Article Title: ***In silico* mouse study identifies tumor growth kinetics as biomarkers for the outcome of anti-angiogenic treatment**

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Supplemental File S1 – Detailed description of computational model

Summarized from [1].

Overview

The model is comprised of three compartments representing the whole mouse (**Figure 1**): normal tissue, blood, and tumor tissue. We include human VEGF isoforms ($VEGF_{121}$ and $VEGF_{165}$) secreted by tumor cells and mouse isoforms ($VEGF_{120}$ and $VEGF_{164}$) secreted by endothelial cells and muscle fibers. The model includes the cell surface VEGF receptors, VEGFR1 and VEGFR2, and the soluble form of VEGFR1 (sVEGFR1). We also include co-receptors called neuropilins (NRP1 and NRP2) that bind VEGF directly and also form tertiary complexes with the VEGFRs. The protease inhibitor α-2-macroglobulin binds VEGF in blood plasma. The VEGF isoforms and sVEGFR1 can be transported between compartments via transendothelial macromolecular permeability and lymphatic flow. Additionally, species are removed from the body via clearance. We consider both the luminal and abluminal endothelial surfaces at the interface between the blood and each tissue compartment. The luminal endothelial cell surface faces blood plasma, and the abluminal surface (outside of blood plasma) faces the tissue interstitium.

Parameters

Geometry (23 parameters). The geometric parameters for the tumor compartment are summarized in **Table S1.1** The tumor cell diameter is assumed to be that of MCF-7 breast tumor cells, 12 μm [2]. Assuming tumor cells are dodecahedral, rather than exactly spherical, we set the tumor cell volume and surface area to be 497 μm^3 and 452 μm^2, respectively. Based on the average luminal diameter of capillaries in growing MCF-7 xenografts, 13.94 μm [3–5], an endothelial cell thickness of 0.5 μm, and the relationship between total perimeter and total cross-sectional area in breast cancer capillaries [6, 7], we estimate the capillary perimeter to be 57.7 μm.

We take the extracellular fluid volume fraction in breast tumor xenografts to be 45%, based on a range of measurements, 33% - 76% [2, 8]. This volume fraction is divided into interstitial space

and intravascular space. Using the capillary dimensions described above and an intravascular volume of 10% [9–11], the capillary density is calculated to be 655 capillaries/mm^2. Based on a cell thickness of 0.5 μm, the volume occupied by the endothelial cells 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 endothelial cell 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 nm 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 is 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 [12–15]. 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 [16], and the same value is used for the ECM and PBM, as it is difficult to distinguish basement membranes and the ECM [17]. The available fluid volume for the ECM, EBM, and PBM are therefore 0.2916, 9.720×10^{-4}, and 5.082×10^{-3} cm^3 / cm^3 tissue, respectively.

Initial concentrations (32 parameters). Receptor densities and ECM binding site densities are listed in **Table S1.2**. VEGFR1, VEGFR2, and NRP1 on the luminal and abluminal surfaces of diseased endothelial cell surfaces and on tumor cells are based on quantitative flow cytometry

measurements in endothelial cells isolated from tumor tissue, as described in [14]. We assume NRP2 surface concentration on tumor cells at the same level as NRP1. The initial concentrations of all other species are zero.

Kinetic parameters (40 parameters). The kinetic rates for VEGF binding to and dissociating from receptors, co-receptors, and glycosaminoglycan (GAG) chains in the ECM and basement membranes are the same as in our previous papers, based on experimental data [12–14, 18] are given in **Table S1.3**. We use experimental data from [19] for the on and off rates of VEGF binding to the anti-VEGF agent, bevacizumab.

Intercompartmental transport (8 parameters). Transport parameters for VEGF, anti-VEGF and the VEGF/anti-VEGF complex are listed in **Table S1.4**. Parameters that govern transport between the normal and blood compartments are the same as in our previous models [15, 18].

Secretion and clearance rates of soluble species (26 parameters). Tumor cells secrete VEGF into the tumor interstitium at a ratio of 50:50 for $VEGF_{121}$:$VEGF_{165}$, based on experimental quantification of mRNA isoform expression levels [20–24]. Here, we also consider VEGF secretion by EC. We set the secretion ratio of $VEGF_{120}$:$VEGF_{164}$ 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. In our previous work [15], we fit the rates of VEGF secretion by muscle fibers, EC, and tumor cells by parameter optimization, fitting to experimental data from Rudge and coworkers [25]. These fitted values are used in the current model.

The model also includes soluble factors sVEGFR1 and a2M. Endothelial cells are a source of sVEGFR1; therefore, sVEGFR1 is secreted in all three compartments. Endothelial cells also secrete a2M; however, due to its large size, a2M is not transported via transendothelial macromolecular permeability and is confined to the blood compartment. The rates of secretion of sVEGFR1 and a2M are given in **Table S1.4** (below).

Molecular species are removed from the system via two mechanisms: plasma clearance and proteolytic degradation. The values of these parameters are in **Table S1.4**. For the normal endothelium, the permeability to sVEGFR1 and VEGF/sVEGFR1 is calculated using an

empirical relation between the Stokes-Einstein radius, a_E, and molecular weight ($a_E = 0.483 \times (MW)^{0.386}$) , the corresponding theoretical macromolecular permeability-surface area product, *PS* [26], and the capillary surface area, *S*. Taking microvascular permeability as *PS/S*, and the calculated value is on the order of 10^{-8} cm/s, between the normal and blood compartments. Since tumor vasculature is more permeable than normal microvessels [27], 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^{-7} cm/s and 3×10^{-7} cm/s for the anti-VEGF and VEGF/anti-VEGF complex. The permeability to sVEGFR1 and VEGF-bound to sVEGFR1 is 1.5×10^{-7} cm/s.

Change in relative volume of the interstitial space

The relative interstitial space decreases as the tumor volume increases and the blood compartment remains constant. A recent study by Christensen and colleagues published data of concentration of cancer cells in MDA-MB-231 E2-Crimson expressing tumors (cancer cells/mm^3 tumor) [28]. Informed by this data, we calculated the total tumor cell (tumor compartment) volume at each time point for the six experimental datasets, assuming an average tumor cell volume of 905 μm^3. The remaining volume (total volume – (blood compartment + tumor compartment)) is calculated as the interstitial space volume. We then individually fit to the relative interstitial space volume of each dataset with an exponential decay function. The equation for how the relative volume of the interstitial space changes as a function of the total tumor volume is unique for each of the datasets (**Table S1.5**).

Dynamic volume of the tumor compartment

Tumor growth is given by an adapted Gompertz model focusing on the exponential and linear phases of the tumor growth, as previously described [29, 30]. We implemented the model such that the volume of the tumor compartment is also dependent on the "angiogenic signal" (*Ang*) produced when VEGF binds to its receptors on endothelial cells in the tumor. This introduces an explicit relationship between the pro-angiogenic VEGF and tumor growth.

The differential equation for the tumor volume is:

$$\frac{dV(t)}{dt} = \frac{k_0 * V(t)}{\left[1+\left(\frac{k_0}{k_1} * V(t)\right)^{\psi}\right]^{\frac{1}{\psi}}} \cdot \left(1 - \frac{Ang_0 - Ang(t)}{Ang_0}\right) \quad (1a)$$

We note that equation (1a) simplifies to:

$$\frac{dV(t)}{dt} = \frac{k_0 * V(t)}{\left[1+\left(\frac{k_0}{k_1} * V(t)\right)^{\psi}\right]^{\frac{1}{\psi}}} \cdot \left(\frac{Ang(t)}{Ang_0}\right) \quad (1b)$$

Here, *V(t)* is the tumor volume in cm^3 at time *t*, k_0 and k_1 are parameters describing the rate of exponential and linear growth, respectively. The units of k_0 and k_1 are s^{-1} and cm^3 tissue/s, respectively. The ψ parameter represents the transition from exponential to linear tumor growth and is unitless. The Ang_0 parameter represents the basal angiogenic signal (at time $t = 0$), and *Ang(t)* is the angiogenic signal at time *t*. The value of *Ang* at any time is calculated as the total concentration of pro-angiogenic VEGF-receptor complexes on tumor endothelial cells. This includes VEGFR1 and VEGFR2 bound to either mouse or human VEGF isoforms, with or without the NRP1 co-receptor. Thus, *Ang(t)* and Ang_0 have units of concentration (mol/cm^3 tissue).

Supplementary Tables

Table S1.1. Geometric parameters

	Value	Units	Reference
Cancer cells			
Tumor cell external diameter	12	μm	[2]
Volume of one cell	905	μm^3	Calculated, see text
Surface area of one cell	497	μm^2	Calculated, see text
Microvessels			
Average luminal diameter	13.9	μm	[3]
Endothelial cell thickness	0.5	μm	Based on normal microvessels [31]
Average external diameter	14.9	μm	Calculated, see text
Cross sectional area of one vessel	175.3	μm^2	Calculated, see text
Perimeter of one vessel	57.7	μm	Calculated, see text
Capillary density	655	capillaries/mm^2	Calculated, see text
Volume fractions			
Interstitial space	35.0%	cm^2/cm^3 tissue	Based on [2, 8]
Cancer cells	53.5%	cm^2/cm^3 tissue	Calculated, see text
Microvessels	11.5%	cm^2/cm^3 tissue	Calculated, see text
of which intravascular space	10.0%	cm^2/cm^3 tissue	Based on [9–11]
Surface areas			
Tumor cells	2939	cm^2/cm^3 tissue	Calculated, see text
Microvessels	378	cm^2/cm^3 tissue	Calculated, see text
Basement membranes (BM)			
Thickness of tumor cell BM	30	nm	Based on [32]
Basement membrane volume (tumor cells)	0.00807	cm^3/cm^3	Calculated, see text
of which available to VEGF	0.00508	cm^3/cm^3 tissue	Calculated, see text
Thickness of microvessel BM	50	nm	Based on [32]
Basement membrane volume (microvessels)	0.00154	cm^3/cm^3 tissue	Calculated, see text
of which available to VEGF	0.000972	cm^3/cm^3 tissue	Calculated, see text
Extracellular matrix volume	0.3375	cm^3/cm^3 tissue	Calculated, see text
of which available to VEGF	0.2892	cm^3/cm^3 tissue	Calculated, see text

Table S1.2. Initial concentrations

Normal compartment		
	Value	**Units**
VEGFR-1		
Abluminal EC	0	dimers/EC
Muscle fibers	0	dimers/fiber
VEGFR-2		
Abluminal EC	0	dimers/EC
Muscle fibers	0	dimers/ fiber
NRP-1		
Abluminal EC	39748	dimers/EC
Muscle fibers	39500	dimers/fiber
NRP-2		
Abluminal EC	0	dimers/EC
Muscle fibers	0	dimers/fiber
ECM binding density	0.75	μM
EBM binding density	13	μM
PBM binding density	13	μM
Blood compartment		
	Value	**Units**
VEGFR-1		
Luminal EC (normal)	3750	dimers/EC
Luminal EC (diseased)	3750	dimers/EC
VEGFR-2		
Luminal EC (normal)	3750	dimers/EC
Luminal EC (diseased)	3750	dimers/EC
NRP-1		
Luminal EC (normal)	3750	dimers/EC
Luminal EC (diseased)	3750	dimers/EC
NRP-2		
Luminal EC (normal)	0	dimers/EC
Luminal EC (diseased)	0	dimers/EC
ECM binding density	0	μM
EBM binding density	0	μM
PBM binding density	0	μM

Tumor compartment		
	Value	**Units**
VEGFR-1		
Abluminal EC	3750	dimers/EC
Tumor cells	1100	dimers/TC
VEGFR-2		
Abluminal EC	300	dimers/EC
Tumor cells	550	dimers/ TC
NRP-1		
Abluminal EC	39748	dimers/EC
Tumor cells	39500	dimers/TC
NRP-2		
Tumor cells	39500	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 S1.3. Kinetic parameters

	Value	Unit	Reference
VEGF binding to VEGFR-1			
k_{on}	3×10^{7}	$M^{-1}s^{-1}$	[12, 13]
k_{off}	10^{-3}	s^{-1}	[12, 13]
K_d	33	pM	[12, 13]
VEGF binding to VEGFR-2			
k_{on}	10^{7}	$M^{-1}s^{-1}$	[12, 13]
k_{off}	10^{-3}	s^{-1}	[12, 13]
K_d	100	pM	[12, 13]
VEGF binding to NRP-1			
k_{on}	3.2×10^{6}	$M^{-1}s^{-1}$	[12, 13]
k_{off}	10^{-3}	s^{-1}	[12, 13]
K_d	312.5	pM	[12, 13]
VEGF binding to GAGs			
k_{on}	4.20×10^{5}	$M^{-1}s^{-1}$	[12, 13]
k_{off}	10^{-2}	s^{-1}	[12, 13]
K_d	24	pM	[12, 13]
Coupling of NRP-1 and VEGFR-1			
k_c	10^{14}	$(mol/cm^2)^{-1}\, s^{-1}$	[12, 13]
k_{off}	10^{-2}	s^{-1}	[12, 13]
Coupling of NRP-1 and VEGFR-2			
$k_{cV165R2,N1}$	3.1×10^{13}	$(mol/cm^2)^{-1}\, s^{-1}$	[12, 13]
$k_{offV165R2,N1}$	10^{-3}	s^{-1}	[12, 13]
$k_{cV165N1,R2}$	10^{14}	$(mol/cm^2)^{-1}\, s^{-1}$	[12, 13]
$k_{offV165N1,R2}$	10^{-3}	s^{-1}	[12, 13]
VEGFR Internalization			
k_{int}	2.8×10^{-4}	s^{-1}	[12, 13]

$VEGF_{121}$ binding to anti-VEGF			
k_{on}	5.4×10^{4}	$M^{-1}s^{-1}$	[19]
k_{off}	2.19×10^{-5}	s^{-1}	[19]
K_d	4456	pM	[19]
$VEGF_{165}$ binding to anti-VEGF			
k_{on}	5.4×10^{4}	$M^{-1}s^{-1}$	[19]
k_{off}	2.19×10^{-5}	s^{-1}	[19]
K_d	4456	pM	[19]
VEGF binding to α2M			
k_{on}	25	$M^{-1}s^{-1}$	Calculated
k_{off}	10^{-4}	s^{-1}	Assumed
K_d	4.0	μM	[33]
VEGF binding to $\alpha 2M_{fast}$			
k_{on}	2.4×10^{2}	$M^{-1}s^{-1}$	Calculated
k_{off}	10^{-4}	s^{-1}	Assumed
K_d	0.42	μM	[33]
sVEGFR1 binding to VEGF			
k_{on}	3×10^{7}	$M^{-1}s^{-1}$	Assumed, based on VEGF binding to VEGFR1
k_{off}	10^{-3}	s^{-1}	Assumed
K_d	33	pM	Assumed
sVEGFR1 binding to NRP-1			
k_{on}	5.6×10^{6}	$M^{-1}s^{-1}$	Calculated
k_{off}	10^{-2}	s^{-1}	Assumed, based on VEGFR1 coupling to NRP1
K_d	1.8	nM	[34]
sVEGFR1 binding to GAGs			
k_{on}	4.20×10^{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 S1.4. Transport, secretion and clearance rates

	Value	Unit	Reference
Permeability between normal and blood			
VEGF	4.0×10^{-8}	cm/s	[13]
Anti-VEGF & VEGF/anti-VEGF complex	3.0×10^{-8}	cm/s	[13]
Soluble VEGFR1	1.5×10^{-8}	cm/s	Calculated, see text
Soluble VEGFR1/VEGF complex	1.5×10^{-8}	cm/s	Calculated, see text
Permeability between tumor and blood			
VEGF	4.0×10^{-7}	cm/s	Assumed, see text
Anti-VEGF & VEGF/anti-VEGF complex	3.0×10^{-7}	cm/s	Assumed, see text
Soluble VEGFR1	1.5×10^{-7}	cm/s	Assumed, see text
Soluble VEGFR1/VEGF complex	1.5×10^{-7}	cm/s	Assumed, see text
Clearance			
VEGF	2.3×10^{-1}	min^{-1}	[35]
Anti-VEGF	8.9×10^{-4}	min^{-1}	[18]
VEGF/anti-VEGF complex	2.8×10^{-4}	min^{-1}	[18]
Soluble VEGFR1	3.0×10^{-4}	min^{-1}	[36]
Soluble VEGFR1/VEGF complex	3.0×10^{-4}	min^{-1}	[36]
Alpha-2-macroglobulin (α2M)	2.6×10^{-3}	min^{-1}	[37]
a2M /VEGF complex	2.6×10^{-3}	min^{-1}	Assumed, based on a2M
a2M /VEGF/anti-VEGF complex	2.6×10^{-3}	min^{-1}	Assumed, based on a2M
Activated alpha-2-macroglobulin ($\alpha 2M_{fast}$)	2.4×10^{-1}	min^{-1}	[38]
a2M /VEGF complex	2.6×10^{-3}	min^{-1}	Assumed, based on $a2M_{fast}$
Degradation			
Soluble VEGFR1	1.2×10^{-2}	min^{-1}	Assumed based on VEGF
Soluble VEGFR1/VEGF complex	1.2×10^{-2}	min^{-1}	Assumed based on VEGF
Synthesis			
$VEGF_{164}$ – Normal cells	9.72×10^{-3}	molecules/cell/s	Estimated in [15]
$VEGF_{164}$ – Endothelial cells	6.72×10^{-3}	molecules/cell/s	Estimated in [15]
$VEGF_{164}$ – Tumor cells	0	molecules/cell/s	Tumor cells only secrete human VEGF isoforms

$VEGF_{120}$ – Normal cells	8.45×10^{-4}	molecules/cell/s	Estimated in [15]
$VEGF_{120}$ – Endothelial cells	7.47×10^{-4}	molecules/cell/s	Estimated in [15]
$VEGF_{120}$ – Tumor cells	0	molecules/cell/s	Tumor cells only secrete human VEGF isoforms
$VEGF_{165}$ – Normal cells	0	molecules/cell/s	Only tumor cells secrete human VEGF isoforms
$VEGF_{165}$ – Endothelial cells	0	molecules/cell/s	Only tumor cells secrete human VEGF isoforms
$VEGF_{165}$ – Tumor cells	4.65×10^{-3}	molecules/cell/s	Estimated in [15]
$VEGF_{121}$ – Normal cells	0	molecules/cell/s	Only tumor cells secrete human VEGF isoforms
$VEGF_{121}$ – Endothelial cells	0	molecules/cell/s	Only tumor cells secrete human VEGF isoforms
$VEGF_{121}$ – Tumor cells	4.65×10^{-3}	molecules/cell/s	Estimated in [15]
Alpha-2-macroglobulin (α2M)	1.8×10^{10}	molecules/cm^3 tissue/s	Calculated, see text
Activated alpha-2-macroglobulin ($\alpha 2M_{fast}$)	1.6×10^{10}	molecules/cm^3 tissue/s	Calculated, see text

Table S1.5. Equations describing change in relative volume of the interstitial space

Dataset	Relative volume of interstitial space (cm^3/cm^3 tissue)
Roland	$Vol_{IS} = 0.8323 \cdot e^{(-0.239 \cdot V(t))}$
Zibara	$Vol_{IS} = 0.8247 \cdot e^{(-0.069 \cdot V(t))}$
Tan	$Vol_{IS} = 0.8343 \cdot e^{(-0.062 \cdot V(t))}$
Volk (2008)	$Vol_{IS} = 0.8628 \cdot e^{(-0.068 \cdot V(t))}$
Volk (2011a)	$Vol_{IS} = 0.8557 \cdot e^{(-0.081 \cdot V(t))}$
Volk (2011b)	$Vol_{IS} = 0.8536 \cdot e^{(-0.068 \cdot V(t))}$

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