Compartment model predicts VEGF secretion and investigates the effects of VEGFTrap in tumor-bearing mice

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INTRODUCTION

Angiogenesis is the formation of new blood capillaries from pre-existing vessels, and is a process involved in physiological function, such as exercise and wound healing, as well as disease conditions, including cancer, peripheral and coronary artery diseases, preeclampsia, and age-related macular degeneration (AMD). The vascular endothelial growth factor (VEGF) family is a key promoter of angiogenesis and vascular development. The VEGF family includes five ligands: VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PIGF). One of the most widely studied members is VEGF-A, commonly referred to as VEGF. Alternative splicing of VEGF produces different isoforms, including VEGF121, VEGF164, VEGF189, and VEGF206 in humans. Expressed rodent isoforms are one amino acid shorter than human isoforms; therefore, the subscripted number is one less. Additionally, there are VEGF164x isoforms, which have been shown to be endogenous anti-angiogenic species (1, 2). VEGF promotes angiogenesis by binding to and activating its receptors VEGFR1 and VEGFR2, and co-receptors called neuropilins (NRPs). Signal transduction through the receptors promotes many cellular processes, including cell proliferation, migration, and survival (3). VEGFR1 and VEGFR2 are expressed on endothelial cells (ECs), cancer cells, and other cell types, including bone marrow-derived cells and neurons [see (4) for review]. NRPs are expressed on various cell types, including ECs, tumor cells, and muscle fibers (4).

Angiogenesis has been targeted to treat diseases characterized by reduced vascularization ("pro-angiogenic therapy") (3, 6) or to inhibit the formation of new blood vessels in conditions leading to hypervascularization ("anti-angiogenic therapy") (7, 8). Of particular importance is anti-angiogenic therapy targeting tumor vascularization. Bevacizumab (9) is a recombinant monoclonal antibody that neutralizes VEGF and is approved by the Food and Drug Administration to treat colorectal cancer, glioblastoma, kidney cancer, and non-small cell lung cancer. Aflibercept
VEGF is secreted by muscle fibers and ECs, as well as the clearance of VEGF, soluble factors that influence VEGF levels, and a dynamic bearing mice (23) did not include EC secretion of VEGF or soluble factors. Therefore, we first refit the expanded two-compartment model that includes these additional features in order to match in vivo experimental data (12). The fitting optimized the values of five parameters: VEGF secretion rate of muscle fibers \(q_{\text{muscle}}\), VEGF secretion rate of ECs \(q_{\text{EC}}\), clearance rate of VEGF Trap \(c_{\text{vA}}\), clearance rate of the VEGF/VEGF Trap complex \(c_{\text{vA}}\), and dissociation constant of VEGF and VEGF Trap \(K_d\). As described in the methods, although the experimental protocol used by Rudge and coworkers utilizes subcutaneous administration of VEGF Trap, we simulate intravenous administration and assume 100% of the reported dose is administered. The fitting procedure allows us to estimate the values of the free parameters using in vivo experimental data.

The optimized parameter values are shown in Table 1, and all raw data from the optimization is given in File 1 in Supplementary Material. The optimized value of \(K_d\) is comparable to the reported in vitro measurement of 0.6 pM (11), providing confidence in the fitting procedure. The optimization predicts the muscle fibers secrete very little VEGF (0.002 molecules/cell/s), and the standard deviation of the optimized values is high. This suggests that the model is not sensitive to the value of \(q_{\text{muscle}}\). To investigate this possibility, we varied muscle secretion from 0 to 0.02 molecules/cell/s and used the model to estimate the concentrations of unbound VEGF Trap and the mouse VEGF (mVEGF)/VEGF Trap complex. This sensitivity study revealed that increasing \(q_{\text{muscle}}\) up to one order of magnitude does not significantly change the fit, as shown in Figure 1. These results indicate that there may not be sufficient data to determine VEGF secretion from muscle fibers. Specifically, it is difficult to separate the contribution of VEGF from muscle fibers, compared to ECs. This result is not specific to the data used here, but more generally that plasma measurements cannot be used to determine endogenous VEGF production from multiple sources.

**Sensitivity analysis reveals model parameters that influence VEGF concentrations**

In the three-compartment model, the values of several parameters are based on characterization of the human VEGF (hVEGF) system due to a lack of quantitative experimental measurements in mice. We previously investigated sensitivity to individual parameters, including vascular permeability, lymphatic drainage, and properties of the anti-VEGF agent (25). In that work, parameters

### Table 1 | Estimated model parameters from optimization of two-compartment model

| Parameter | Units | Optimal value | Standard deviation |
|-----------|-------|---------------|--------------------|
| Normal secretion | Molecules/cell/s | 0.002 | 0.003 |
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| Tumor secretion | Molecules/cell/s | N/A | N/A |
| Clearance of free VEGF Trap | s\(^{-1}\) | \(1.3 \times 10^{-5}\) | \(2 \times 10^{-7}\) |
| Clearance of bound VEGF Trap | s\(^{-1}\) | \(2.5 \times 10^{-6}\) | \(2 \times 10^{-7}\) |
| \(K_d\) of VEGF Trap | pM | 0.29 | 0.011 |

**RESULTS**

**RE-CALIBRATION OF TWO-COMPARTMENT MODEL CAPTURES DYNAMICS OF BOUND AND COMPLEXED VEGF TRAP**

The previous two-compartment model simulating non-tumor-bearing mice (23) did not include EC secretion of VEGF or soluble factors. Therefore, we first refit the expanded two-compartment model that includes these additional features in order to match in vivo experimental data (12). The fitting optimized the values of five parameters: VEGF secretion rate of muscle fibers \(q_{\text{muscle}}\), VEGF secretion rate of ECs \(q_{\text{EC}}\), clearance rate of VEGF Trap \(c_{\text{vA}}\), clearance rate of the VEGF/VEGF Trap complex \(c_{\text{vA}}\), and dissociation constant of VEGF and VEGF Trap \(K_d\). As described in the methods, although the experimental protocol used by Rudge and coworkers utilizes subcutaneous administration of VEGF Trap, we simulate intravenous administration and assume 100% of the reported dose is administered. The fitting procedure allows us to estimate the values of the free parameters using in vivo experimental data.

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**SENSITIVITY ANALYSIS REVEALS MODEL PARAMETERS THAT INFLUENCE VEGF CONCENTRATIONS**

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were varied one by one. Here, we perform a modular sensitivity analysis, where we investigate how variability in three sets of parameters (model inputs) influence mouse and hVEGF concentrations and sVEGFR1 levels in normal tissue, blood, and tumor (model outputs). Specifically, we investigated the effect of VEGF receptor expression, transport parameters, and kinetic parameters using the extended Fourier Amplitude Sensitivity Test (eFAST), as described in the Section “Materials and Methods.” Two indices provide an estimate of the sensitivity of the model output to model parameters. The first FAST index quantifies the variance of a model output with respect to the variance of each input. The total FAST index quantifies the variance of a model output with respect to the variances of each input and covariances between all combinations of inputs. If total FAST indices are larger than the first FAST indices, it means that the parameter is more important in combination with other parameters rather than individually.

The FAST indices for each set of model inputs are shown in Figure 2. When investigating the effect of tumor cell receptor expression, VEGF and sVEGFR1 concentrations are sensitive to the density of NRP co-receptors. Additionally, the level of VEGFR1 is an important determinant of hVEGF concentration in the tumor. In the transport module, the rate of lymphatic flow from normal or tumor tissue in concert with other transport parameters is estimated to influence hVEGF levels in plasma and normal tissue. Soluble VEGFR1 concentrations, as well as mVEGF levels in plasma and normal tissue, are particularly sensitive to the permeability of the normal tissue to VEGF and VEGF/sVEGFR1 complexes. Individual parameters investigated in the kinetic module are predicted to influence VEGF and sVEGFR1 concentrations, rather than in combination with other kinetic parameters. VEGF and sVEGFR1 levels are particularly sensitive to VEGF164 and VEGF165 binding to NRP co-receptors and VEGF binding to VEGFR1. These results aid in our understanding of how uncertainty in the values of particular parameters influence the model output. Additionally, the sensitivity analysis provides quantitative data to support obtaining additional experimental measurements of specific parameters that significantly influence model outputs.

THE RATE OF VEGF SECRETION BY HUMAN TUMOR CELLS IS DEPENDENT ON THE TUMOR MICROENVIRONMENT

Tumor cells are a source of VEGF; however, there is a lack of in vivo data for VEGF secretion rates. Therefore, we have used in vivo experimental data on the plasma concentration of free VEGF Trap and VEGF Trap bound to mouse and hVEGF to determine VEGF
FIGURE 2 | Sensitivity indices of model parameters. The extended Fourier Amplitude Sensitivity Test (eFAST) was used to estimate the variance in the model output with respect to variance in individual model inputs (first FAST indices) and covariances in combinations of model inputs (total FAST indices). A modular approach was used to investigate the sensitivity to (A), tumor receptor expression; (B), transport parameters; and (C), kinetic parameters.
secretion rates in mice bearing human tumor xenografts. Here, we use the clearance rates of unbound and complexed VEGF Trap predicted in the two-compartment model and experimentally determined VEGF binding affinity. However, the VEGF secretion rates \( q_{\text{VEGF}_m} \), \( q_{\text{VEGF}_{\text{EC}}} \), and tumor VEGF secretion, \( q_{\text{VEGF}_t} \), were optimized to fit experimental data. We optimize the VEGF secretion rates since there is large variability in the predicted rate of muscle secretion obtained using the two-compartment model.

The VEGF secretion rates were predicted using the optimization algorithm, assuming the tumors follow either the average (baseline) or fast tumor growth profiles. We use data from Rudge et al. (12), where tumors were allowed to grow to \( \sim 100 \text{ mm}^3 \), and then the tumor-bearing mice were injected with VEGF Trap ("anti-VEGF") twice weekly for 2 weeks. Various dosages of VEGF Trap were used, and the concentrations of free VEGF Trap and the mVEGF/VEGF Trap complex and hVEGF/VEGF Trap complex in the blood were measured. These measurements can be directly compared to model estimates where the anti-VEGF agent is administered intravenously. The optimized model provides a good fit to the experimental data, as shown in Figure 3. The average and standard deviation of the predicted VEGF secretion rates from the optimization runs are in Figure 4 and Table 2, and File 1 in Supplementary Material contains the raw data.

**CIRCULATING LEVELS OF VEGF TRAP AND HUMAN VEGF/VEGF TRAP COMPLEX AND MAXIMUM CONCENTRATION OF TOTAL VEGF TRAP VARY WITH DOSE**

To our knowledge, the dynamic levels of free and complexed VEGF Trap in tumor-bearing mice have not been reported. These data are useful in elucidating the mechanism of action of VEGF Trap and to determine if the dosage is sufficient to neutralize VEGF secreted by the tumor. Therefore, we used the optimized model for A673 rhabdomyosarcoma human xenograft to predict the concentration profiles for free VEGF Trap and VEGF Trap bound to hVEGF (Figure 5). The level of VEGF Trap bound to hVEGF is more than an order of magnitude lower than the concentration of mVEGF complexed with VEGF Trap. This result is consistent with the finding that normal production of VEGF eclipses the production from tumors, as described by Rudge and co-authors (12). Additionally, the level of free VEGF Trap remains well above the level of the hVEGF/VEGF trap complex for up to 14 days. This indicates effective dosing, as the VEGF-neutralizing agent is able to neutralize all VEGF secreted by the tumor. The HT1080 fibrosarcoma tumor response is similar (data not shown).

**VEGF TRAP IS PREDICTED TO DEPLETE UNBOUND VEGF IN THE BODY**

The optimized model of a tumor-bearing mouse provides a framework with which to study the concentration of unbound VEGF before and after administration of VEGF Trap. As expected, endogenous levels of unbound VEGF are highest in the normal tissue and plasma, and the concentration of hVEGF is highest in the tumor, based on the source of mouse and hVEGF. Before any injection, mVEGF concentration is estimated to range from 0.17 to 1.47 pM in mice with A673 tumors, based on 1 SD above and below the average predicted VEGF secretion rates (Table 3). Unbound hVEGF in the tumor is estimated to be \( \sim 0.5 \text{ pM} \). We also present free VEGF concentration during twice-weekly injections of VEGF Trap at 2.5 mg/kg (Figures 6A,B). The model estimates that free VEGF in the body is first depleted before increasing slightly before the next injection. Thus, the model can be used to understand the effect of anti-VEGF agents on systemic and tissue levels of VEGF.

In addition to using the model to estimate the concentration of unbound VEGF, we have also determined the percentage of free VEGF in the form of VEGF\(_{164}\) or VEGF\(_{165}\). The isoform secretion ratio for VEGF\(_{164}\):VEGF\(_{121}\) in muscle is 92:8 and 90:10 in EC, and the secretion ratio for VEGF\(_{165}\):VEGF\(_{121}\) in tumor cells is 50:50, as described in the Section “Materials and Methods.” These ratios determine the fraction of VEGF\(_{164}\) or VEGF\(_{165}\) in the compartments; and, the fractions at which the isoforms are present change with time and drug dose. Here, we consider a dosage of 2.5 mg/kg. After the first anti-VEGF injection, the percentage of free mVEGF...
in the form of VEGF₁₆₄ is ≈90% in all compartments (Figure 6C, left). The percentage of hVEGF in the form of VEGF₁₆₅ in tumor is slightly lower than the percentage of VEGF₁₆₅ in normal tissue and plasma (44–49%, as compared to 55%; Figure 6D). These types of model predictions can aid in biomarker identification, as the concentration of specific VEGF isoforms may predict tumors that will respond to anti-VEGF treatment or other anti-angiogenic therapies.

We also apply the model to investigate the total levels of circulating VEGF in plasma. The soluble factors sVEGFR1 and α₂-macroglobulin (α₂M) bind to VEGF and contribute to circulating levels of VEGF. Thus, total circulating VEGF is comprised of free VEGF, VEGF bound to sVEGFR1, and α₂M-bound VEGF (both the native and active forms). VEGF bound to the VEGF Trap drug is also included. We again allow the tumors to reach a volume of 100 mm³ before simulating twice-weekly injections of VEGF Trap at varying doses. Before the first injection, the relative amounts of free, sVEGFR1-bound, and α₂M-bound circulating VEGF are 80, 4, and 16%, respectively. One day after the first injection of VEGF Trap, the composition of the circulating VEGF changes, depending on the drug dose (data not shown). If we consider a drug dose of 2.5 mg/kg, the relative amounts of free, sVEGFR1-bound, α₂M-bound, and VEGF Trap-bound VEGF are 0.6, 0.03, 5, and 94%, respectively. Thus, the VEGF Trap displaces the soluble factors bound to VEGF.

**DISCUSSION**

We have developed a compartment model of VEGF distribution in tumor-bearing mouse. The model incorporates tumor-specific properties, including the rate of tumor growth and VEGF secretion. We have used *in vivo* experimental data for the levels of free and bound VEGF Trap in mice bearing human tumor xenografts in order to predict the endogenous rate of VEGF secretion by myocytes and ECs and compared them to the predicted secretion rates in normal mice. We also predicted the rate at which cells from different human tumor xenografts secrete VEGF. To our knowledge, VEGF secretion rates can only be obtained from *in vitro* experiments and cannot be directly measured *in vivo*; however, VEGF concentrations that depend on the secretion rates can be measured experimentally, although such interstitial measurements are presently not available. Therefore, this work provides new insight into VEGF levels in a pre-clinical *in vivo* model of cancer. In addition, using the optimized model for tumor-bearing mice, we have estimated the concentration of VEGF in the mouse following administration of VEGF Trap, as well as the distribution of VEGF in mice and circulating levels of VEGF Trap and the VEGF/VEGF Trap complex. These results show that the concentration of free VEGF in the tumor depends on the tumor-specific properties such as the rate of tumor growth and the amount of VEGF secreted by tumor cells. Lastly, we used the predicted level of VEGF Trap and hVEGF/VEGF Trap complex to compare various dosages. The model predicted that all hVEGF originating from the tumor is neutralized at higher doses of the drug. This demonstrates an important application of the model: to incorporate tumor-specific properties and investigate the efficacy of different drug doses.

We used the two-compartment model to estimate VEGF secretion rates, clearance of free and bound VEGF Trap, and the binding affinity of VEGF Trap for normal mice. The value of binding affinity of VEGF Trap estimated by the model is comparable to the experimentally measured value (11). Additionally, the estimated EC secretion is comparable to the experimentally determined value of 0.028 molecules/cell/s (27). However, the predicted rate at which muscle cells secrete VEGF is very low, and varying this parameter over one order of magnitude does not significantly change the fit. In contrast, EC secretion can be specified and changing this parameter drastically influences the fit to experimental data (results not shown). These results may indicate that the rate of VEGF secretion from muscle and ECs cannot be simultaneously estimated using the available experimental data. That is, measurements of free and bound VEGF Trap in plasma do not allow us to distinguish how muscle and ECs contribute to VEGF levels. Additional experimental measurements such as interstitial levels of VEGF in

| Tumor | Baseline tumor growth profile | Fast growth profile |
|-------|------------------------------|---------------------|
|       | Normal                       | EC                  | Tumor               | Normal                       | EC                  | Tumor               |
| A673  | 0.011 ± 0.007                | 0.009 ± 0.008       | 0.009 ± 5 × 10⁻⁵   | 0.009 ± 0.006              | 0.009 ± 0.008       | 0.007 ± 4 × 10⁻⁵   |
| HT1080| 0.007 ± 0.006                | 0.008 ± 0.008       | 0.023 ± 3 × 10⁻⁴   | 0.007 ± 0.006              | 0.008 ± 0.008       | 0.017 ± 3 × 10⁻⁵   |

*Secretion rate is given in molecules/cell/s. We report the mean ± SD of the 20 optimization runs.*
The optimized model was applied to predict the time course of free VEGF Trap (black), mouse VEGF bound to VEGF Trap (blue), and human VEGF bound to VEGF Trap (red) in the mouse plasma after a single intravenous injection of VEGF Trap at (A), 0.5 mg/kg; (B), 2.5 mg/kg; (C), 10 mg/kg; and (D), 25 mg/kg in the A673 rhabdomyosarcoma human tumor xenograft. We use the mean (solid lines) and standard deviation (dashed lines) of the fitted secretion rates.

Table 3: Estimated concentrations of free VEGF before VEGF Trap injection.

| Tumor      | Mouse Range of free VEGF (pM)* | Human Range of free VEGF (pM)* |
|------------|--------------------------------|---------------------------------|
|            | Normal | Plasma | Tumor | Normal | Plasma | Tumor |
| A673       | 0.17–1.47 | 0.04–0.61 | 0.002–0.02 | 5.03 $\times$ 10^{-5}–5.30 $\times$ 10^{-5} | 1.18 $\times$ 10^{-3}–1.20 $\times$ 10^{-3} | 0.49–0.50 |
| HT1080     | 0.07–1.27 | 0.02–0.54 | 0.001–0.02 | 1.26 $\times$ 10^{-4}–1.34 $\times$ 10^{-4} | 2.95 $\times$ 10^{-3}–3.05 $\times$ 10^{-3} | 1.23–1.26 |

*Calculated using (mean ± SD).

skeletal muscle are needed in order to predict VEGF secretion by muscle fibers with confidence. Currently, interstitial VEGF concentrations are only available in human tissue (28–33); however, similar studies in mice are of great interest.

We found that fitted parameters from normal mice were not sufficient to match the levels of unbound and complexed VEGF Trap in the model of tumor-bearing mice. We first attempted to use the fitted parameters from the two-compartment model in the model of tumor-bearing mice and use in vivo experimental data to fit the rate of VEGF secretion from tumor cells. However, the model overestimated the amount of VEGF Trap complexed with mVEGF (results not shown). We are able to more closely fit the experimental data for the tumor-bearing mice by optimizing the three-compartment model independent of the optimized model for
normal mice. This indicates that endogenous VEGF secretion may be different in normal and tumor-bearing mice (Tables 1 vs 2). Experimental studies are needed to validate these results; however, evidence shows that VEGF secretion is reduced following administration of VEGF Trap (34) or other anti-angiogenic therapies (35–37).

The three-compartment model predicted that the in vivo tumor VEGF secretion rates needed to fit experimental data are lower than data obtained from in vitro measurements. In vitro experimental measurements of the VEGF secretion rate vary widely: 0.03–2.65 molecules/cell/s (38–41). We predicted that human tumors secrete VEGF at rates ranging from 0.007 to 0.023 molecules/cell/s. Interestingly, there is little variability in the predicted tumor secretion rate, as indicated by the small standard deviation (~10^{-5} molecules/cell/s). Having experimental measurements of the plasma concentration of VEGF Trap bound to hVEGF (i.e., VEGF originating from the tumor) enables us to predict the rate at which the tumor secretes VEGF in vivo. In this way, xenograft models are preferable to syngeneic tumor models, in which VEGF derived from tumor and other tissues are indistinguishable. Similarly, plasma measurements in human patients would not be sufficient to specify tumor VEGF. Thus, xenograft models provide unique insight into the effects of anti-angiogenic therapies and are relevant to human studies.

Tumor VEGF secretion is predicted to depend on the tumor microenvironment. HT1080 tumors are predicted to secrete ~2-fold more VEGF than A673 tumors. Additionally, average- and fast-growing tumors are predicted to secrete different amounts of VEGF, where VEGF secretion in fast-growing tumors is slightly lower than that of tumors that grow at an average rate. To our knowledge, experimental data for VEGF secretion rates is limited to in vitro measurements. Therefore, the ability to use the model to determine the VEGF secretion from in vivo data and track and quantify normal and tumor VEGF are important features of the model.
Using the optimized model, it is possible to estimate VEGF concentrations in the mouse before and after VEGF Trap administration. In the model, we allowed the tumor to grow for 2 weeks before the VEGF Trap injection. Just before the injection, the estimated plasma VEGF levels are within the range of experimental measurements in mouse of 0.3–1.4 pM (42, 43). The model indicates that plasma VEGF depends on properties of the tumor, such as volume, a result that is validated by experimental evidence (44). Using the model, free VEGF in muscle interstitium is predicted to range from 0.2 to 1.5 pM. To our knowledge, interstitial VEGF in normal tissues has only been quantified in human samples. Interstitial muscle VEGF in humans ranges from 0.3 to 3 pM (28–33, 45). It is not clear how this concentration range varies across species. However, since the range of plasma VEGF measurements is similar between mice and humans, where human plasma VEGF is measured to be 0.4–3 pM (46), it is possible that interstitial VEGF is also comparable in mice and humans. Thus, our model results and predictions provide a framework to compare VEGF distribution in different species and can be experimentally validated. Additionally, we are able to predict the concentration of specific VEGF isoforms (i.e., the percentage of free VEGF in the form of VEGF164 or VEGF165, as compared to the shorter isoforms VEGF120 or VEGF121). These results may be useful in identifying predictive biomarkers for anti-VEGF treatment, where the level of VEGF121 is being evaluated as a biomarker (47, 48). We also applied the model to estimate the relative contribution of sVEGFR1-bound and α2M-bound VEGF to total circulating VEGF. The soluble factors compete with anti-VEGF agents; therefore, it is of interest to investigate the effect of sVEGFR1 on the response to anti-VEGF treatment. In this way, the model complements studies evaluating sVEGFR1 as a potential biomarker to predict resistance to anti-VEGF treatment (49).

We can also compare the estimated levels of plasma VEGF generated by the model following administration of VEGF Trap with experimental studies. In vivo studies of mice with breast tumor xenografts indicate the plasma VEGF is reduced following VEGF Trap treatment, particularly at the higher doses (34). Additionally, Hoff and coworkers report that VEGF Trap is able to bind all free VEGF 11 days after treatment in an experimental model of rat glioma (50). These studies support the computational model predictions. However, we are not aware of animal studies that provide the time course of VEGF and VEGF/VEGF Trap concentration, which is an important contribution of the model and can complement pre-clinical studies that investigate the efficacy of VEGF Trap.

We show that interstitial tumor VEGF levels depend on specific properties of the tumor. To our knowledge, there are no experimental measurements for interstitial tumor VEGF concentrations. However, a sampling of available experimental measurements of total VEGF in tumor tissue (free and bound VEGF, both intracellular and extracellular) reveals a wide range of values, depending on tumor type and size. File 1 in Supplementary Material shows a compilation of measurements of tumor VEGF for various tumor types. Experimental studies to measure free VEGF in tumor tissue in mouse models would provide much needed quantitative data to test and validate the model predictions presented here.

**MODEL LIMITATIONS**

We consider the model presented here to be a minimal model that accurately reproduces experimental data, both qualitatively and quantitatively. The model includes several assumptions, which may be addressed as experimental data become available. For example, we assume the normal tissue is skeletal muscle, although other tissues and organs secrete and contain VEGF (51), but are not as well-characterized as muscle. We include two major VEGF isoforms (VEGF120/VEGF121 and VEGF164/VEGF165); however, other isoforms such as VEGF188/VEGF189 (52) and VEGF189 (53, 54) also influence angiogenesis and may impact anti-VEGF therapies. Recent studies also show that other VEGF ligands and receptors contribute to angiogenesis (55–57), and the model can be expanded in the future to include these molecular species. Additionally, although platelets contain large amounts of VEGF and contribute to angiogenesis (58), we have not included them in the model as the rate and conditions under which they secrete or unload VEGF are unknown. We assume that as the tumor grows, the relative proportions of interstitial space, vascular volume, and tumor cells remain constant. However, experimental studies indicate that these proportions should change as the tumor grows (59). Finally, we have not included the effects of anti-VEGF treatment on tumor volume or vascular permeability. Pre-clinical studies show tumor growth inhibition and even regression of the tumor following anti-angiogenic therapy that targets VEGF. We have performed preliminary studies where the tumor volume is constant after 1 week of anti-VEGF treatment since experimental studies indicate that tumor growth is halted during 2 weeks of twice-weekly VEGF Trap injections (34). We found that the predicted tumor secretion rate is slightly larger when accounting for tumor growth stagnation. This is because the tumor is smaller and consists of fewer cells. Therefore, the amount of VEGF that must be secreted on a per cell basis in order to obtain a certain level of VEGF or VEGF/VEGF Trap complex is higher. Tumor permeability may decrease with anti-angiogenic therapy, as the tumor normalizes neovasculature and it begins to resemble normal vessels; however, we have not included that effect in the current model. In a human model of VEGF transport and kinetics, we considered “low” and “high” vascular permeability between the tumor and blood (22). Interestingly, the model predicts that tumor VEGF can increase above the pre-treatment level depending on properties of the tumor microenvironment, even when tumor permeability is high. Future computational studies may investigate the effect of anti-VEGF treatment on tumor volume and vascular permeability in greater detail.

**CONCLUSION**

The compartment model presented here provides a framework to investigate the action of VEGF-targeting agents for particular types of tumors. The physiologically based and experimentally validated model, based on currently available animal data, predicted the dynamic concentrations of molecular species and other biological parameters that are difficult to quantify experimentally. Thus, the model complements pre-clinical experiments, can aid in the development of agents that target VEGF and inhibit angiogenesis, and may be useful in evaluating biomarkers of anti-angiogenic therapies. The model can be extended to human patients; this is
particularly important since in 2012 aflibercept has been approved to treat metastatic colorectal cancer in humans (60).

**MATERIALS AND METHODS**

**COMPUTATIONAL MODEL**

We have expanded the two-compartment model of VEGF distribution in the mouse (23) to include tumor tissue (“tumor compartment”). The model is illustrated in Figure 7. Geometric and kinetic parameters for the normal and blood compartments have been fully detailed in (23). By simulating a human tumor xenograft (tissue that grows from human cancer cells that have been injected into the mouse), we also incorporate hVEGF isoforms and cross-species reactions between ligands and receptors. Specifically, we include VEGF$_{121}$ and VEGF$_{165}$, which are secreted by tumor cells. The human isoforms can bind to human receptors present on tumor cells, as well as mouse receptors on endothelial surfaces in the body (normal and tumor EC) and muscle fibers in the normal compartment. Additionally, the mouse isoforms bind to mouse receptors on muscle fibers and ECs and human receptors on tumor cells. The model can also be adapted to simulate mouse syngeneic tumors, where the tumor cells secrete VEGF$_{120}$ and VEGF$_{164}$; in this case, only mVEGF is present in the model. In this work, however, we have focused on human tumors. The molecular interactions between VEGF and its receptors are illustrated in Figure 8.

In addition to introducing the tumor compartment, we include VEGF interactions with two soluble factors: soluble VEGFR1 (sVEGFR1) and α2M and introduce VEGF secretion by ECs. Soluble VEGFR1 is secreted by ECs and transported throughout the body, enabling it to interact with VEGF in all compartments. The soluble factor α2M is present in two forms: native and active (α2M$_{	ext{act}}$) (61). Both forms are present at high concentrations (nanomolar to micromolar levels) (62), and due to their size (720 kDa MW), we assume that both forms are confined to the blood compartment. The model predicts the levels of free VEGF in the tissue interstitium and in plasma. These soluble factors interfere with assays that measure VEGF concentration, making it difficult to distinguish between VEGF that is truly free versus VEGF that is bound to trapping molecules (63). Both sVEGFR1 and α2M can sequester VEGF and reduce the levels of free VEGF. Therefore, it is important to include these factors in the model.

We have also included VEGF secretion by ECs, as experimental studies demonstrate that EC are a source of VEGF (64, 65). The luminal and abluminal endothelial surfaces secrete VEGF, and luminal secretion is predicted to be a major determinant of plasma VEGF. Due to EC secretion of VEGF, the compartments are relatively autonomous, since the concentration of VEGF in each compartment is determined primarily by the secretion rate in that compartment, as well as the microenvironmental variables of the compartment; however, transport between compartments is also important.

The model is described by 258 non-linear ordinary differential equations (ODEs), including 53 for the normal compartments, 126 for the blood, and 79 for the tumor compartment. In addition to the ODEs that describe how the species’ concentrations vary with time, we include an equation for the tumor volume, such that the model simulates VEGF distribution in tumor-bearing mice, immediately following inoculation of tumor cells. The initial tumor volume is $10^{-6}$ cm$^3$. A sampling of experimental data for the volume of xenografts generated from MCF-7 and MDA-MB-231 breast cancer cells (66–74) reveals various growth profiles. We fit the data to exponential curves, accounting for a range of tumor growth profiles (Figure 9). The growth curves fit experimental data well, within the time scales used in the model (i.e., <6 weeks). In cases where the model is run for longer times, different growth
FIGURE 8 | Molecular interactions. The interactions of VEGF₁₂₀ and VEGF₁₆₄ are illustrated. VEGF₁₂₁ is involved in the same binding reactions as VEGF₁₂₀. Similarly, the interactions for VEGF₁₆₅ are the same as VEGF₁₆₄. Differences in the interactions of VEGF₁₂₀/₁₂₁ as compared to VEGF₁₆₄/₁₆₅ are due to differential exon splicing.

FIGURE 9 | Tumor growth profiles. We investigate the growth profiles of two categories of tumors: average (blue) and fast-growing (green) tumors, based on available experimental data. The data are fit to exponential curves, and the growth equations are given in the File 2 in Supplementary Material.

curves should be used in order to capture the full range of tumor growth dynamics for the desired time scale. The complete set of equations, chemical reactions, and glossary of terms are given in File 2 in Supplementary Material.

SIMULATION OF ADMINISTRATION OF VEGF TRAP

Experimental studies utilize a subcutaneous injection of VEGF Trap ("anti-VEGF"); however, the authors of the experimental study state that the bioavailability of the drug is the same whether injected subcutaneously or intravenously (12). The current model does not include a subcutaneous compartment; therefore, we simulate an intravenous injection, which inherently assumes that all of the drug appears in the blood. Injection lasts for 1 min (the duration does not affect the results, within limits) and is performed once the tumor reaches a particular volume, according to experimental methods described by Rudge et al. (12). Various doses of VEGF Trap are used, as reported by Rudge and coworkers (12) (0.5, 1, 2.5, 10, and 25 mg/kg).

SENSITIVITY ANALYSIS

In order to understand the impact of various parameters, we perform variance-based global sensitivity analyses using the eFAST (75). The eFAST method estimates the sensitivity of model outputs (i.e., VEGF concentration) with respect to variations in model
The three-compartment model is run multiple times with different parameter sets, where all parameters are varied from their baseline values. Variance for a parameter $i$ is:

$$D_i = 2 \sum_{p=1}^{\infty} \left( A_{pj}^2 + B_{pj}^2 \right)$$

where $A_j$ and $B_j$ are the Fourier coefficients of the cosine series and sine series, respectively, for the frequency, $j$, associated with the parameter $i$ and include harmonics, $p$, of the base frequency. The total variance in the output is:

$$D_{total} = 2 \sum_{j=1}^{\infty} \left( A_j^2 + B_j^2 \right)$$

The variances are used to estimate two indices that provide a measure the sensitivity: first-order FAST indices, $S_i$, and the total FAST indices, $S_{Ti}$. The first-order indices measure the local sensitivity and do not account for interactions with other parameters:

$$S_i = \frac{D_i}{D_{total}}$$

The Total FAST indices measure of global sensitivity and take into account second- and higher-order interactions between parameters. $S_{Ti}$ are calculated by excluding the effects of the complementary set of other parameters:

$$S_{Ti} = 1 - \frac{D_{Ti}}{D_{total}}$$

The eFAST method has been applied to systems biology models (76), and our laboratory has previously used the method to investigate the sensitivity of VEGFR2 signaling (77). In this work, we apply eFAST to investigate the sensitivity of steady state VEGF concentrations with respect to kinetic parameters, transport parameters, and receptor expression levels. We use Simlab 2.2 from Econometrics and Applied Statistics Unit EAS at the Joint Research Centre of the European Commission to implement eFAST.

**Numerical Implementation**

The model equations were implemented in MATLAB using the SimBiology toolbox and were solved with the Sundials solver. The model is available in SBML format at: http://www.jhu.edu/apopel/software.html

**Parameters**

*Geometry*

The geometric parameters for the tumor compartment are summarized in Table A1 in Appendix. The tumor cells are assumed to have the same volume as the MCF-7 breast tumor cells, which have a mean diameter of 12 $\mu$m (78). A sphere of this diameter would have a volume and surface area of 905 $\mu$m$^3$ and 452 $\mu$m$^2$, respectively. However, since tumor cells are not spherical, we assume a dodecahedral cell of the same volume, which has a surface area of 497 $\mu$m$^2$. The average luminal diameter of capillaries in growing MCF-7 xenografts is 13.94 $\mu$m (79), and imaging of tumor vasculature supports this value (80). We assume an EC thickness of 0.5 $\mu$m, which would yield a cylindrical cross-sectional area of 175 $\mu$m$^2$ and an outer perimeter of 46.9 $\mu$m. However, microvessels are not cylindrical. Therefore, to find the true perimeter, we used a relationship between total perimeter and total cross-sectional area in breast cancer capillaries, where the increase in perimeter is 23% (81, 82), yielding a capillary perimeter of 57.7 $\mu$m.

The extracellular fluid volume fraction in the breast tumor xenografts has been shown to range from 33 to 76% (78). Another measurement reports the extracellular fluid volume in MCF-7 tumors to be 40% (83). We assume a value of 45%, which is divided into interstitial space and intravascular space. We set the volume fraction of intravascular space to be 10%, which is within the range of available experimental data (84–86). Given the capillary dimensions described above and an intravascular volume of 10%, the capillary density is calculated to be 655 capillaries/mm$^2$. Based on a cell thickness of 0.5 $\mu$m, the volume occupied by the ECs of the microvessels is 1.5%. Cancer cells occupy the remaining tissue volume of 53.5%. The volume fractions of microvessels and tumor cells are then used to calculate the total surface area of all vessels and tumor cells per unit volume of tissue: 378 cm$^2$ EC surface/cm$^3$ tissue and 2939 cm$^2$ tumor cell surface/cm$^3$ tissue.

The interstitial space is composed of extracellular matrix (ECM), and basement membranes associated with the microvessels (endothelial basement membrane, EBM) and tumor cells (parenchymal basement membrane, PBM). The thickness of the basement membranes is assumed to be 50 and 30 nm, for the EBM and PBM, respectively, yielding volume fractions of 0.0081 and 0.0015 cm$^3$/cm$^3$ tissue. The remaining volume of the interstitial space is the ECM volume (34.04%).

Each region of the interstitial space can be represented as a porous medium that contains a solid fraction composed primarily of collagen that is unavailable to VEGF, and a fluid fraction that is accessible to VEGF. The size of the pores further limits the volume available for VEGF to diffuse. Therefore, the available volume in the ECM and basement membranes is calculated as the product of the volume, fluid fraction, and partition coefficient. The fluid fraction is the non-collagen fraction and is calculated by using the total collagen content in interstitial space. Given limited data for this measurement, we used 5%, the same value as in our previous models (24, 25, 87). The ratio of basement membrane collagen to total body collagen is assumed to be 0.3, which yields 0.0482 for the ratio of ECM collagen to total body collagen. The fluid fractions are then 0.7 for the basement membranes and 0.9318 for the ECM. The partition coefficient is the ratio of available fluid volume to interstitial fluid volume. We take 0.9 for the partition coefficient for the EBM (88), and the same value is used for the ECM and PBM, as it is difficult to distinguish basement membranes and the ECM (89). The available fluid volume for the ECM, EBM, and PBM are therefore 0.2916, 9.720 $\times$ 10$^{-4}$, and 5.082 $\times$ 10$^{-3}$ cm$^3$/cm$^3$ tissue, respectively.

**Concentrations**

Receptor densities and ECM binding site densities are listed in Table A2 in Appendix. VEGFR1, VEGFR2, and NRP1 on the
luminal and abluminal surfaces of diseased EC surfaces and on tumor cells are based on quantitative flow cytometry measurements in ECs isolated from tumor tissue, as described in (25). We assume NRP2 surface concentration on tumor cells at the same level as NRP1.

**Kinetics**

To our knowledge, there are no data for the kinetics of mVEGF isoforms binding to glycosaminoglycan (GAG) chains or mouse receptors or cross-reactions between human and mouse isoforms and receptors. Therefore, we assume the kinetic rates for VEGF binding to and dissociation from receptors, co-receptors, and GAG chains in the ECM and basement membranes are the same as in our previous papers, based on experimental data (23–25, 87) and are given in Table A3 in Appendix. We use experimental data from Papadopoulos (11) for the on and off rates of VEGF binding to VEGF Trap.

**Transport**

Transport parameters for VEGF, anti-VEGF, and the VEGF/anti-VEGF complex are listed in Table A4 in Appendix. Parameters that govern transport between the normal and blood compartments are the same as in our previous model (23). Here, we explain specific transport parameters required for the addition of soluble factors sVEGFR1 and α2M and the tumor compartment. As in the previous model, myocytes are a source of VEGF and secrete the VEGF isoforms VEGF<sub>120</sub> and VEGF<sub>164</sub> at a ratio of 8:92 (90, 91). Additionally, tumor cells secrete VEGF into the tumor interstitium at a ratio of 50:50 for VEGF<sub>121</sub>:VEGF<sub>165</sub>. Based on experimental quantification of mRNA isoform expression levels (92–96). Here, we also consider VEGF secretion by EC. We set the secretion ratio of VEGF<sub>120</sub>:VEGF<sub>164</sub> by EC to be 10:90, similar to the isoform ratio in muscle tissue, since to our knowledge, this ratio has not been determined experimentally. Additionally, we assume normal and tumor EC secrete the same amount of VEGF; tumor EC are a small fraction of the total EC in the body, thus this assumption should not affect VEGF distribution. The rates of VEGF secretion by muscle fibers, EC, and tumor cells are determined by parameter optimization, fitting to experimental data from Rudge and coworkers (12).

This expanded model includes soluble factors sVEGFR1 and α2M. ECs are a source of sVEGFR1, and the rates of secretion by normal EC was set to 6 × 10<sup>−9</sup> molecules/cell/s. Similar to VEGF secretion, we assume that sVEGFR1 secretion rate is the same for tumor EC. At steady state, the model estimated the distribution of sVEGFR1 in the body to be 0.4, 2.1, and 0.04 pM in the normal, blood, and tumor compartments, respectively. The level of sVEGFR1 in the plasma is within the range of experimental measurements, which range from 1 to 10 pM (97, 98). The clearance of α2M was set at 2.62 × 10<sup>−3</sup> min<sup>−1</sup>, based on experimental measurements of the half-life, t<sub>1/2</sub> (99), using ln(2)/t<sub>1/2</sub>. The synthesis of α2M was then estimated from mass balance at steady state, where the concentrations of native and active α2M are 1.4 μM (62) and 14 nM, respectively. We assume that the concentration of active α2M is 100-fold lower than that of the native form, based on experimental data for humans (100–102).

Molecular species are removed from the system via two mechanisms: plasma clearance and proteolytic degradation. The values of these parameters are in Table A4 in Appendix. For the normal endothelium, the permeability to sVEGFR1 and VEGF/sVEGFR1 is calculated using an empirical relation between the Stokes–Einstein radius, a<sub>E</sub>, and molecular weight [a<sub>E</sub> = 0.483 × (MW)<sup>0.386</sup>], the corresponding theoretical macro-molecular permeability-surface area product, PS (103), and the capillary surface area, S. Taking microvascular permeability as PS/S, and the calculated value is on the order of 10<sup>−9</sup> cm/s, between the normal and blood compartments. Since tumor vasculature is more permeable than normal microvessels (104), we assume that the microvascular permeability between the tumor and blood is an order of magnitude higher than permeability between normal and blood for both VEGF and the anti-VEGF or complex. Therefore, the permeability to VEGF is 4 × 10<sup>−7</sup> and 3 × 10<sup>−7</sup> cm/s for the anti-VEGF and VEGF/anti-VEGF complex. The permeability to sVEGFR1 and VEGF bound to sVEGFR1 is 1.5 × 10<sup>−7</sup> cm/s.

**Parameter estimation**

The estimation of the VEGF secretion by muscle fibers, ECs, and tumor cells was achieved using the “lsqnonlin” function in MATLAB, as previously described (23). This algorithm solves the non-linear least squares problem using the trust-region-reflective optimization algorithm (105, 106), minimizing the weighted sum of the squared residuals (WSSR):

\[
\text{min WSSR}(\theta) = \min \sum_{i=1}^{n} \left[ W_i (C_{\text{experimental},i} - C_{\text{simulation},i}(\theta)) \right]^2
\]

where C<sub>experimental</sub><sub>,i</sub> is the i<sup>th</sup> experimentally measured plasma concentration data point, C<sub>simulation</sub><sub>,i</sub>(θ) is the i<sup>th</sup> simulated plasma concentration at the corresponding time point, W<sub>i</sub> is the weight taken to be 1/|C<sub>experimental</sub><sub>,i</sub>−1, and n is the total number of experimental measurements. The minimization is subject to the upper and lower bounds of the free parameters, θ.

The two-compartment model was used to determine the rate of VEGF secretion by muscle fibers and ECs (“normal” and “EC” secretion, respectively), clearance of free and bound VEGF Trap, dissociation constant of VEGF and VEGF Trap. These five free parameters were fit to experimental data for the concentration profiles of VEGF/VEGF Trap complex and unbound VEGF Trap in mice at different doses of VEGF Trap (12), with a total of 58 data points. The initial value of the secretion rates was generated within the upper and lower bounds of 6 × 10<sup>−8</sup> molecules/cell/h and was set based on the limit of detection of standard ELISA kits used to measure (63). The half-life of VEGF Trap in mouse serum has been reported as 72 h (107), which corresponds to a clearance rate of 1.6 × 10<sup>−4</sup> min<sup>−1</sup>, assuming clearance rate is equal to ln(2)/half-life. The upper and lower bounds of the clearance rates were one order of magnitude above and below this value, respectively. The upper and lower bounds for the dissociation constant were set to 0.25 and 5 pM, based on experimental data (11, 12). The baseline value of permeability of the normal tissue to VEGF Trap is 3 × 10<sup>−8</sup> cm/s, as described above, and the bounds were one order...
of magnitude above and below this value. The optimal parameter values are reported as the mean and standard deviation of the 20 runs.

We used the three-compartment model to determine the rate at which VEGF is secreted by tumor cells (“tumor secretion”) and permeability of diseased tissue to free and complexed VEGF Trap. Tumor secretion was optimized to fit experimental data for the system VEGF Trap levels (free and complexed) reported by Rudge et al. (12). Experimental data for two human tumor xenografts (A673 rhabdomyosarcoma and HT1080 fibrosarcoma) were used separately; the total number of data points was 11 for A673 tumors and 10 for HT1080 tumors. Twenty runs were performed for each tumor, which either followed the average (baseline) or fast growth profile. This yields two conditions for each tumor type. The optimal secretion rates are reported as the mean and standard deviation of the 20 runs and should be interpreted as a range of values, where the values are dependent on the tumor microenvironment, tumor type, and growth profile.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/Molecular_and_Cellular_Oncology/10.3389/fonc.2013.00196/abstract

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### TABLE A1 | Geometric parameters.

| Parameter                               | Value | Units     | Reference                           |
|-----------------------------------------|-------|-----------|-------------------------------------|
| **CANCER CELLS**                        |       |           |                                     |
| Tumor cell external diameter            | 12    | µm        | Paran et al. (78)                   |
| Volume of one cell                      | 905   | µm$^3$    | Calculated (see manuscript)         |
| Surface area of one cell                | 497   | µm$^2$    | Calculated (see manuscript)         |
| **MICROVESSELS**                        |       |           |                                     |
| Average luminal diameter                | 13.9  | µm        | Schaefer et al. (79)                |
| Endothelial cell thickness              | 0.5   | µm        | Based on normal microvessels (108)  |
| Average external diameter               | 14.9  | µm        | Calculated (see manuscript)         |
| Cross-sectional area of one vessel      | 175.3 | µm$^2$    | Calculated (see manuscript)         |
| Perimeter of one vessel                 | 57.7  | µm        | Calculated (see manuscript)         |
| Capillary density                       | 655   | Capillaries/mm$^2$ | Calculated (see manuscript) |
| **VOLUME FRACTIONS**                    |       |           |                                     |
| Interstitial space                      | 35.0% | cm$^2$/cm$^3$ tissue | Based on (78, 83) |
| Cancer cells                            | 53.5% | cm$^2$/cm$^3$ tissue | Calculated (see manuscript)         |
| Microvessels of which intravascular space | 11.5% | cm$^2$/cm$^3$ tissue | Calculated (see manuscript)         |
|                                          | 10.0% | cm$^2$/cm$^3$ tissue | Based on (84–86)                   |
| **SURFACE AREAS**                       |       |           |                                     |
| Tumor cells                             | 2939  | cm$^2$/cm$^3$ tissue | Calculated (see manuscript)         |
| Microvessels                            | 378   | cm$^2$/cm$^3$ tissue | Calculated (see manuscript)         |
| **BASEMENT MEMBRANES (BM)**             |       |           |                                     |
| Thickness of tumor cell BM              | 30    | nm        | Based on (109)                      |
| Basement membrane volume (tumor cells) of which available to VEGF | 0.00807 | cm$^3$/cm$^3$ tissue | Calculated (see manuscript)         |
|                                          | 0.00508 | cm$^3$/cm$^3$ tissue | Calculated (see manuscript)         |
| Thickness of microvessel BM             | 50    | nm        | Based on (109)                      |
| Basement membrane volume (microvessels) of which available to VEGF | 0.00154 | cm$^3$/cm$^3$ tissue | Calculated (see manuscript)         |
|                                          | 0.000972 | cm$^3$/cm$^3$ tissue | Calculated (see manuscript)         |
| Extracellular matrix volume of which available to VEGF | 0.3375 | cm$^3$/cm$^3$ tissue | Calculated (see manuscript)         |
|                                          | 0.2892 | cm$^3$/cm$^3$ tissue | Calculated (see manuscript)         |

### TABLE A2 | Concentrations in tumor compartment.

| Protein      | Value | Units     |
|--------------|-------|-----------|
| VEGFR1       |       |           |
| Luminal EC   | 3750  | Dimers/EC |
| Abluminal EC | 3750  | Dimers/EC |
| Tumor        | 1100  | Dimers/TC |
| VEGFR2       |       |           |
| Luminal EC   | 300   | Dimers/EC |
| Abluminal EC | 300   | Dimers/EC |
| Tumor        | 550   | Dimers/TC |
| NRP1         |       |           |
| Luminal EC   | 39,748| Dimers/EC |
| Abluminal EC | 39,748| Dimers/EC |
| Tumor        | 39,500| Dimers/TC |
| NRP2         |       |           |
| Tumor        | 39,500| Dimers/TC |
| ECM binding density | 0.75  | µM        |
| EBM binding density | 13    | µM        |
| PBM binding density | 13    | µM        |

*EC,* endothelial cell; *TC,* tumor cell.
Table A3 | Kinetic parameters.

| Table A3 | Kinetic parameters. |
|----------|---------------------|
| **VEGF BINDING TO VEGFR1** | |
| \(k_{\text{on}}\) & \(3 \times 10^7\) M\(^{-1}\)s\(^{-1}\) & Mac Gabhann and Popel (87), Stefanini et al. (24) |
| \(k_{\text{off}}\) & \(10^{-3}\) s\(^{-1}\) & Mac Gabhann and Popel (87), Stefanini et al. (24) |
| \(K_d\) & 33 pM & Mac Gabhann and Popel (87), Stefanini et al. (24) |
| **VEGF BINDING TO VEGFR2** | |
| \(k_{\text{on}}\) & \(10^7\) M\(^{-1}\)s\(^{-1}\) & Mac Gabhann and Popel (87), Stefanini et al. (24) |
| \(k_{\text{off}}\) & \(10^{-3}\) s\(^{-1}\) & Mac Gabhann and Popel (87), Stefanini et al. (24) |
| \(K_d\) & 100 pM & Mac Gabhann and Popel (87), Stefanini et al. (24) |
| **VEGF BINDING TO NRP1** | |
| \(k_{\text{on}}\) & \(3.2 \times 10^6\) M\(^{-1}\)s\(^{-1}\) & Mac Gabhann and Popel (87), Stefanini et al. (24) |
| \(k_{\text{off}}\) & \(10^{-3}\) s\(^{-1}\) & Mac Gabhann and Popel (87), Stefanini et al. (24) |
| \(K_d\) & \(312.5\) pM & Mac Gabhann and Popel (87), Stefanini et al. (24) |
| **VEGF BINDING TO GAGs** | |
| \(k_{\text{on}}\) & \(4.20 \times 10^5\) M\(^{-1}\)s\(^{-1}\) & Mac Gabhann and Popel (87), Stefanini et al. (24) |
| \(k_{\text{off}}\) & \(10^{-2}\) s\(^{-1}\) & Mac Gabhann and Popel (87), Stefanini et al. (24) |
| \(K_d\) & \(24\) pM & Mac Gabhann and Popel (87), Stefanini et al. (24) |
| **COUPLING OF NRP1 AND VEGFR1** | |
| \(k_c\) & \(10^{14}\) (mol/cm\(^2\))\(^{-1}\)s\(^{-1}\) & Mac Gabhann and Popel (87), Stefanini et al. (24) |
| \(k_{\text{off}}\) & \(10^{-2}\) s\(^{-1}\) & Mac Gabhann and Popel (87), Stefanini et al. (24) |
| **COUPLING OF NRP1 AND VEGFR2** | |
| \(k_{\text{on}}\) & \(3.1 \times 10^3\) (mol/cm\(^2\) s\(^{-1}\)) & Mac Gabhann and Popel (87), Stefanini et al. (24) |
| \(k_{\text{off}}\) & \(10^{-3}\) s\(^{-1}\) & Mac Gabhann and Popel (87), Stefanini et al. (24) |
| \(K_d\) & \(100\) pM & Mac Gabhann and Popel (87), Stefanini et al. (24) |
| **VEGFR INTERNALIZATION** | |
| \(k_{\text{int}}\) & \(2.8 \times 10^{-4}\) s\(^{-1}\) & Mac Gabhann and Popel (87), Stefanini et al. (24) |
| **VEGF\(_{121}\) BINDING TO ANTI-VEGF** | |
| \(k_{\text{on}}\) & \(3.75 \times 10^6\) M\(^{-1}\)s\(^{-1}\) & Calculated |
| \(k_{\text{off}}\) & \(1.35 \times 10^{-5}\) s\(^{-1}\) & Papadopoulos et al. (11) |
| \(K_d\) & \(0.36\) pM & Papadopoulos et al. (11) |
| **VEGF\(_{165}\) BINDING TO ANTI-VEGF** | |
| \(k_{\text{on}}\) & \(4.10 \times 10^7\) M\(^{-1}\)s\(^{-1}\) & Calculated |
| \(k_{\text{off}}\) & \(2.01 \times 10^{-5}\) s\(^{-1}\) & Papadopoulos et al. (11) |
| \(K_d\) & \(0.49\) pM & Papadopoulos et al. (11) |
| **VEGF\(_{120}\) BINDING TO ANTI-VEGF** | |
| \(k_{\text{on}}\) & \(2.15 \times 10^7\) M\(^{-1}\)s\(^{-1}\) & Calculated |
| \(k_{\text{off}}\) & \(1.23 \times 10^{-5}\) s\(^{-1}\) & Papadopoulos et al. (11) |
| \(K_d\) & \(0.572\) pM & Papadopoulos et al. (11) |
| **VEGF\(_{164}\) BINDING TO ANTI-VEGF** | |
| \(k_{\text{on}}\) & \(2.80 \times 10^7\) M\(^{-1}\)s\(^{-1}\) & Calculated |
| \(k_{\text{off}}\) & \(1.64 \times 10^{-5}\) s\(^{-1}\) & Papadopoulos et al. (11) |
| \(K_d\) & \(0.586\) pM & Papadopoulos et al. (11) |
| **VEGF BINDING TO α2M** | |
| \(k_{\text{on}}\) & \(25\) M\(^{-1}\)s\(^{-1}\) & Calculated |
| \(k_{\text{off}}\) & \(10^{-4}\) s\(^{-1}\) & Assumed |
| \(K_d\) & \(4.0\) µM & Bhattacharjee et al. (110) |
| **VEGF BINDING TO α2M\(_{\text{FAST}}\)** | |
| \(k_{\text{on}}\) & \(2.4 \times 10^2\) M\(^{-1}\)s\(^{-1}\) & Calculated |
| \(k_{\text{off}}\) & \(10^{-4}\) s\(^{-1}\) & Assumed |
| \(K_d\) & \(0.42\) µM & Bhattacharjee et al. (110) |

(Continued)
Table A3 | Continued

| Value | Unit | Reference |
|-------|------|-----------|
| $k_{on}$ | $3 \times 10^7$ M$^{-1}$ s$^{-1}$ | Assumed, based on VEGF binding to VEGFR1 |
| $k_{off}$ | $10^{-3}$ s$^{-1}$ | Assumed |
| $K_d$ | 33 pM | Assumed |

$VEGFR_1$ BINDING TO $NRP_1$

| $k_{on}$ | $5.6 \times 10^6$ M$^{-1}$ s$^{-1}$ | Calculated |
| $k_{off}$ | $10^{-2}$ s$^{-1}$ | Assumed, based on VEGFR1 coupling to NRP1 |
| $K_d$ | 1.8 nM | Fuh et al. (111) |

$VEGFR_1$ BINDING TO GAGs

| $k_{on}$ | $4.20 \times 10^5$ M$^{-1}$ s$^{-1}$ | Assumed, based on VEGF$^{165}$ binding to GAG |
| $k_{off}$ | $10^{-2}$ s$^{-1}$ | Assumed |
| $K_d$ | 24 pM | Assumed |

Table A4 | Transport parameters.

| Value | Unit | Reference |
|-------|------|-----------|
| PERMEABILITY BETWEEN NORMAL AND BLOOD |
| VEGF | $4.0 \times 10^{-8}$ cm/s | Stefanini et al. (24) |
| Anti-VEGF and VEGF/anti-VEGF complex | $3.0 \times 10^{-8}$ cm/s | Stefanini et al. (24) |
| Soluble VEGFR1 | $1.5 \times 10^{-8}$ cm/s | Calculated, see text |
| Soluble VEGFR1/VEGF complex | $1.5 \times 10^{-8}$ cm/s | Calculated, see text |

| PERMEABILITY BETWEEN TUMOR AND BLOOD |
| VEGF | $4.0 \times 10^{-7}$ cm/s | Assumed, see text |
| Anti-VEGF and VEGF/anti-VEGF complex | $3.0 \times 10^{-7}$ cm/s | Assumed, see text |
| Soluble VEGFR1 | $1.5 \times 10^{-7}$ cm/s | Assumed, see text |
| Soluble VEGFR1/VEGF complex | $1.5 \times 10^{-7}$ cm/s | Assumed, see text |

| CLEARANCE |
| VEGF | $2.3 \times 10^{-1}$ min$^{-1}$ | Folkman (112) |
| Anti-VEGF | $8.9 \times 10^{-4}$ min$^{-1}$ | Yen et al. (23) |
| VEGF/anti-VEGF complex | $2.8 \times 10^{-4}$ min$^{-1}$ | Yen et al. (23) |
| Soluble VEGFR1 | $3.0 \times 10^{-4}$ min$^{-1}$ | Wu et al. (113) |
| Soluble VEGFR1/VEGF complex | $3.0 \times 10^{-4}$ min$^{-1}$ | Wu et al. (113) |
| $\alpha_2M$-VEGF complex | $2.6 \times 10^{-3}$ min$^{-1}$ | Hudson et al. (69) |
| $\alpha_2M$-VEGF/anti-VEGF complex | $2.6 \times 10^{-3}$ min$^{-1}$ | Assumed, based on $\alpha_2M$ |
| Activated alpha-2-macroglobulin ($\alpha_2M_{fast}$) | $2.4 \times 10^{-1}$ min$^{-1}$ | Imber and Pizzo (114) |
| $\alpha_2M$-VEGF complex | $2.6 \times 10^{-3}$ min$^{-1}$ | Assumed, based on $\alpha_2M_{fast}$ |

| DEGRADATION |
| Soluble VEGFR1 | $1.2 \times 10^{-2}$ min$^{-1}$ | Assumed based on VEGF |
| Soluble VEGFR1/VEGF complex | $1.2 \times 10^{-2}$ min$^{-1}$ | Assumed based on VEGF |

| SYNTHESIS |
| Alpha-2-macroglobulin | $1.8 \times 10^{10}$ Molecules/cm$^3$ tissue/s | Calculated, see text |
| Activated alpha-2-macroglobulin | $1.6 \times 10^{10}$ Molecules/cm$^3$ tissue/s | Calculated, see text |
FIGURE A1 | Predicted systemic VEGF Trap levels for fast-growing tumors. The model predicts the plasma levels of free VEGF Trap (black lines), mouse VEGF bound to VEGF Trap (blue lines), and human VEGF bound to VEGF Trap (red lines) for fast-growing tumors. VEGF Trap was administered twice per week for 2 weeks at doses of 0.5, 1, 2.5, 10, and 25 mg/kg. The simulated results are shown for the optimized model where the secretion rates of VEGF by myocytes, EC, and tumor cells were fit to experimental data (circles). We use the mean (solid lines) and 1 SD (dashed lines) of the fitted secretion rates. (A) A673 tumor; and (B) HT1080 tumor.

A

A673 - Fast

B

HT1080 - Fast

FIGURE A2 | Optimized VEGF secretion rates. The model parameters were optimized to fit experimental data, and the values of normal, EC, and tumor VEGF secretion rates were determined. The mean optimal secretion rates and standard deviation of 20 optimization runs for fast-growing tumors are shown.

Fast-growing tumors

| Muscle fibers | Endothelial cells | Tumor cells |
|--------------|------------------|------------|
| A673         | 0.025            | 0.020      |
| HT1080       | 0.015            | 0.010      |

FIGURE A2 | Optimized VEGF secretion rates. The model parameters were optimized to fit experimental data, and the values of normal, EC, and tumor VEGF secretion rates were determined. The mean optimal secretion rates and standard deviation of 20 optimization runs for fast-growing tumors are shown.