporosis. Journal of Medicinal Chemistry. 2003; 46:4790–8. [PubMed: 14561098]
37. Murphy MG, Cerchio K, Stoch SA, Gottesdiener K, Wu M, Recker R. Effect of L-000845704, an alphaVbeta3 integrin antagonist, on markers of bone turnover and bone mineral density in postmenopausal osteoporotic women. The Journal of Clinical Endocrinology and Metabolism. 2005; 90:2022–8. [PubMed: 15687321]
38. Okabe R, Inaba M, Nakatsuka K, Miki T, Naka H, Moriguchi A, et al. Significance of serum CrossLaps as a predictor of changes in bone mineral density during estrogen replacement therapy; comparison with serum carboxyterminal telopeptide of type I collagen and urinary deoxypyridinoline. J Bone and Mineral Metabolism. 2004; 22:127–31.
39. Okabe R, Nakatsuka K, Inaba M, Miki T, Naka H, Masaki H, et al. Clinical evaluation of the Elecsys beta-CrossLaps serum assay, a new assay for degradation products of type I collagen C-tlopeptides. Clin Chemistry. 2001; 47:1410–4.
40. Schmidt-Gayk H, Spanuth E, Kotting J, Bartl R, Felsenberg D, Pfeilschifter J, et al. Performance evaluation of automated assays for beta-CrossLaps, N-MID-Osteocalcin and intact parathyroid hormone (BIOROSE Multicenter Study). Clin Chem Lab Medicine: CCLM / FESCC. 2004; 42:90–5.
41. Maitland ML, Xu CF, Cheng YC, Kistner-Griffin E, Ryan KA, Karrison TG, et al. Identification of a variant in KDR associated with serum VEGFR2 and pharmacodynamics of pazopanib. Clin Cancer Res. 2014
42. Thomeas V, Chow S, Gutierrez JO, Karovic S, Wroblewski K, Kistner-Griffin E, et al. Technical considerations in the development of circulating peptides as pharmacodynamic biomarkers for angiogenesis inhibitors. J Clin Pharmacol. 2014; 54:682–7. [PubMed: 24374901]
43. Karovic S, Wen Y, Karrison TG, Bakris GL, Levine MR, House LK, et al. Sorafenib dose escalation is not uniformly associated with blood pressure elevations in normotensive patients with advanced malignancies. Clinical Pharmacol Ther. 2014; 96:27–35. [PubMed: 24637941]
44. Sleijfer S, Gorlia T, Lamers C, Burger H, Blay JY, Le Cesne A, et al. Cytokine and angiogenic factors associated with efficacy and toxicity of pazopanib in advanced soft-tissue sarcoma: an EORTC-STBSG study. British J Cancer. 2012; 107:639–45.
45. Wagner JA. Strategic approach to fit-for-purpose biomarkers in drug development. Annual Rev Pharmacol Toxicol. 2008; 48:631–51. [PubMed: 17937595]

46. Lee JW, Devanarayan V, Barrett YC, Weiner R, Allinson J, Fountain S, et al. Fit-for-purpose method development and validation for successful biomarker measurement. Pharmaceutical Research. 2006; 23:312–28. [PubMed: 16397743]

47. Kopetz S, Hoff PM, Morris JS, Wolff RA, Eng C, Glover KY, et al. Phase II trial of infusional fluorouracil, irinotecan, and bevacizumab for metastatic colorectal cancer: efficacy and circulating angiogenic biomarkers associated with therapeutic resistance. J Clin Oncol. 2010; 28:453–9. [PubMed: 20008624]

48. Nixon AB, Pang H, Starr MD, Friedman PN, Bertagnolli MM, Kindler HL, et al. Prognostic and predictive blood-based biomarkers in patients with advanced pancreatic cancer: results from CALGB80303 (Alliance). Clin Cancer Res. 2013; 19:6957–66. [PubMed: 24097873]

49. Willett CG, Duda DG, di Tomaso E, Boucher Y, Ancukiewicz M, Sahani DV, et al. Efficacy, safety, and biomarkers of neoadjuvant bevacizumab, radiation therapy, and fluorouracil in rectal cancer: a multidisciplinary phase II study. J Clin Oncol. 2009; 27:3020–6. [PubMed: 19470921]

50. Mourad JJ, des Guetz G, Debbabi H, Levy BI. Blood pressure rise following angiogenesis inhibition by bevacizumab. A crucial role for microcirculation. Annals of Oncology. 2008; 19:927–34. [PubMed: 18056916]

51. Smith DC, Smith MR, Sweeney C, Elfiky AA, Logothetis C, Corn PG, et al. Cabozantinib in patients with advanced prostate cancer: results of a phase II randomized discontinuation trial. J Clin Oncol. 2013; 31:412–9. [PubMed: 23169517]

52. Smith MR, Sweeney CJ, Corn PG, Rathkopf DE, Smith DC, Hussain M, et al. Cabozantinib in chemotherapy-pretreated metastatic castration-resistant prostate cancer: results of a phase II nonrandomized expansion study. J Clin Oncol. 2014; 32:3391–9. [PubMed: 25225437]

STATEMENT OF 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.

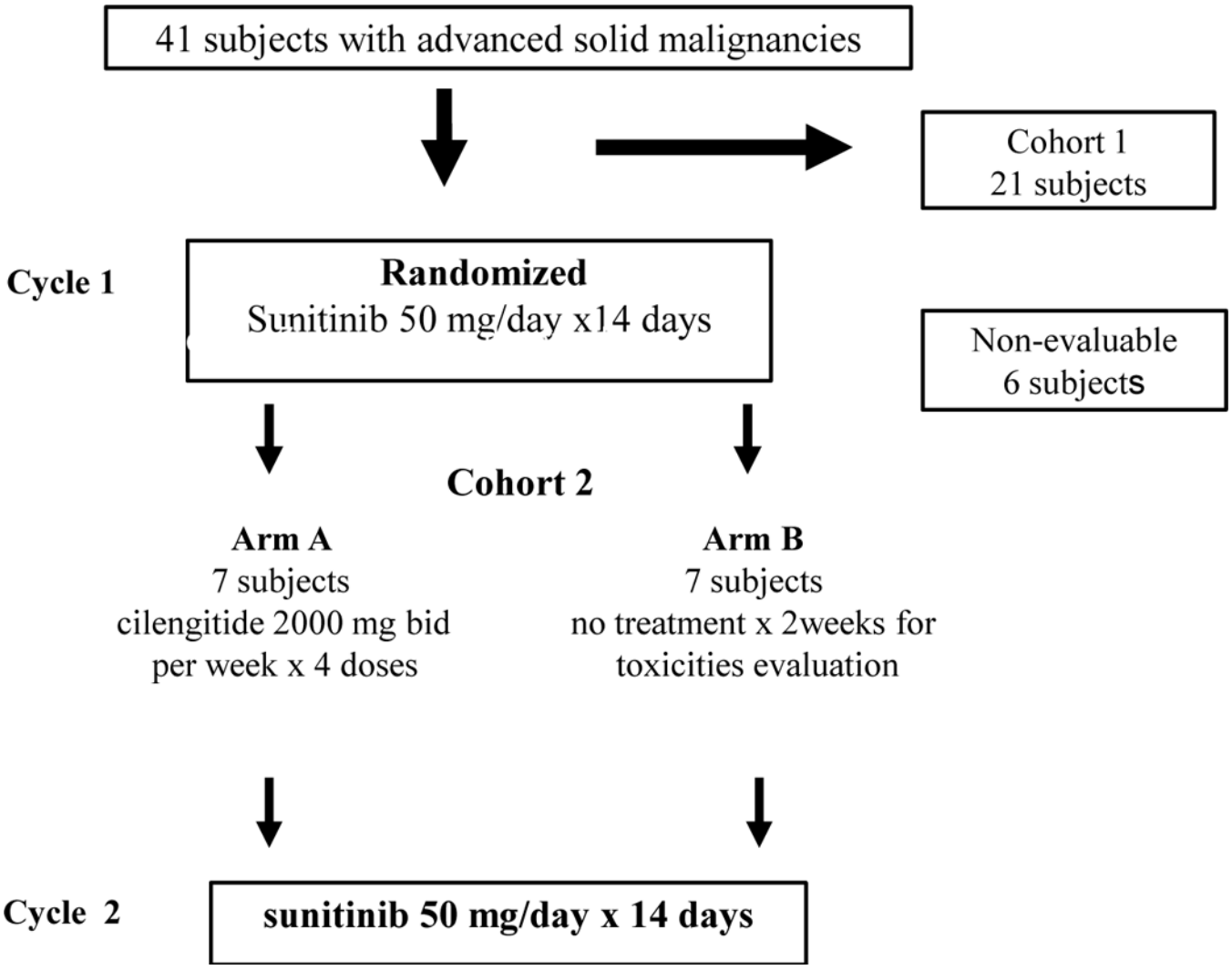

Figure 1. Study Schema

Two-arm, randomized study design schema during 2 first cycles of the treatment

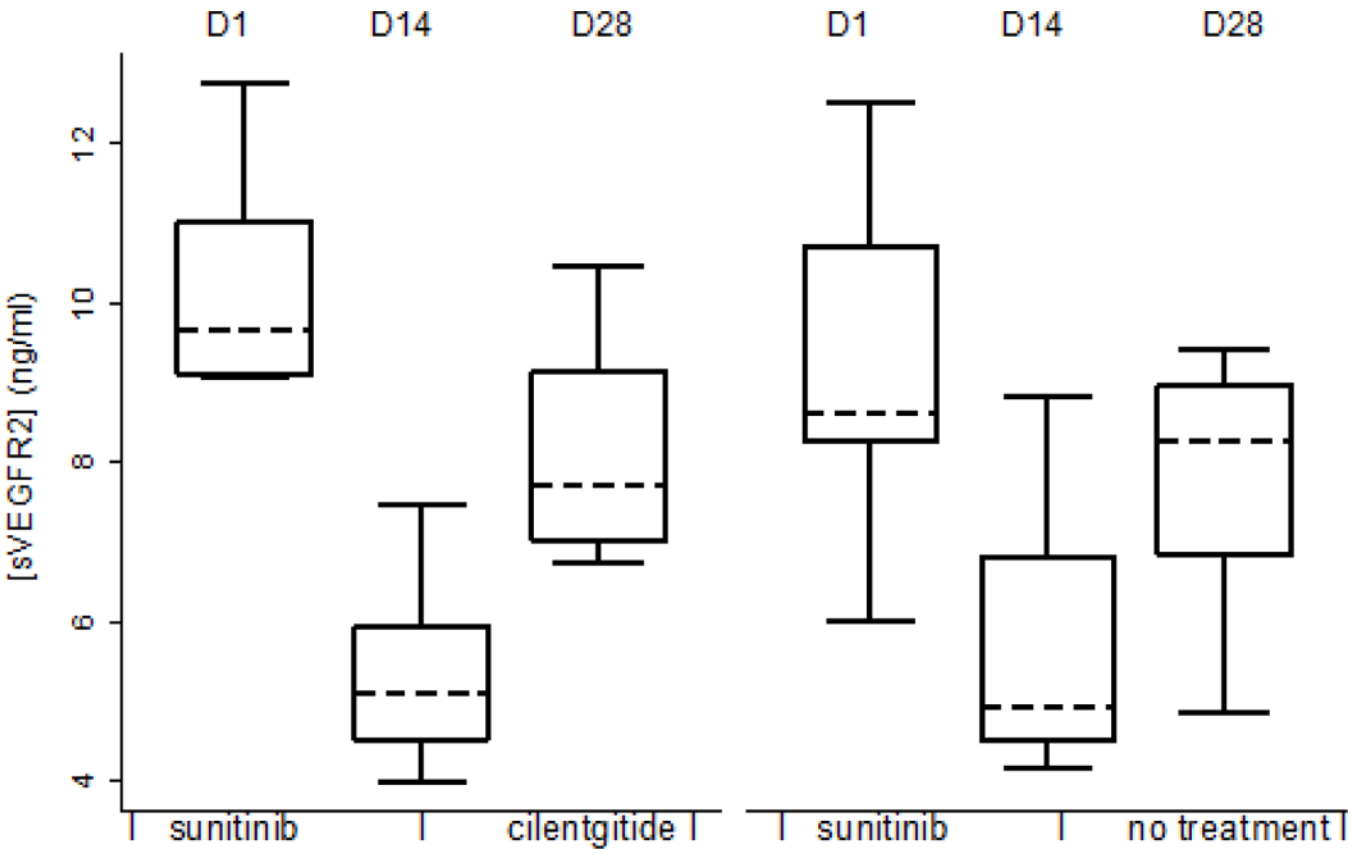

Figure 2. [sVEGFR2] response to sunitinib and cilengitide administration

Boxplots depict minimum, first quartile, median (dash lines), third quartile and maximum of [sVEGFR2] for each study arm (sunitinib/cilengitide and sunitinib/no treatment) at Day 1 (D1), Day 14 (D14), and Day 28 (D28); the intervals for each treatment administered are marked on the horizontal axis. s[VEGFR2] = soluble vascular endothelial growth factor receptor-2, ng/mL = nanograms/milliliter

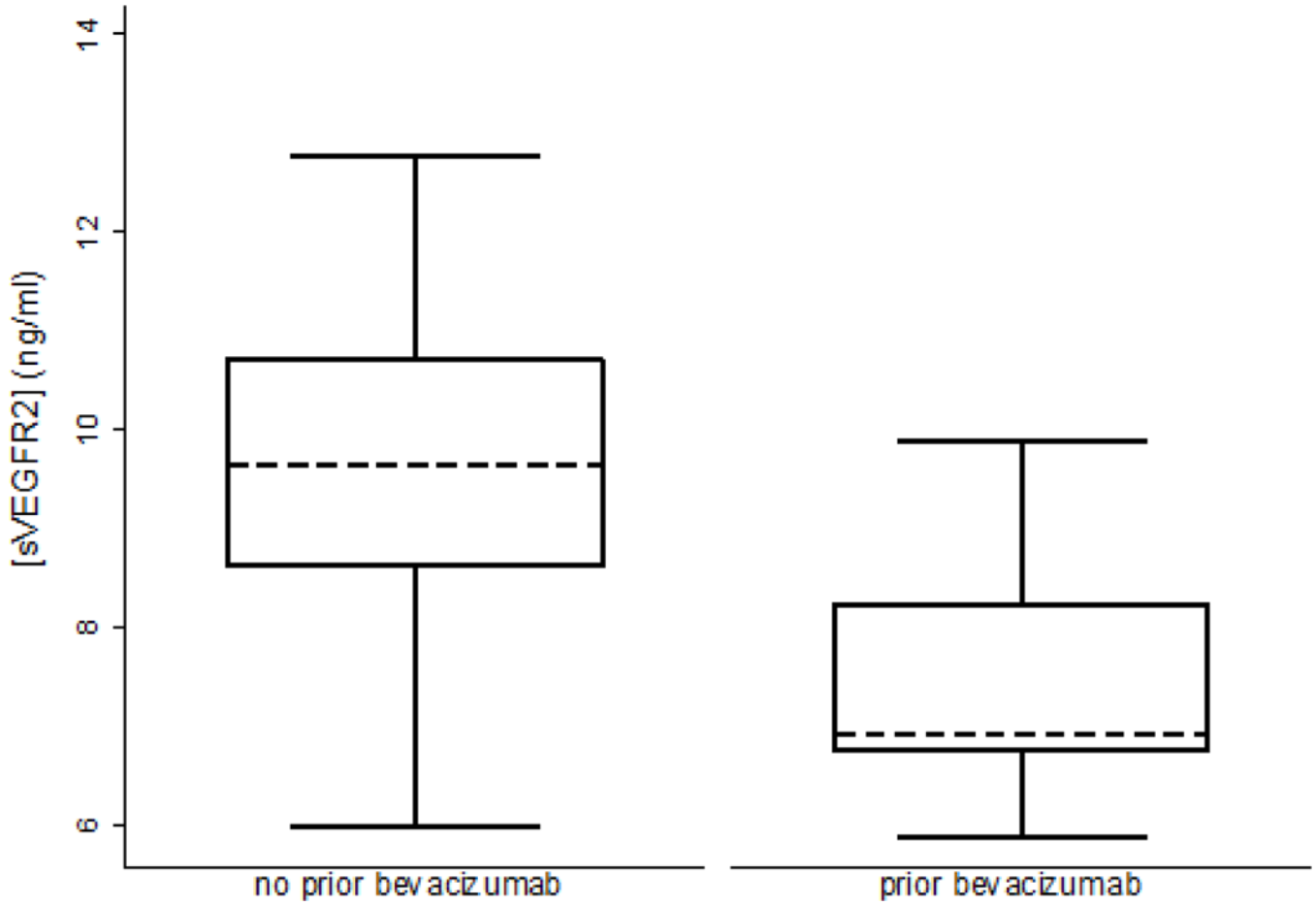

Figure 3. Pre-sunitinib [sVEGFR2] with or without prior bevacizumab therapy

Boxplots depict minimum, first quartile, median (dash lines), third quartile and maximum of [sVEGFR2] for each study group (no prior bevacizumab and prior bevacizumab) at Day 1 (D1); [sVEGFR2] = soluble vascular endothelial growth factor receptor-2, ng/mL = nanograms/milliliter

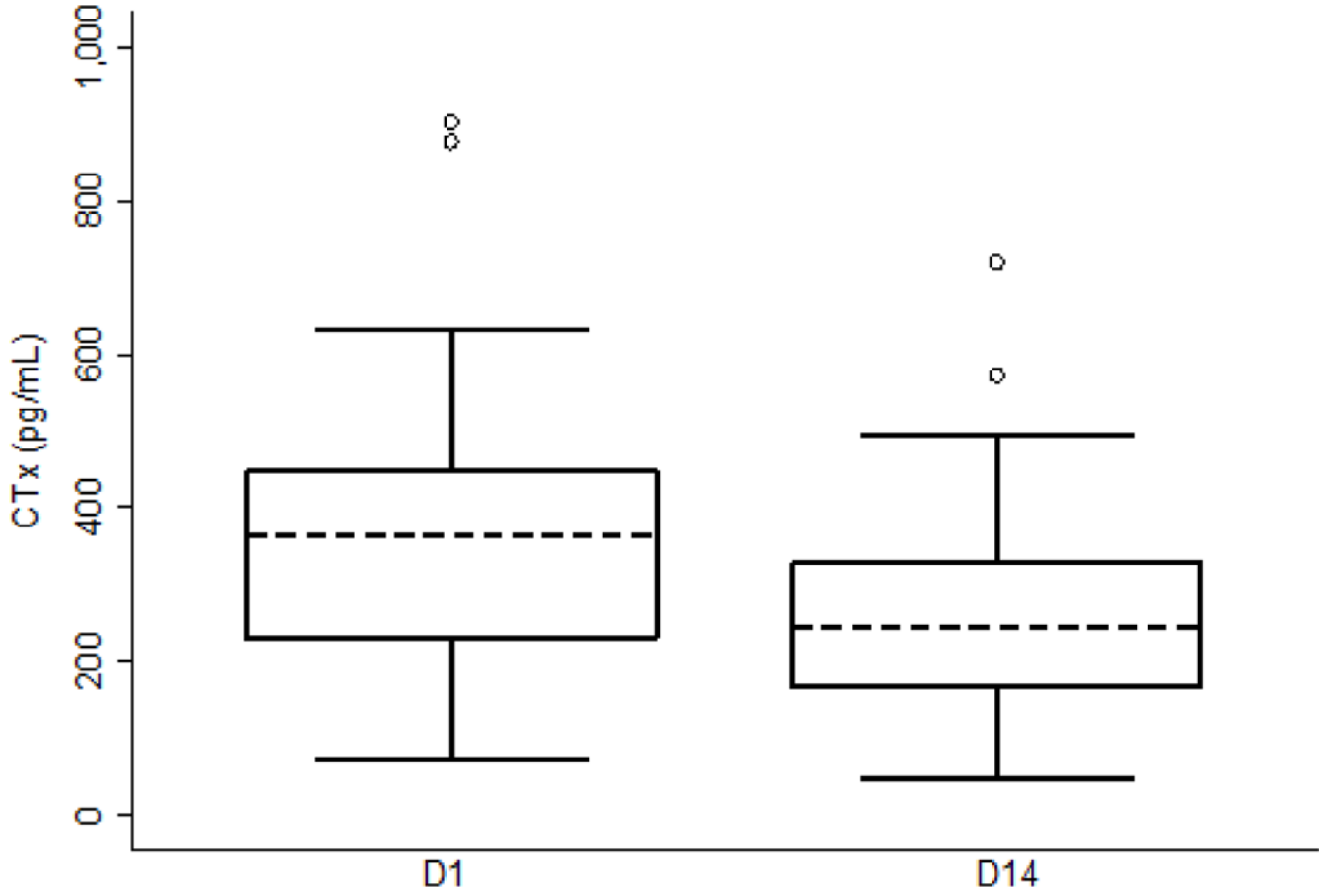

Figure 4. CTx response to sunitinib administration

Boxplots depict minimum, first quartile, median (dash lines), third quartile and maximum of CTx at Day 1 (D1) and Day 14 (D14); CTx = C-telopeptide crosslinks, pg/mL = picograms/milliliter

Table 1

Characteristics of 41 enrolled study subjects

Characteristics	N	%
Sex		
Women	20	49
Men	21	51
Age (median, range)	60 (31–81)	
Self-reported race/ethnicity		
Black non-Hispanic	4	10
White non-Hispanic	31	75
White-Hispanic	4	10
East Asian	2	5
Tumor Type		
Esophageal	6	15
Uterine/Cervical/Fallopian	5	12
Colorectal	5	12
Lung	4	10
Adenoid Cystic Carcinoma	4	10
Primary Brain Tumor	4	10
Renal	3	7
Sarcoma	3	7
Thyroid	3	7
Carcinoid/Neuroendocrine	2	6
Thymic carcinoma	1	2
Melanoma	1	2