ORIGINAL ARTICLE

Quantitative Insight in Utilizing Circulating Angiogenic Factors as Biomarkers for Antiangiogenic Therapy: Systems Pharmacology Approach

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Circulating angiogenic factors (CAF) like vascular endothelial growth factor (VEGF), placental growth factor (PlGF), and sVEGFR2 have potential as biomarkers for antiangiogenic therapy. The interpretation of changes in CAF is complicated by the dynamic nature of the tumor and host cells emanating CAF in response to VEGF pathway inhibition. We developed a systems pharmacology model of anti-VEGF agents to investigate CAF modulation by tumor and host cells, and the relationship between overall CAF changes in response to sunitinib and antitumor efficacy. This model distinguishes between the tumor cells’ contributions from tumor-independent response to therapy and total plasma CAF correlating with antitumor activity. Altered VEGF is more likely to serve as a useful biomarker reflecting tumor responses in cancer patients whose pretreatment VEGF is higher than baseline VEGF in healthy subjects. Our findings provide a mechanistic insight into tumor modulation of angiogenic molecules, and may explain the inconsistent results found in previous biomarker studies.

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Although therapies targeting the vascular endothelial growth factor (VEGF) signaling pathway can be effective, not all patients benefit from treatment. Patients who do respond show transient response, illustrating the need for validated biomarkers.1 Biomarkers allow us to monitor treatment responses, select patients who are likely to respond, determine the optimum biological dose of treatment, design combination therapies, and identify resistance to therapies.2–5

Circulating angiogenic factors (CAF) are potential biomarkers for antiangiogenic therapies. Many govern angiogenesis, and are upregulated or downregulated following anti-VEGF therapies. These changes are observed as a class effect of antiangiogenic therapies, and are detectable systemically. VEGF, PlGF, and sVEGFRs were frequently evaluated in early research: results were inconsistent.4 These CAF are produced by tumor, cells within the tumor microenvironment, and host body cells. Host cells contribute to overall CAF as seen in non-tumor-bearing mice5 and healthy subjects2 receiving sunitinib. This complicates correlating CAF with treatment outcome in cancer patients. Distinguishing between tumor- and host-produced CAF is possible in mouse xenograft models, but not in humans. To determine tumor contribution to CAF changes following antiangiogenic therapies, we used a systems pharmacology approach integrating the mechanistic details of disease targets, pathways, response, and biomarker data into a quantitative framework.8,9

We first used mouse xenograft data and quantitatively described time- and dose-dependent CAF modulation following sunitinib with a systems pharmacology model delineating VEGF-induced VEGFR2 activation, signal transduction, tumor growth, antitumor activity, and feedback compensatory mechanisms. We then applied the model to explain VEGF modulation in cancer patients experiencing different treatment outcomes,10 and outlined the importance of baseline VEGF and its correlation to treatment outcome.

RESULTS
Establishing a systems pharmacology model for anti-VEGF agents
Increased levels of circulating VEGF ligands are a class effect of anti-VEGF agents. Circulating soluble VEGFR-2 and -3 decrease after VEGFR-targeting treatments like sunitinib. These return to basal levels after treatment stops. This change reflects modulation of defined biological targets and is a potential pharmacodynamic biomarker, emphasizing the need to evaluate CAF dynamics quantitatively. We developed a systems pharmacology model of CAF’s relationship with signaling activity in the tumor microenvironment and host cells. It describes their connections to anti-VEGF therapy outcomes. Our model characterizes (i) time- and dose-dependent angiogenic factor modulation, (ii) tumor progression after treatment with anti-VEGF agents, and (iii) host and tumor contributions to total CAF (Figure 1). Tumor cells secrete angiogenic factors, including VEGF. VEGF binds to its receptors (VEGFR2) which are expressed on endothelial cells, producing active phosphorylated receptor forms. The active signal (Sv) initiates canonical downstream cascades (Ras/Raf/MEK/ERK), causing endothelial cell proliferation and prolonged cell survival. Although different signaling cascades exist, we omitted the details of other intermediates, for simplicity. Sunitinib inhibits VEGFR2 activation in endothelial cells, exerting antiproliferative effect on tumors, and is cytotoxic at moderate to high concentrations. The
model reflects these modes of action. CAF changes after anti-VEGF treatment occur as compensatory mechanisms to overcome VEGF inhibition. These are mediated by increased transcription factor HIF-1α to overcome VEGF inhibition. These are mediated by anti-VEGF treatment occur as compensatory mechanisms to overcome VEGF inhibition. These are mediated by increased transcription factor HIF-1α to overcome VEGF inhibition. These are mediated by anti-VEGF treatment occur as compensatory mechanisms to overcome VEGF inhibition. These are mediated by increased transcription factor HIF-1α to overcome VEGF inhibition. These are mediated by increased transcription factor HIF-1α to overcome VEGF inhibition.

The host body contains normal cells that produce angiogenic factors (Figure 1). We assumed the same conserved mechanisms exist in CAF modulation following sunitinib treatment. We presumed sunitinib inactivates VEGF-mediated signaling pathways, but does not affect a normal cell pool. Angiogenic factor turnover, signal transduction, and tumor growth kinetics were described using a series of ordinary differential equations (see Methods section). We used a step-wise approach with increasing model complexity to improve parameter identifiability.

Tumor-independent CAF changes in non-tumor-bearing mice

We first examined host-derived CAF modulation in non-tumor-bearing mice using data from the literature. Plasma VEGF, PI GF, and sVEGFR2 levels in normal mice receiving various sunitinib treatments (Figure 2 Supplementary Figure S1) were fitted simultaneously to the truncated model without tumor microenvironment (i.e., host body only). Following daily 60 mg/kg sunitinib, circulating VEGF and PI GF increased within 24 h and plateaued by day 5 (Figure 2a,b). Their change patterns coincided with PK profiles resulting from daily administration. The time to reach plateau matched the time taken to reach the PK steady-state. Such cyclical profiles reflect the drug elimination process compared with the biological VEGF-ligand family circulation half-lives (~3.5 h; Table 1). sVEGFR-2 levels changed slowly without fluctuating (Figure 2c), probably reflecting its longer circulation half-life (22 h). VEGF and PI GF remained elevated for the duration of therapy and returned to pretreatment values within 1–2 days of treatment withdrawal. Consistent with its turnover rate, sVEGFR2 levels returned slowly. Signal transmission from receptor phosphorylation to nucleus occurs in minutes. We observed a clear dose-dependency in modulating plasma CAF following 7.5 to 120 mg/kg/day sunitinib (Figure 2d–f). Doses below 30 mg/kg produced no significant changes. Doses 60 mg/kg or higher increased VEGF and PI GF and decreased sVEGFR2, suggesting a nonlinearity in sunitinib-mediated modulation with a high Hill Coefficient (nH = 6). VEGF and PI GF had similar baseline values (40 pg/ml). The overall PI GF increase was two times higher (β = 20-fold) than VEGF (α = 10-fold). At the highest dose (γ = 0.62), sVEGFR2 decreased 62 percent from baseline (70 ng/ml).

Simulation for tumor-contributed VEGF in xenograft mice

We then simulated the tumor’s contribution to systemic VEGF, using the tumor growth dynamic model describing sunitinib’s antiangiogenic and cytotoxic effects. The tumor pharmacodynamic model explained the A431 tumor growth inhibition profile from sunitinib doses of 20–80 mg/kg/day (Figure 3a). Lower doses of sunitinib (20–40 mg/kg/day) decreased tumor VEGF, compared with VEGF in untreated animals. Higher doses (80 mg/kg/day) produced higher tumor VEGF than control baseline (Figure 3b). Compared with the increased VEGF by host cells (~400 pg/ml), tumor VEGF was low (Figure 3c). Overall, tumor-contributed VEGF accounted for a fraction (Figure 3d) of total plasma VEGF, consistent with reported values.
**DISCUSSION**

We developed a systems pharmacology model to understand the relationship between CAF dynamics and in vivo antitumor activity in response to anti-VEGF agents. Antiangiogenic therapies target the stroma, not the tumor. Because these agents are cytostatic, monitoring clinical treatment outcomes is challenging and produces inconclusive results.4 Contributing to these inconsistencies are tumor growth’s dynamic nature,17 compensatory mechanisms,11,18 CAF production by healthy cells,4,6,7,19 and PK variability.20 There is a growing need for a computational modeling tool that can integrate these complex systems in a quantitative framework to enable translating knowledge about CAF changes into improved therapeutic outcomes for patients. Hansson et al. used a PK/PD model to correlate CAF changes with treatment outcome.21 Their results suggest sVEGFR3 as a potential predictive biomarker. However, their model does not explain the mechanistic basis of biomarker modulation in tumors or host cell contributions.

Host-derived CAF and how these might confound correlating CAF changes with treatment outcomes is a major concern, since angiogenesis is a conserved physiological process in healthy cells,22 which respond similarly to antiangiogenic therapy.7 Distinguishing between tumor-derived VEGF and host-derived VEGF is impossible with current technologies. Using xenograft mouse models is advantageous. Tumor- (human-) derived VEGF can be distinguished from host- (mouse-) derived VEGF. We used a mouse xenograft model and characterized host-derived CAF modulation in non-tumor-bearing mice following sunitinib treatment. The tumor’s contribution was added with model assumptions based on the literature. Notably, estimates of the stimulation/inhibition capacity of VEGF (α), sVEGFR2 (γ), and the hill coefficient (n1) in mice were 10.2, 0.62, and 3.143, respectively. These values are similar to those reported in healthy volunteers: 10.2, 0.62, and 3.143, respectively, supporting the idea that system-specific parameters for conserved physiological processes like angiogenesis are comparable across species.

Our model provides a quantitative understanding of baseline CAF and an explanation for inconsistent results regarding CAF as predictive biomarkers. Hansson et al. showed that relative changes in sVEGFR3 from baseline predicted the GIST
patients' survival following treatment. Changes in VEGF and sVEGFR2 did not. Baseline sVEGFR3 was approximately threefold higher than that of healthy volunteers. VEGF and sVEGFR2 values were close to their baseline values in healthy volunteers. Similarly, Kontovinis et al. reported VEGF as a predictive biomarker. Baseline VEGF was again approximately threefold higher than baseline VEGF in normal participants. VEGF was not a predictive biomarker in hepatocellular carcinoma or RCC. This is consistent with our finding that CAF could be effective predictive markers when baseline values are higher than host-produced CAF. Although higher baseline CAF have potential as predictive and prognostic biomarkers, they also suggest higher tumor burden and aggressiveness of disease. Studies have reported that patients with higher baseline VEGF have shorter progression-free survival or overall survival compared with patients with lower baseline VEGF.

Our findings suggest that CAF's biological circulation time is a factor in selecting suitable biomarkers and designing sampling times. VEGF ligands with short half-lives fluctuate and show high variability over time. Soluble receptors with longer half-lives were less sensitive to sampling schedules with lower variability. Hence, soluble receptors were better suited as biomarkers. Measuring CAF at the end of the washout period could relate levels to treatment efficacy and tumor burden. Future biomarker research should seek factors selectively secreted only by tumors, to identify successful predictive biomarkers for antiangiogenic therapies.

Optimum antiangiogenic drug dosing leads to normalization of blood vessels and better therapeutic management of solid tumors. Higher doses lead to excessive blood vessel pruning producing increased hypoxia, leading to compensatory increase in proangiogenic signals. This is consistent with our findings that, at lower drug doses, tumor VEGF declined compared with controls (Figure 3b). Despite greater tumor suppression at 80 mg/kg doses, tumor VEGF increased. The therapeutic gain from increasing the dose above 40 mg/kg was modest, compared with the corresponding tumor VEGF elevation.

A bidirectional change in tumor VEGF following anti-VEGF therapy has been proposed. CAF upregulation occurs following anti-VEGF therapy, is dose dependent, and might contribute towards mechanisms helping the tumors escape VEGF inhibition. A higher dose might be counterproductive. This underlines the importance of the right treatment dose. A “VEGF Inhibition Index” using CAF changes would be valuable to determine the optimum drug dose balancing therapeutic benefits and excessive pruning. Our model could be used in such efforts.

Several signaling pathways mediate cell proliferation, migration, survival, and permeability. We focused on VEGF-VEGFR2 interaction and its canonical Ras/Raf/Mek/Erk signaling pathway, as this is the major pathway involved in endothelial cell proliferation. Our primary objective was to delineate time- and dose-dependent changes in CAF, determine their origin, and link these to anti-VEGF effects on tumor growth. Although we focused on the VEGFR2-mediated signaling pathway, we recognize the potential contribution of other VEGFR isoforms and families omitted from our model. VEGFR1 and soluble receptors can influence the ligand-receptor binding process by acting as negative regulators, since they have a higher binding affinity to VEGF than VEGFR2. Similarly, co-receptors increase VEGF's binding affinity to VEGFR, influencing binding kinetics. The estimate of Kd (131 pg/ml) from this study likely represents the overall average contributed from the isoforms of VEGF-VEGFR family present in vivo, and thus could be different from the binding affinity (Kd) estimated in vitro. Few studies have reported an in vitro estimation of Kd value: those that do report estimates of 1–400 pmol/l for VEGF to VEGFR1 and VEGFR2. The VEGF trap consists of the Ig domain of human VEGFR1 and VEGFR2, which binds to VEGF-A and its isoforms, VEGF-B, and PI GF. The Kd of the VEGF trap might better mimic the in vivo estimation. The reported Kd value for the VEGF trap is 0.36–29.3 pmol/l and is comparable with the estimate derived from our model. Popel et al. have contributed to a quantitative understanding of VEGFR2 receptor trafficking, transport, and kinetics of the VEGF ligand family, receptors, and co-receptors involved in angiogenesis. Knowledge from these studies will help us to expand our model.

Cancer signaling networks are complex. Knowledge about these networks is evolving. Recent work by Kirovac and others have contributed to our understanding of the role of CAF as a biomarker for antiangiogenic therapy. The development of novel biomarker models, such as our Systems Pharmacology Model of Angiogenic Biomarker, provides a framework for the integration of multiple pathways and their interactions. This model can be used to predict therapeutic outcomes and guide treatment decisions in clinical settings.

### Table 1: Pharmacodynamic model parameters for angiogenic factors and tumor growth kinetics

| Parameter (unit) | Definition | Estimate | % CV |
|------------------|------------|----------|------|
| E<sub>max</sub>VEGF | Michaelis-Menten capacity constant of VEGF with VEGFR1 | 445.6 | 7.26 |
| K<sub>d</sub>VEGF (pg/ml) | Michaelis-Menten affinity constant of VEGF with VEGFR1 | 131 | 4.59 |
| Kout<sub>VEGF</sub>(day<sup>-1</sup>) | VEGF degradation rate constant | 4.807 | 7.27 |
| Kout<sub>sVEGFR2</sub>(day<sup>-1</sup>) | sVEGFR2 degradation rate constant | 0.373 | 7.78 |
| τ<sub>r</sub>(day<sup>-1</sup>) | Time delay for signal transduction | 0.001 | Fixed |
| τ<sub>n</sub>(day<sup>-1</sup>) | Time delay for translation of angiogenic proteins | 1.327 | 8.1 |
| α | Maximum stimulation for VEGF | 10.2 | 5.15 |
| β | Maximum stimulation for PI GF | 21.87 | 6.62 |
| γ | Maximum inhibition for sVEGFR2 | 0.62 | 4 |
| σ<sub>0</sub> | Stimulation constant for VEGF | 0.53 | 4.76 |
| β<sub>0</sub> | Stimulation constant for PI GF | 0.501 | 5.84 |
| τ<sub>0</sub> | Inhibition constant for sVEGFR2 | 0.425 | 7.42 |
| n<sub>1</sub> | Hill Coefficient for signal inhibition by sunitinib | 3.143 | 6.09 |
| n<sub>2</sub> | Hill Coefficient for stimulation of VEGF, PI GF, and sVEGFR2 change | 6 | Fixed |
| IC<sub>50</sub> (pg/ml) | Inhibition constant for VEGF signal by sunitinib | 2.065 | 4.79 |
| VEGF<sub>n</sub> (pg/ml) | Host VEGF baseline in mice | 40 | Fixed |
| PI GF<sub>n</sub> (pg/ml) | Host PI GF baseline in mice | 40 | Fixed |
| sVEGFR<sub>n</sub> (mg/ml) | Host sVEGFR2 baseline in mice | 70 | Fixed |
| Tumor growth | | | |
| k<sub>1</sub>(day<sup>-1</sup>) | Exponential growth rate of tumor | 0.36 | Fixed |
| k<sub>2</sub>(mm<sup>3</sup>/day) | Linear growth rate of tumor | 94.38 | Fixed |
| W<sub>T</sub> (mm<sup>3</sup>) | Initial weight of tumor | 0.1 | Fixed |
| k<sub>2</sub>(day<sup>-1</sup>) | Maximum cell killing rate constant | 0.272 | 0.05 |
| k<sub>2</sub>(μg/ml) | Inhibitory constant of cell killing | 1.602 | 0.01 |
| W<sub>L</sub> | Sensitivity coefficient of antiangiogenic effect | 0.424 | 0.18 |
| k<sub>δ</sub>(day<sup>-1</sup>) | Time delay for cell killing | 4.942 | 0.13 |
| n<sub>3</sub> | Hill Coefficient for cell killing | 5 | Fixed |

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**CPT: Pharmacometrics & Systems Pharmacology**

Sharan and Woo
Zhang et al.\(^4\) highlights systems pharmacology modeling approaches integrating diverse information about biomarkers and network activity to predict therapeutic response and identify key targets for inhibition.

We developed a base model which can be refined and improved with additional data and mechanistic information. The goal of the systems pharmacology model is also to generate hypotheses which can be further explored and tested.\(^4\) The model framework can be adapted for other antiangiogenic drugs showing a class effect.\(^4\)

Our systems pharmacology model illustrates angiogenesis biomarker modulation in response to anti-VEGF therapy. We must recognize the relative contributions from host-derived CAF over tumor-derived CAF. Our findings will aid in biomarker study design and determining optimum drug doses, and highlight baseline CAF as a potential confounding factor.

**METHODS**

**Data collection and computational approaches**

All datasets were obtained from the literature (Supplementary Table S1) and digitized using GetData Graph Digitizer (version 2.26.0.20). The model was constructed in a step-wise manner, connecting (i) host-derived CAF in non-tumor-bearing mice, (ii) tumor growth profile in xenograft mice, (iii) CAF emanating from xenografted tumor, and (iv) VEGF changes in healthy participants and cancer patients. Parameters obtained by fitting CAF modulation in host cells by sunitinib treatment were fixed for modeling tumor growth data. All model fittings used the maximum likelihood algorithm in ADAPT 5 (Biomedical Simulations Resource, CA Supplementary Data).\(^4\) For simulations, we used Berkeley Madonna (version 8.3.18, University of California at Berkeley, CA).

**Pharmacokinetics**

Preclinical PK parameters were estimated using a one-compartment model (Supplementary Table S2) from the plasma profile of sunitinib in rats.\(^4\) PK parameters were fixed in subsequent pharmacodynamic modeling. For humans, mean population PK parameters for sunitinib and its metabolite SU12662, estimated with a two-compartment model, were obtained.\(^4\) Plasma protein binding of sunitinib and the metabolite was assumed to be 95 and 90%.\(^7\)

**Ligand-receptor interactions and anti-VEGF effects**

VEGF binds to and activates receptor tyrosine kinases. The VEGF-VEGFR family includes five ligands (VEGF-A through -D and PlGF), three receptors (VEGFR-1, -2, and -3), and two non-signaling co-receptors (NRP-1 and NRP-2).
VEGF total toward VEGFR. Before sunitinib administration, the inactivation was explained by a combination of competitive binding in fully stimulated cells, proportional to the steady-state VEGFR activation ($S_{v0}$) by baseline VEGF total, $C_{\text{max}}$ is the maximum VEGFR activation by VEGF

$$S_v = \frac{E_{\text{max,VEGF}} \cdot \text{VEGF}_{\text{tot}}}{K_d_{\text{VEGF}} + \text{VEGF}_{\text{tot}}} \left(1 - \frac{I_{\text{max}} \cdot C^{n_1}}{IC_{50}^{n_1} + C^{n_1}}\right)$$  \hspace{1cm} (1)

where $E_{\text{max,VEGF}}$ is the maximum VEGFR activation by VEGF binding in fully stimulated cells, proportional to the steady-state VEGFR amount, and $K_d_{\text{VEGF}}$ is an affinity constant of VEGF total toward VEGFR. Before sunitinib administration, the steady-state VEGFR activation ($S_{v0}$) by baseline VEGF total is defined as:

$$S_{v0} = \frac{E_{\text{max,VEGF}} \cdot \text{VEGF}_{\text{tot}}}{K_d_{\text{VEGF}} + \text{VEGF}_{\text{tot}}}$$  \hspace{1cm} (1a)

C represents sunitinib concentration. We assumed VEGFR activation is fully inhibited by sunitinib ($I_{\text{max}} = 1$). IC$_{50}$ is the sunitinib concentration producing 50% of maximum VEGFR inactivation and $n_1$ is the Hill Coefficient for signal inhibition and VEGF total represents the sum of VEGF from host and tumor.

**Signal transduction**

VEGF$_2$ activation initiates signaling pathways. Several intermediate steps are involved in signal transmission from active receptor to nucleus. We simplified this to the Ras/Raf/MEK/ERK downstream pathway. The signal transmits from the active receptor to the serial kinases within 15 min. The intermediaries are not experimentally measured. So, the extracellular ligand-induced Ras/Raf/MEK/ERK cascade activation was described by the signal transduction model. Cascades are initiated from phosphorylated receptor relative to the steady-state baseline ($S_v/S_{v0}$), converting into normalized intracellular signals. Signal transit compartments represent the activated kinases as a ratio of phosphorylated/total kinase (normalized to 1 at baseline). Phosphorylated ERK translocates to the nucleus (NUC) to regulate nuclear transcription factors and cellular proteins.

$$d(RAS) = \frac{1}{\tau_1} \cdot \left(\frac{S_v}{S_{v0}} - \text{RAS}\right)$$  \hspace{1cm} (2)

$$d(\text{RAF1}) = \frac{1}{\tau_1} \cdot (\text{RAS} - \text{RAF1})$$  \hspace{1cm} (3)

$$d(\text{MEK}) = \frac{1}{\tau_1} \cdot (\text{RAF1} - \text{MEK})$$  \hspace{1cm} (4)

$$d(\text{ERK}) = \frac{1}{\tau_1} \cdot (\text{MEK} - \text{ERK})$$  \hspace{1cm} (5)

$$d(\text{NUC}) = \frac{1}{\tau_1} \cdot (\text{ERK} - \text{NUC})$$  \hspace{1cm} (6)

where ANG represents the angiogenic signal in the transcriptional level, induced by VEGF-VEGFR interaction, prompting VEGF-mediated angiogenic effects. Two delay time parameters accounted for delayed signal transmission from phosphorylated-receptor to translocation to nucleus ($\tau_i$) and delayed transcriptional factor regulation for angiogenic proteins ($\tau_j$). The initial condition is 1 for Eqs. 2–6 and $\tau_j/\tau_i$ for Eq. 7.

**Host- and tumor-driven CAF modulation by feedback regulation following sunitinib**

Continuous VEGF-pathway blockade leads to vessel pruning, which can produce hypoxia and CAF modulation as an acute phase functional adaptation. Time course and CAF modulation were described by indirect response models with non-linear feedback stimulation or inhibition function.
Figure 5 Simulated plasma concentration time profiles of VEGF in cancer patients with partial response (PR; upper panels): either (a) with low VEGF levels ($\text{VEGF}_{\text{TOTAL}} = 88$ pg/ml) or (b) with high VEGF levels ($\text{VEGF}_{\text{TOTAL}} = 320$ pg/ml); in cancer patients with progressive disease (PD: lower panels) either (c) with low VEGF levels or (d) with high VEGF levels. The low VEGF ($\text{VEGF}_{\text{TOTAL}} = 88$ pg/ml) was defined as tumor VEGF levels tenfold lower than endogenous VEGF in healthy subjects ($\text{VEGF}_{\text{HOST}} = 80$ pg/ml), whereas the high VEGF ($\text{VEGF}_{\text{HOST}} = 320$ pg/ml) was defined as tumor VEGF levels threefold higher than baseline VEGF in healthy subjects.

$$\frac{d\text{VEGF}_{\text{total}}}{dt} = (\text{Ksyn}_{\text{VEGF(h)}} + \text{Ksyn}_{\text{VEGF(tm)}}) \left(1 + \frac{\alpha \cdot \text{FBs}}{\alpha_{50} + \text{FBs}_{50}^2}\right) - \text{Kout}_{\text{VEGF}} \cdot \text{VEGF}_{\text{total}}$$

(8)

$$\frac{d\text{PIGF}_{\text{total}}}{dt} = (\text{Ksyn}_{\text{PIGF(h)}} + \text{Ksyn}_{\text{PIGF(tm)}}) \left(1 + \frac{\beta \cdot \text{FBs}}{\beta_{50} + \text{FBs}_{50}^2}\right) - \text{Kout}_{\text{PIGF}} \cdot \text{PIGF}_{\text{total}}$$

(9)

$$\frac{d\text{sVEGFR2}_{\text{total}}}{dt} = (\text{Ksyn}_{\text{sVEGFR2(h)}} + \text{Ksyn}_{\text{sVEGFR2(tm)}}) \left(1 - \frac{\gamma \cdot \text{FBs}}{\gamma_{50} + \text{FBs}_{50}^2}\right) - \text{Kout}_{\text{sVEGFR2}} \cdot \text{sVEGFR2}_{\text{total}}$$

(10)

where $X_{\text{total}}$ is the total concentration of angiogenic factors produced from the host body ($X_{\text{h}}$) and tumor ($X_{\text{tm}}$). At the steady-state (before treatment), the baseline concentration of these factors $X_{\text{total}}^{0}$ is defined as:

$$\text{VEGF}_{\text{total}}^{0} = \text{VEGF}_{\text{h}}^{0} + \text{VEGF}_{\text{tm}}^{0}$$

(11a)

$$\text{PIGF}_{\text{total}}^{0} = \text{PIGF}_{\text{h}}^{0} + \text{PIGF}_{\text{tm}}^{0}$$

(11b)

$$\text{sVEGFR2}_{\text{total}}^{0} = \text{sVEGFR2}_{\text{h}}^{0} + \text{sVEGFR2}_{\text{tm}}^{0}$$

(11c)

$\text{Ksyn}_{\text{h}}$ and $\text{Ksyn}_{\text{tm}}$ refer to the zero-order production rate for host- and tumor-originated factors. The degradation rate constant (Kout$_{\text{h}}$) is assumed the same for both host- and tumor-originated proteins.

Tumors, the microenvironment, and host cells contribute to these compensatory changes: similar patterns were observed in non-tumor-bearing mice receiving sunitinib. We expressed treatment-induced angiogenic factor modulations as a functional adaptation process mediated via a transcriptional feedback regulation. This feedback regulatory circuit (FB) is operated by the change in VEGF-VEGFR-mediated angiogenic signal relative to its baseline:

$$\text{FB} = \frac{\text{ANG}_{0} - \text{ANG}}{\text{ANG}_{0}}$$

(12)

where $\text{ANG}_{0}$ is the basal angiogenic signal produced by VEGF-VEGFR interaction. As ANG is perturbed by anti-VEGF treatment, it produces a feedback signal and stimulates or inhibits angiogenic protein production. The $\alpha$, $\beta$, $\gamma$ parameters represent maximum fold changes: $\alpha_{50}$, $\beta_{50}$, $\gamma_{50}$ are the FB signal, producing 50% of the maximum changes. The Hill Coefficient (n2) was common for all three factors. We assumed feedback regulation works similarly for tumor and host cells.
Host-driven CAF changes in non-tumor-bearing mice

We characterized dynamic changes in plasma concentrations of VEGF, PIGF, and sVEGFR-2 over time in non-tumor-bearing mice receiving sunitinib as reported by Ebos et al.6 In non-tumor-bearing mice, the tumor contribution toward CAF production was set to zero \( X_{(m)} = 0 \) and \( \text{Ksyn}_{(v)} = 0 \) in Eqs. 8–11. The host-driven CAF production rate (\( \text{Ksyn}_{(v,h)} \)) was expressed as a secondary parameter as:

\[
\text{Ksyn}_{(v,h)} = \text{Kout}_{(v)} \cdot \text{VEGF}_{(t)}(h_0) \quad (13a)
\]

\[
\text{Ksyn}_{(v,h)} = \text{Kout}_{(v)} \cdot \text{PIGF}_{(h_0)} \quad (13b)
\]

\[
\text{Ksyn}_{(v,h)} = \text{Kout}_{(v)} \cdot \text{sVEGFR}_{(h_2)} \quad (13c)
\]

Tumor growth in xenograft mice

To characterize tumor contribution to total CAF, dynamic tumor size changes following anti-VEGF therapy must be linked to VEGF turnover. VEGF production changes as a function of tumor weight.15 The tumor growth model described sunitinib’s antitumor effects in A431 xenograft mice.18,40 Tumor growth was explained by rate of exponential (\( k_e \)) and linear growth (\( k_l \)). Sunitinib’s antiangiogenic effect was expressed as overall growth suppression resulting from angiogenic signal inhibition with a sensitivity index (\( W_t \)). Cell death occurring at high concentrations was explained by a non-linear irreversible function where \( k_{2,50} \) is the sunitinib concentration at 50% of maximum cell death (\( k_3 \), n3 is the Hill Coefficient, k3 is a first-order transduction rate constant for cell death, and \( W_t = 20 \)), which leads to the transition from exponential to linear tumor growth.49

\[
\frac{dw(t)}{dt} = \frac{k_0 \cdot w(t)}{1 + \left( \frac{k_2 \cdot W_t}{k_3} \right)^n} \left( 1 - \frac{\text{ANG}_{0} - \text{ANG}_{b}}{\text{ANG}_{b}} \right) \quad (14)
\]

\[
\frac{dw(t)}{dt} = \frac{k_2 \cdot C^{n3}}{k_2^{n3} + C^{n3}} \cdot w(t) \quad (15)
\]

\[
\frac{dw(t)}{dt} = k_3 \cdot w_2(t) - k_3 \cdot w_2(t) \quad (16)
\]

\[
\frac{dw(t)}{dt} = k_3 \cdot w_2(t) - k_3 \cdot w_2(t) \quad (17)
\]

\[
w(t) = w_1(t) + w_2(t) + w_3(t) + w_4(t) \quad (18)
\]

Tumor-driven VEGF changes as a function of tumor growth in xenograft mice

The aforementioned dynamic change in VEGF production rate by tumor (\( \text{Ksyn}_{(v)} \); Eq. 8) was expressed as a function of tumor size (\( w(t) \)).

\[
\text{Ksyn}_{(v)} = \text{Ksyn}_{(v)(t)} \cdot w(t) \quad (19)
\]

where \( \text{Ksyn}_{(v)} \) is an intrinsic tumor VEGF production rate per tumor volume. This rate \( \text{Ksyn}_{(v)} \) was calculated on the terminal tumor volume \( w_{(last)} \) and plasma VEGF concentration (\( \text{VEGF}_{(t)}(t) \)) in control mice at study termination (\( w_{(last)} = 1,600 \text{mm}^3 \)).

\[
\text{Ksyn}_{(v)} = \frac{\text{Kout}_{(v)} \cdot \text{VEGF}_{(m)}(t)_{(last)}}{w_{(last)}} \quad (20)
\]

The advantage of using \( \text{VEGF}_{(t)}(t)_{(last)} \) and \( w_{(last)} \) is these values are measurable, unlike initial tumor volume \( (W_t) \) which is mathematically extrapolated. With small tumors, no tumor VEGF is detectable in mouse plasma. The baseline value of tumor-dependent VEGF (\( \text{VEGF}_{(t)}(m) \)) (Eq. 11a) before treatment changes over time as the tumor grows, and is approximately based on tumor volume at treatment initiation (\( w_{(start)} \)).

\[
\text{VEGF}_{(t)}_{(m)}(t) = \frac{\text{VEGF}_{(t)}_{(m)_{(last)}}}{w_{(start)}} \quad (21)
\]

In mice xenografted with human cancer cells, the human VEGF form is tumor-originated and different from mouse VEGF (host-driven VEGF). We used \( \text{VEGF}_{(m)_{(last)}} = 40 \text{pg/ml} \) in plasma from control SKOV3 xenograft mice using an ELISA kit specific to human VEGF (data not shown).

Dynamic VEGF changes in humans

We explored whether our model would translate into clinical settings to understand VEGF changes accompanying sunitinib treatment. We started with host-driven VEGF changes following sunitinib treatment in healthy volunteers, by setting the tumor VEGF contribution to zero. Tumor growth and tumor VEGF turnover process were added to account for cancer patients. To permit preclinical to clinical translation, we used the mean baseline VEGF in healthy subjects (\( \text{VEGF}_{(h)}_{(baseline)} = 80 \text{pg/ml} \)). Accounting for interspecies differences, we kept VEGF stimulation capacity (\( \alpha = 10 \)) the same, but changed the sensitivity constant (\( \kappa_{(t)} \)), allowing a twofold VEGF increase in healthy subjects taking sunitinib, per a previous study.7 We modified the tumor growth model and intrinsic VEGF production rate to account for tumor cells’ contribution to VEGF changes in cancer patients. Instead of preclinical VEGF turnover (\( \text{VEGF}_{(t)}_{(m)_{(last)}} \)) and tumor volume (\( w_{(last)} \)) measured at xenograft study termination (Eq. 20), the intrinsic tumor VEGF production rate in cancer patients (\( \text{Ksyn}_{(v)} \)) was calculated based on baseline tumor VEGF (\( \text{VEGF}_{(t)}_{(m)_{(last)}} \)) and tumor volume (\( \text{W}_{(t)} \)) at treatment initiation, both accessible, relevant parameters. The VEGF production rate from tumor (\( \text{Ksyn}_{(v)} \)) was expressed as a function of tumor volume (\( \text{W}_{(t)} \)). Tumor-derived VEGF (\( \text{VEGF}_{(t)}_{(m)_{(last)}} \)) in total VEGF in cancer patients (\( \text{VEGF}_{(t)}_{(total)} \)) was determined based on the difference from baseline VEGF in healthy volunteers (\( \text{VEGF}_{(h)}_{(baseline)} = 80 \text{pg/ml} \)).

\[
\text{Ksyn}_{(v)} = \text{Ksyn}_{(v)} \cdot \text{WT}_{(t)} \quad (23)
\]

\[
\text{Ksyn}_{(v)} = \text{Kout}_{(v)} \cdot \text{VEGF}_{(t)}_{(m)_{(last)}} / \text{WT}_{(t)} \quad (24)
\]

\[
\text{VEGF}_{(t)}_{(m)_{(last)}} = \text{VEGF}_{(t)}_{(total)} - \text{VEGF}_{(h)}_{(baseline)} \quad (25)
\]

Clinical tumor growth \( \text{WT}_{(t)} \) was modeled by a simple equation, using the first-order tumor growth rate (\( K_{(g)} \)) from baseline tumor (\( \text{WT}_{(BL)} \)) before treatment.
We performed simulations using Response Evaluation Criteria In Solid Tumors guidelines, to mimic clinical outcomes. Tumor VEGF and total VEGF time profiles were simulated for stable disease assuming no tumor growth (Kg = 0), partial response, and PD. Kg values were modified to allow a 30% decrease in the sum of tumor lesion diameters in partial response (Kg = −0.012 day^{-1}), and 20% increase in the sum of tumor lesion diameters in PD (Kg = 0.006 day^{-1}).

Simulations were performed for PD and partial response with two VEGF baseline concentrations: (i) tumor VEGF threefold higher than endogenous VEGF in healthy volunteers (VEGF_{\text{Baseline}} = 320 pg/ml), and (ii) tumor VEGF tenfold lower than endogenous VEGF (VEGF_{\text{Baseline}} = 88 pg/ml). Percentage change in tumor diameter was used to calculate percentage change in tumor volume, assuming the tumor to be spherical.

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Conflict of Interest. The authors declared no conflict of interest.

Study Highlights

**WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?**

CAFs levels are modulated in response to anti-VEGF agents and are potential biomarkers. Studies have produced mixed results.

**WHAT QUESTION DID THIS STUDY ADDRESS?**

A systems pharmacology model was developed to explain time- and dose-dependent CAF modulation, the distinction between host and tumor-derived VEGF, and the correlation of CAF modulation with response to anti-VEGF therapy.

**WHAT THIS STUDY ADDS TO OUR KNOWLEDGE**

- The study provides a mechanistic view of dose-dependent CAF modulation and underlines the importance of host-produced CAF as a confounder. CAF could be effective predictive markers for antiangiogenic therapy's antitumor activity when baseline CAF values are higher than host-produced CAF.

**HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS**

- This study will help in designing better biomarker sampling design, determining the optimum biological dose, and will improve understanding and utilization of baseline CAF values as predictive or prognostic biomarkers.

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