Pharmacodynamic Study Using FLT PET/CT in Patients with Renal Cell Cancer and Other Solid Malignancies Treated with Sunitinib Malate

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Abstract

Purpose: To characterize proliferative changes in tumors during the sunitinib malate exposure/withdrawal using 3'-deoxy-3'-[18F]fluorothymidine (FLT) positron emission tomography (PET)/computed tomography (CT) imaging.

Patients and Methods: Patients with advanced solid malignancies and no prior anti-VEGF exposure were enrolled. All patients had metastatic lesions amenable to FLT PET/CT imaging. Sunitinib was initiated at the standard dose of 50 mg p.o. daily either on a 4/2 or 2/1 schedule. FLT PET/CT scans were obtained at baseline, during sunitinib exposure, and after sunitinib withdrawal within cycle #1 of therapy. VEGF levels and sunitinib pharmacokinetic (PK) data were assessed at the same time points.

Results: Sixteen patients (8 patients on 4/2 schedule and 8 patients on 2/1 schedule) completed all three planned FLT PET/CT scans and were evaluable for pharmacodynamic imaging evaluation. During sunitinib withdrawal (change from scans 2 to 3), median FLT PET standardized uptake value (SUVmean) increased +15% (range: −14% to 277%; P = 0.047) for the 4/2 schedule and +19% (range: −5.3% to 200%; P = 0.047) for the 2/1 schedule. Sunitinib PK and VEGF ligand levels increased during sunitinib exposure and returned toward baseline during the treatment withdrawal.

Conclusions: The increase of cellular proliferation during sunitinib withdrawal in patients with renal cell carcinoma and other solid malignancies is consistent with a VEGF receptor (VEGFR) tyrosine kinase inhibitor (TKI) withdrawal flare. Univariate and multivariate analysis suggest that plasma VEGF is associated with this flare, with an exploratory analysis implying that patients who experience less clinical benefit have a larger withdrawal flare. This might suggest that patients with a robust compensatory response to VEGFR TKI therapy experience early "angiogenic escape." Clin Cancer Res; 17(24): 7634–44. ©2011 AACR.

Introduction

In 1971, Judah Folkman hypothesized that angiogenesis plays an integral role in tumor growth (1). As a result, inhibiting angiogenesis was considered a reasonable strategy to prevent tumor progression and metastasis. Currently, the VEGF signaling pathway is the best described target for antiangiogenic treatment. Since 2004, multiple VEGF signaling pathway (VSP) inhibitors have been approved by the U.S. Food and Drug Administration based on improvements in overall survival (OS) and/or progression-free survival (PFS) validating this target. The first of these drugs approved was bevacizumab, a humanized monoclonal antibody against VEGF. Although its single-agent activity in most solid malignancies was low (2, 3), it is approved for use in metastatic colorectal cancer (CRC; refs. 4, 5) and non-small cell lung carcinoma (NSCLC; ref. 6) due to improvements in OS when combined with cytotoxic chemotherapy. In comparison, newer agents that target the VEGF receptor (VEGFR) tyrosine kinase inhibitor (TKI) withdrawal flare have been approved as mono- or combination therapies. Multiple VEGFR TKIs, including sorafenib, sunitinib, and pazopanib, are now approved as monotherapy in metastatic renal cell carcinoma (mRCC; refs. 7–11) and gastrointestinal stromal tumors (12). Despite relatively modest OS improvements, all of these clinical studies confirm that targeting angiogenesis is a validated treatment strategy.

Sunitinib malate is a receptor TKI that exerts its key mechanism of action in mRCC through inhibition of
During sunitinib exposure and temporary withdrawal, there might be an increase in tumor cell proliferation (instead of an inflammatory or vascular effect). On the basis of our clinical observations and available clinical trial and preclinical data, we hypothesized that there is a period of rapid tumor progression in most solid tumors following acute cessation of sunitinib. This study was designed to explore this hypothesis by molecular imaging to better understand the pharmacodynamic (PD) changes with sunitinib. As sunitinib is administered using an "intermittent" schedule, it allows imaging assessment both during treatment exposure and during acute treatment withdrawal. Better understanding of the PD changes with sunitinib would also provide necessary understanding to test the second hypothesis of synergistic combination of VEGFR TKI with cytotoxic therapy.

### FLT PET/CT in Patients Treated with Sunitinib

**Translational Relevance**

Inhibiting the VEGF signaling pathway is important when treating patients with advanced cancer. Despite the benefits shown when combining bevacizumab with cytotoxic chemotherapy in certain solid malignancies, investigations combining VEGF receptor (VEGFR) tyrosine kinase inhibitors (TKI) with cytotoxic chemotherapy have not shown similar promise despite higher single-agent activity of VEGFR TKIs than bevacizumab. This has raised questions whether concurrent VEGFR TKIs may be diminishing the effect of cytotoxic chemotherapy and whether alternative dosing strategies may be better. Given the number of newer VEGFR TKIs in development, understanding of the pharmacodynamic (PD) effects of VEGFR TKIs in patients is needed. Here, we present a PD study using 3'-deoxy-3'-[18F]fluorothymidine positron emission tomography/computed tomography in patients treated with sunitinib. This study provides new insight into the VEGFR TKI withdrawal flare and how this knowledge may be incorporated into more optimal sequencing strategies with these agents. In addition, this study highlights the power of using functional imaging to assist in early drug development.

### FLT PET/CT Imaging

- **VEGFRs -1, -2, and -3** and platelet-derived growth factor receptors -α and -β on tumor vascular endothelium (13).
- The approved schedule is 50 mg by mouth daily for 4 weeks followed by a 2-week break (4/2 schedule); however, sunitinib has also been studied on a 2/1 schedule (14).
- In treatment-naive mRCC patients, a randomized phase III trial showed improved median PFS with sunitinib as compared to cytokines in patients with an investigator-reported objective response rate (ORR) for sunitinib of 47% (3% complete responses; ref. 10). Reports in the literature of complete response (CR) in mRCC patients treated with sunitinib have remained consistently low (15–17). In contrast with sunitinib, single-agent bevacizumab seems to produce a relatively modest ORR and PFS of 10% to 13% and 4.8 to 8.5 months, respectively (18, 19).
- Overall, all patients eventually progress on antiangiogenic therapy. This has generated many questions about how patients become resistant to antiangiogenic drugs and led many investigators to empirically combine sunitinib with other agents in an attempt to prolong benefit.

We have observed that patients with mRCC treated with sunitinib on the 4/2 schedule can achieve clinical improvement in sites of painful metastases during sunitinib exposure, but can develop recurrent pain within 4 to 5 days of stopping sunitinib. We suspected that this VEGFR TKI withdrawal flare may be associated with a period of rapid, transient tumor regrowth (20, 21) and that the pain would decrease with resumption of therapy. We suggested that during this "flare," the tumor would be more engaged in S phase of the cell cycle (proliferation) and thus more responsive to cytotoxic chemotherapy. However, several key questions remain: (i) whether the VEGFR TKI withdrawal flare is common, (ii) whether it is specific to clear cell renal cell carcinoma as opposed to other solid tumor types, and (iii) whether the flare represents a true increase in tumor cell proliferation (instead of an inflammatory or vascular effect). On the basis of our clinical observations and available clinical trial and preclinical data, we hypothesized that there is a period of rapid tumor progression in most solid tumors following acute cessation of sunitinib. This study was designed to explore this hypothesis by molecular imaging to better understand the pharmacodynamic (PD) changes with sunitinib. As sunitinib is administered using an "intermittent" schedule, it allows imaging assessment both during treatment exposure and during acute treatment withdrawal. Better understanding of the PD changes with sunitinib would also provide necessary understanding to test the second hypothesis of synergistic combination of VEGFR TKI with cytotoxic therapy.

### FLT PET/CT Imaging

- **3'-Deoxy-3'-[18F]fluorothymidine (FLT)** is a tracer used for imaging tumor proliferation by positron emission tomography (PET; refs. 22–26).
- Direct correlation between FLT uptake and proliferation as assessed by Ki-67 labeling index has also been observed when full kinetic analysis is carried out (27–30). As a result, use of FLT PET/computed tomography (CT) scanning was ideal for this study, as it allowed noninvasive assessment of proliferation PDs in patients treated with sunitinib.

This study was designed and conducted at the University of Wisconsin Carbone Cancer Center. The goal was to use FLT PET/CT to characterize and quantify changes in tumor proliferation during sunitinib exposure and temporary withdrawal, explore PD changes that may yield insight into predicting treatment response/failure, and gain further insight into the physiologic effects of VEGFR TKI in patients with advanced malignancies. In addition to clinical outcomes, the aim was to correlate FLT PET/CT imaging results with both sunitinib pharmacokinetic (PK) and plasma VEGF levels as potential explanations for the withdrawal flare.

### Patients and Methods

#### Patient selection

Patients were required to have histologically or cytologically confirmed renal cell cancer or any other solid malignancy (excluding lymphoma) that was metastatic or unresectable and for which no standard curative therapy existed. For the renal cell cancer subset, a component of clear cell histology was required. Other key inclusion criteria included the following: measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST) 1.0 guidelines (31), appropriate target lesions for FLT PET/CT assessment (minimum 1.5 cm, located in a region of body with low motion artifact, reasonable ability to delineate tumor boundaries on CT and PET, nonhepatic due to high background FLT uptake), controlled blood pressure (<140/90 mmHg) at enrollment, and normal organ and bone marrow function.

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**Translational Relevance**

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Patients with prior anti-VEGF treatment (for example, bevacizumab or VEGFR TKI) were excluded, as were patients taking inducers or moderate or strong inhibitors of the CYP3A4 liver enzyme. All patients signed informed consent documents approved by the Institutional Review Board at the University of Wisconsin. Additional approval by the Radioactive Drug Research Committee at the University of Wisconsin was obtained given use of an experimental tracer. This study was conducted in accordance with the Declaration of Helsinki.

**Drug administration and study design**

Sunitinib was supplied in 12.5, 25, and 50 mg capsules. Two treatment schedules were assessed (Fig. 1). On treatment schedule A (4/2 schedule), patients initially took one 50 mg capsule of sunitinib daily for 4 consecutive weeks followed by 2 weeks (drug withdrawal) with no sunitinib (6-week cycle). On treatment schedule B (2/1 schedule), patients took one 50 mg capsule of sunitinib daily for 2 consecutive weeks followed by 1 week (drug withdrawal) with no sunitinib. This 2/1 schedule was administered every 3 weeks (repeated once) during the 6-week cycle. PD and PK time points, described in greater detail below and depicted in Fig. 1, included the following: (i) baseline (before sunitinib treatment), (ii) maximum sunitinib exposure (near end of consecutive dosing period), and (iii) maximum sunitinib washout (near end of drug withdrawal period).

**FLT PET/CT imaging**

To minimize uncertainties inherent in PET imaging, strict imaging procedures were followed, including employing optimized and standardized image acquisition and reconstruction protocols and conducting comprehensive image analysis procedures. At the beginning of each imaging session, a CT scan was obtained on a Discovery LS (General Electric) PET/CT scanner. Dynamic PET imaging was carried out for the first 30 minutes over the predefined region (15 cm field of view) to obtain necessary data for kinetic analysis. After the dynamic scan, a static whole body scan (6 scanning positions ~90 cm total length) was initiated at 60 minutes post injection. All scans were acquired in a 2-dimensional mode and reconstructed on a 256 × 256 reconstruction grid using OSEM iterative reconstruction algorithm with 5 mm postfiltering. The dynamic FLT PET/CT imaging data were used to conduct kinetic analysis of the imaging data, thus allowing increased correlation to the biological parameters (cell proliferation index) and extraction of vasculature data (e.g., perfusion/permeability and blood volume). The static whole body FLT PET/CT imaging data were employed to identify primary tumor and metastases. The CT data were analyzed to establish anatomic changes in tumor size. Each metastasis was identified by an experienced nuclear medicine physician and segmented by a combination of semiautomatic segmentation methods using Amira (Visage Imaging Inc.) software with up to 5 metastases used in image analysis. As the whole body kinetic analysis is impractical, we conducted analysis on various standardized uptake value (SUV) measures of FLT uptake. $SUV_{mean}$, $SUV_{max}$, $SUV_{peak}$, and $SUV_{total}$ were recorded and analyzed. Multiple SUV measures were investigated to capture intraleSION response heterogeneity [e.g., most proliferative parts of the tumor ($SUV_{max}$) might not respond, even though tumor as whole ($SUV_{mean}$) does respond]. To cross-validate our results, FLT PET SUV measures were compared with measures extracted from the full kinetic analysis. All image analysis was conducted at the University of Wisconsin Image Analysis Core (IMAC) facility.

**Sunitinib PK and plasma VEGF**

Plasma PK levels of sunitinib were drawn before initiating sunitinib therapy on day 1 and on the days PET scans were carried out. To monitor changes in serum VEGF levels,
samples were collected: on day 1 before dosing, weekly during cycle 1, and on day 1 of each subsequent cycle.

Plasma VEGF levels were evaluated using a commercially available 96-well plate quantitative sandwich immunoassay (Quantikine human VEGF; R&D Systems). Sunitinib and the active metabolite SU12662 in plasma were evaluated by liquid chromatography/tandem mass spectrometry as previously described (32). The analytic system consisted of an HPLC coupled directly to a model API 4000 triple quadrupole mass spectrometer equipped with a Turbo V atmospheric pressure ionization source fitted with the electrospray probe (Applied Biosystems/MDS Sciex). The assay was validated in our laboratory and showed a linear range of 3.12 to 100 ng/mL, with $r^2 = 0.998$, with an intraday variability of 3.37% for sunitinib and 6.55% for SU12662. Interday variability was 2.20%/4.03% for the low sunitinib/SU12662 standard of 6.25/3.12 ng/mL; it was 0.45%/0.99% for the 100/50 ng/mL. The lower limit of quantification was 3.12 ng/mL for both sunitinib and SU12662. Recovery based on standard addition was 98.3% for sunitinib and 93.6% for SU12662.

Treatment response evaluation

Patients were evaluated for response and progression after every 2 cycles (every 12 weeks) of therapy using RECIST 1.0 guidelines (31). Those with CR or partial response (PR) as the best response by RECIST were classified as objective responders. Those with stable disease or progressive disease as the best response by RECIST were classified as objective nonresponders.

An exploratory, unplanned analysis was added to categorize patients by clinical benefit (CB) status (yes/no). Patients who discontinued sunitinib prior to month 6 for any reason (including progression, toxicity, patient/physician discretion, etc.) were categorized as having no clinical benefit (NCB) and those who remained on sunitinib or after 6 months were categorized as having CB. Given the heterogeneous patient population (different tumor types, variable prior therapies received, etc.) in this PD trial, this endpoint of CB was felt to capture additional information not included in the objective response status. The objective response and CB status were correlated with PD FLT PET imaging data.

Statistical methods

The study was powered to detect a mean change of 33% in the SUVs parameters at the 5% significance level, assuming an anticipated SD ranging from 20% to 30%. A sample size of 10 evaluable patients was planned for each of the 2 treatment schedules. With a sample size of 10 evaluable patients per treatment schedule, an anticipated mean change of 33% in the SUVs parameters would have been detected with 87% power at the 2-sided 5% significance level, assuming an SD of 30%. We anticipated a drop-out rate of up to 20% and, hence, accrued a total of 25 patients.

FLT PET imaging PD measures, plasma VEGF, and PK levels of sunitinib and its metabolite were summarized in terms of medians and ranges for each assessment time point. As the distribution of FLT PET SUV values are known to be left skewed (33), nonparametric tests were used for carrying out statistical inference, and median profile plots were used to represent the data graphically. Specifically, changes from baseline were evaluated by a nonparametric Wilcoxon signed-rank test. Associations between changes in the various PK and imaging PD parameters were analyzed using Spearman’s rank correlation ($r_s$) analysis. Furthermore, to adjust the associations between changes in PD parameters for changes in sunitinib concentration, partial Spearman rank correlation analysis ($r_{ps}$) was conducted. The comparison of changes in PD parameters between responders and nonresponders was carried out using a nonparametric Wilcoxon rank test. All analyses were conducted by SAS software (version 9.2; SAS Institute). All statistical tests were 2-sided and $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

From June 2007 to September 2009, 25 patients were enrolled at the University of Wisconsin Carbone Cancer Center. To be evaluable for PK/PD analysis, a patient must have taken the full cycle of sunitinib as planned and received a set of all 3 FLT PET/CT scans of adequate quality. Of 25 enrolled patients, 16 were evaluable. Technical problems, which included radiotracer instability and the cyclotron going offline, resulted in 2 patients not receiving the first FLT PET/CT scans. An additional 7 patients who received at least 1 FLT PET/CT scan were unevaluable for various reasons (FLT production not meeting quality control standard, PET/CT problem, treatment tolerability, etc.), leaving 8 evaluable patients each in both treatment schedules. One patient with mRCC (systemic treatment-naïve) on the 2/1 schedule remains on study after 22 cycles of therapy.

Median age was 60 (range: 42–76), whereas 75% of subjects were male. As specified by the protocol, nearly half ($n = 7$) of evaluable patients had mRCC (4/2 schedule: $n = 5$; 2/1 schedule: $n = 2$). The remainder represented a diverse group of tumor types: esophagus ($n = 2$), liver (hepatocellular; $n = 2$), prostate ($n = 1$), sarcoma ($n = 1$), small cell lung ($n = 1$), thymus ($n = 1$), and uterine carcinosarcoma ($n = 1$). Six of 7 patients with mRCC had prior nephrectomy. The median number of prior systemic therapy regimens was 1 (range: 0–4). All subjects were naïve to anti-VEGF therapy, as specified by the protocol. Seven subjects (44%) had been treated with prior chemotherapy, whereas 3 subjects (19%) had received investigational treatment. One subject with mRCC had undergone treatment with an investigational agent and immunotherapy with interleukin-2, whereas the other 6 patients with mRCC were systemic treatment-naïve.

FLT PET/CT imaging

Figure 2 shows examples of the tumor flare phenomenon on FLT PET/CT in various tumor types. Quantitative assessment of the FLT PET/CT imaging parameters are
summarized in Table 1 and Fig. 3. Even though the comprehensive image analysis included many imaging parameters, we report here only the mean standardized uptake value (SUVmean) and maximum standardized uptake value (SUVmax) because these likely represent distinct parameters of cellular proliferation distribution. The average pretreatment SUVmean across patient population was 3.2 (range: 1.4–7.9), decreasing to 2.1 (range: 0.6–3.8), and rebounding back to 3.1 (range: 0.7–8.3). The average pretreatment SUVmax across patient population was 10.1 (range: 4.1–30.2), decreasing to 6.0 (range: 1.5–13.4), and rebounding back to 10.1 (range: 2.6–32.1). Note that all the SUV data used were normalized to the pretreatment scan for each patient, as the overall objective was to quantify the

### Table 1. Changes in PD and PK parameters during the sunitinib treatment and withdrawal periods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>4/2 Schedule (n = 8)</th>
<th>2/1 Schedule (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment</td>
<td>Withdrawal</td>
</tr>
<tr>
<td><strong>Median change (range)</strong></td>
<td><strong>P</strong></td>
<td><strong>Median change (range)</strong></td>
</tr>
<tr>
<td>Sunitinib, Css (ng/mL)</td>
<td>63 (37–184)</td>
<td>0.005</td>
</tr>
<tr>
<td>Sunitinib met, Css (ng/mL)</td>
<td>20 (14–39)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VEGF (pg/mL)</td>
<td>168 (51–461)</td>
<td>0.008</td>
</tr>
<tr>
<td>SUVmean (% change)</td>
<td>–16 (–100 to 0)</td>
<td>0.031</td>
</tr>
<tr>
<td>SUVmax (% change)</td>
<td>–34 (–100 to 4)</td>
<td>0.016</td>
</tr>
</tbody>
</table>

NOTE: The Wilcoxon signed-rank test was used for comparing the change between time points during each period. Percentage change was used for SUVmean and SUVmax, whereas absolute changes were used for all other values.

Abbreviations: Css, concentration at steady state; met, metabolite.

Figure 2. Examples of the tumor flare phenomenon on FLT PET/CT in various tumor types. ccRCC, clear cell renal cell carcinoma. SCLC, small cell lung cancer.
Sunitinib PKs and plasma VEGF

The median change from baseline in plasma sunitinib concentrations between baseline and the second PET/CT scan was 63 ng/mL (range: 37–184 ng/mL) in the 4 of 2 cohort and 62 ng/mL (range: 32–162 ng/mL) in the 2 of 1 cohort. The median change from the baseline in plasma sunitinib metabolite concentrations were 20 ng/mL (range: 14–39 ng/mL) in the 4 of 2 cohort and 21 ng/mL (range: 4.8–52 ng/mL) in the 2 of 1 cohort. After sunitinib withdrawal, and at the time of the third PET/CT scan, plasma concentrations declined to near baseline levels. Changes in levels of sunitinib and its metabolite were statistically significant during both the exposure and withdrawal period and in both treatment groups.

Plasma VEGF increased during sunitinib exposure in both treatment groups, as expected. Likewise, plasma VEGF decreased during sunitinib withdrawal in both treatment groups. Changes in plasma VEGF were statistically significant during the exposure and withdrawal period and in both treatment groups.

Objective response evaluation

There were 16 total patients evaluable for objective response per RECIST 1.0, with an overall ORR of 36% (95% CI: 16–61). The response rate (PR) for the 4/2 schedule (n = 8) was 38% and the response rate for the 2/1 schedule (n = 8) was 33%. No complete responses were observed. Of the 16 patients evaluable for FLT PET/CT imaging, only 14 of the patients were evaluable for objective response per RECIST. 1.0. Of the 2 un evaluable patients, 1 patient with esophageal cancer was taken off study before the planned week 12 disease assessment secondary to a cerebral vascular accident. The second patient (uterine carcinosarcoma) withdrew consent after 48 days of study participation for personal reasons.

Correlation between PK, PD, and response parameters

The Spearman rank correlations (rₛ) for the changes from baseline to the end of the treatment and withdrawal period between the various PK and PD parameters (i.e., sunitinib concentration, VEGF, and SUV) were calculated and are shown in Table 2. Statistically significant negative correlations were observed between VEGF and SUV₁₀₀₀ and VEGF and SUV₅₀₀ during sunitinib treatment on the 4/2 treatment schedule: rₛ = −0.92 (P = 0.003) and rₛ = −0.79 (P = 0.036), respectively.

Parameters were also analyzed with the 2 groups combined. After adjusting for sunitinib concentration in the combined group, VEGF was found to be negatively correlated with both SUV₁₀₀₀ and SUV₅₀₀ during sunitinib treatment with partial Spearman rank correlations of rₛ = −0.69 (P = 0.0041) and rₛ = −0.71 (P = 0.0030), respectively. Similarly, VEGF was found to be negatively correlated with both SUV₁₀₀₀ and SUV₅₀₀ during sunitinib withdrawal after adjusting for sunitinib concentration in the combined group (rₛ = −0.82, P = 0.0002; rₛ = −0.85, P = 0.0001). Multivariate analysis, involving both changes in VEGF and sunitinib concentration as predictors for

Figure 3. PK and PD parameters at time points throughout the sunitinib cycle. Thin dotted lines show individual patient results. ccRCC, clear cell renal cell carcinoma.
changes in $\Delta$SUV$_{\text{mean}}$ showed that changes in VEGF independently predicted change in SUV$_{\text{mean}}$ during the sunitinib treatment period ($P = 0.003$).

Furthermore, the association between changes in PD parameters and clinical response was evaluated. When both treatment groups were combined, objective nonresponders ($n = 9$) had statistically significant median increases in SUV$_{\text{mean}}$ ($+29\%$, range: $-5\%$ to $277\%$, $P = 0.012$) and SUV$_{\text{max}}$ ($+36\%$, range: $-11\%$ to $221\%$, $P = 0.039$) during sunitinib withdrawal. Objective responders ($n = 5$) seemed to have a trend toward unchanged SUV$_{\text{mean}}$ ($+0.00\%$, range: $-14\%$ to $8\%$, $P = 0.648$) and SUV$_{\text{max}}$ ($+2.6\%$, range: $-33\%$ to $31\%$, $P = 0.963$) during sunitinib withdrawal. However, the differences between the responder and nonresponder groups were not statistically significant by the Wilcoxon-rank sum test.

In an exploratory, unplanned analysis, the association between the changes in SUV parameters and the CB status was evaluated. The CB rate for both groups combined was $32\%$: $43\%$ for the 4/2 schedule and $18\%$ for the 2/1 schedule. Figure 4A and B shows the median change in SUV$_{\text{mean}}$ and SUV$_{\text{max}}$ over different scan periods for the CB and NCB groups. Subjects with CB showed only a trend toward no change in SUV$_{\text{mean}}$ ($+0.7\%$, $P = 0.238$) and a trend toward increase in SUV$_{\text{max}}$ ($+21\%$, $P = 0.278$). Figure 4 visually suggests that relative lack of withdrawal flare (peak exposure to washout) might predict CB, whereas a larger withdrawal flare seemed to correlate with a decreased chance of CB (e.g., NCB). However, the differences in median SUV change during sunitinib withdrawal between the CB and NCB groups were not statistically significant by the Wilcoxon-rank sum test.

### Discussion

Antiangiogenic strategies are incorporated as treatment for many oncologic diseases, and studies to further optimize their use are ongoing. Despite hundreds of clinical trials, we still do not have a complete understanding of why some patients fail to respond to VSP inhibitors and other patients, despite initially responding to treatment, later progress on VSP therapy. We have learned through trial and error that bevacizumab is most effective when combined with other agents (e.g., cytotoxic chemotherapy). Most surprising is that despite higher single-agent activity with VEGFR TKI agents (compared with bevacizumab alone), trials reported to date combining VEGFR TKI agents with cytotoxic chemotherapy have not shown the same promise. Phase III trials of carboplatin/paclitaxel with or without sorafenib in both advanced melanoma (34) and NSCLC (35), gemcitabine with or without axitinib in pancreatic cancer (36), and sunitinib with or without FOLFIRI in mCRC (37) have all failed to show an improvement in the primary endpoint with the addition of a VEGFR TKI. Furthermore, patients with squamous histology in the NSCLC trial seemed to have
increased mortality on the sorafenib-chemotherapy arm compared with the placebo-chemotherapy arm.

It was postulated by Teicher in 1996 that combined administration of antiangiogenic and cytotoxic therapies would yield maximal benefit because such combinations would destroy 2 separate compartments of tumors—cancer cells and endothelial cells (38). This hypothesis was supported by the clinical data showing that bevacizumab treatment resulted in a significant increase of 5 months in OS in patients with metastatic CRC, when given in combination with standard chemotherapy (4), whereas as a single agent, it produced only modest objective responses (2, 18). Jain in 2001 also proposed that judicious application of antiangiogenic agents can normalize the abnormal tumor vasculature, resulting in more efficient delivery of drugs and oxygen to the targeted cancer cells, and therefore enhancing the effectiveness of chemotherapy and radiation (39). However, excessive vascular regression may be counterproductive as it compromises drug and oxygen delivery.

A normalization window, or a period during which the blood vessels become normalized, is the theoretically optimal time when the addition of chemotherapy or radiotherapy to antiangiogenic therapy should yield the best therapeutic outcome (40). A novel study using dynamic-contrast enhanced MRI showed improved tumor perfusion, indicating normalization of blood vessels, in a murine RCC model after 3 days of sunitinib administration, but not after 1 day of sunitinib administration; mice were subsequently treated with weekly gemcitabine (41). This study suggests that chemotherapy combination with antiangiogenic therapy may be optimized through the use of functional imaging. Understanding how VEGFR TKI therapy may antagonize cytotoxic chemotherapy was another important goal during the analysis of our study, as ongoing trials combining newer VEGFR TKIs with chemotherapy continue.

Here, we show that nearly all patients with advanced solid malignancies have some initial reduction in tumor proliferation as measured by FLT PET/CT after 4 weeks of sunitinib treatment. This reduction was more apparent on the 4/2 schedule versus 2/1 schedule, and more significant for the FLT PET SUV_{max} than SUV_{mean}, which is likely because of a greater duration of sunitinib exposure. Nevertheless, in both schedules, during the brief treatment break patients experienced a relative increase in FLT uptake consistent with an increase in tumor proliferation during the treatment withdrawal period. This finding supports our hypothesis and was seen in both the patients with renal cell cancer as well as other solid malignancies. Because of the longer exposure to sunitinib and longer treatment break, we would have predicted that the 4/2 schedule would have a larger "withdrawal flare," but this was not the case. This may simply be due to the low number of patients assessed; however, a complex relationship between the clinical flare, sunitinib PK, and circulating VEGF could also be playing a role. We are addressing this question in currently ongoing trials using a VEGFR TKI with a shorter half-life (e.g., NCT00859118).

During sunitinib exposure, it was observed that most (but not all) patients had some decline in SUV (Fig. 3). Looking at the individual patients (dotted lines), one can begin to appreciate the differences in response. Possible categories include those patients that experience no change or a small increase in SUV (proliferation) while on sunitinib but still experience a sunitinib withdrawal flare in which SUV greatly increases; individuals in which an initial decline in SUV is seen during sunitinib exposure but during withdrawal have an increase in proliferation; and individuals in which no apparent change in SUV is seen during/after sunitinib treatment. In an attempt to link these observations with clinical outcomes (challenging given heterogeneous disease population and low numbers of patients), we conducted an exploratory analysis to evaluate the association between changes in SUV and the CB. As shown in Fig. 4A and B, changes in SUV parameters suggest that those with lack of CB have a larger proliferative flare. Looking at the individual patient data (dotted lines), one can appreciate that initial steep declines in SUV during sunitinib treatment do not

Figure 4. Change in SUV_{max} (A) and SUV_{mean} (B; normalized to baseline) by CB group. Note that the 4/2 and 2/1 schedules are combined so that the abscissa is scaled to sunitinib exposure (and scan time points) instead of time. ccRCC, clear cell renal cell carcinoma.
always correlate with CB. Our explanation for these findings is that sunitinib has both antiangiogenic and antitumor activity in most solid tumors as evidenced by a decrease in FLT PET uptake in most patients. Treatment leads to increased physiologic hypoxia, which results in a compensatory increase in proangiogenic factors, resulting in angiogenic escape. Thus, the greater the ability of the host (patient) to compensate for treatment-induced hypoxia, the more likely that the patient will experience early sunitinib treatment failure. On the contrary, if the host cannot robustly compensate for treatment-induced hypoxia, then angiogenic escape is less likely to occur. The implications of this are enormous: this may provide a rationale to test antiangiogenic combinations to ameliorate this compensatory response and personalize treatment by appropriate dosing schedule.

Our data suggest that the sunitinib withdrawal flare correlates with shorter duration of CB and that the largest contributor on univariate and multivariate analysis to drive the flare is plasma VEGF ligand level. It has been observed that baseline VEGF levels gradually increase with chronic sunitinib exposure (42). Although this may be an explanation for eventual treatment failure, the obvious implication is that we do have agents that can target VEGF ligand (e.g., bevacizumab). Although studies testing the concurrent combination of sunitinib with bevacizumab have raised concerns about overlapping toxicities (43, 44), suggestions of increased antitumor activity were seen. This raises the question of whether a "sequential" approach can be used to minimize overlapping toxicity and prolong benefit. Our current data have resulted in an ongoing study titled “Phase I Study of Sequential Sunitinib and Bevacizumab in Patients with Metastatic Renal Cell Carcinoma and Other Advance Solid Malignancies” (NCT01243359) testing sequential sunitinib with bevacizumab. Sunitinib is administered on the standard 4/2 schedule, with low-dose bevacizumab given on day 29 (at start of the 2-week treatment withdrawal) to suppress flare and cycles repeated every 6 weeks. FLT PET/CT imaging is being planned to see whether the addition of low-dose bevacizumab will suppress the proliferative flare during sunitinib withdrawal.

One question that might be raised is whether the change in FLT PET uptake we observe is a reflection of tumor proliferation or simply vascular effect (e.g., decreased perfusion in tumor results in decreased tracer delivery resulting in lower FLT uptake). To address this question, we carried out dynamic FLT PET/CT imaging. Using compartmental modeling (intravascular, extravascular, and intracellular), one can calculate $K_i$, which reflects the proliferative rate when corrected for $K_p$, which represents the vascular permeability/perfusion (45). In summary, the FLT PET SUV and FLT PET $K_i$ trends were the same, indicating that FLT PET SUV analysis does adequately represent proliferative activity, which is consistent with both clinical and anatomic imaging observations.

In summary, we have shown that during sunitinib treatment on both the 4/2 and 2/1 schedules, there are statistically significant increases in median sunitinib concentrations and median serum VEGF levels, and a median decrease in cellular proliferation as measured by $SUV_{mean}$ and $SUV_{max}$. Change in VEGF during sunitinib treatment predicted change in $SUV_{mean}$ during sunitinib withdrawal, which fits with the biologically plausible hypothesis that the rise in VEGF ligand during treatment may drive tumor flare during the withdrawal period. Finally, there was a suggestion of a more brisk proliferative flare in nonresponders compared with responders. This suggests that patients with a robust compensatory response to treatment-induced hypoxia (e.g., large flare) might develop early treatment failure as a result.

Disclosure of Potential Conflicts of Interest

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