# Data-driven modeling and characterization of anti-angiogenic molecule effects on tumoral vascular density

J.-B. Tylcz<sup>a,b</sup>, K. El Alaoui-Lasmaili<sup>a,b</sup>, E.-H. Djermoune<sup>a,b</sup>, N. Thomas<sup>a,b</sup>, B. Faivre<sup>a,b</sup>, T. Bastogne<sup>a,b,c</sup>

<sup>a</sup> Université de Lorraine, CRAN, UMR 7039, Vandæuvre-lès-Nancy, France <sup>b</sup> CNRS, CRAN, UMR 7039, Vandæuvre-lès-Nancy, France <sup>c</sup> INRIA, BIGS, France

## Abstract

Angiogenesis is the phenomenon by which new blood vessels are created from preexisting ones. But this natural process is also involved, in a chaotic way, in tumor development. Many molecules have shown particular efficiency in inhibiting this phenomenon, hopefully leading to either: (i) a reorganization of the neovessels allowing a better tumor uptake of cytotoxic molecules (as chemotherapy) or (ii) a deprivation of the tumor vascular network with the view to starve it. However, characterizing the anti-angiogenic effects of a molecule remains difficult, mainly because the proposed physical modeling approaches have barely been confronted to *in vivo* data, which are not directly available. This paper presents an original approach to characterize and analyze the anti-angiogenic responses in cancerology that allows biologists to account for spatial and dynamical dimensions of the problem. The proposed solution relies on the association of a specific biological *in vivo* protocol using skinfold chambers, image processing and dynamic system identification. An empirical model structure of the anti-angiogenic effect of a tested molecule is selected according to experimental data. Finally the model is identified and its parameters are used to characterize and compare responses of the tested molecule.

*Keywords:* data-driven modeling, system identification, image processing, cancer, angiogenesis

# 1. Introduction

Angiogenesis is a normal and necessary phenomenon consisting in creating new blood vessels from preexisting ones. It concerns many physiological processes during life cycle as, for example, wound healing, development of new tissues, embryonic maturation, menstrual cycles, *etc*.

Unfortunately, it is also involved in tumor development, especially in tumor neovascularization. As a matter of fact, at the very beginning of tumor development (*i.e.* before it reaches 1 to  $3 \text{ mm}^3$ ) oxygen and nutrients uptakes can

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be done by a simple diffusion process. But beyond a  $3 \text{ mm}^3$  threshold, to carry

on their development, cancer cells over-express physiological pro-angiogenic fac-10 tors, as Vascular Endothelial Growth Factor (VEGF), in order to stimulate the angiogenesis process in favor of the tumor [1]. Nearby endothelial cells (blood vessel constituting cells) will then be activated by VEGF and will get out of their quiescent state and start the construction of a new vessel in the angiogenic signal source direction.

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Due to the permanent excess of physiological pro-angiogenic factors, the newly created blood vessels have an aberrant architecture, non stabilized, permeable and tortuous. Therefore the tumor irrigation is not optimal and creates hypoxia areas, leading to a continuous tumor angiogenesis stimulation. Such

conditions could make the tumor treatment-resistant to chemo- or radiothera-20 pies since the lack of suitable vascularization prevents a good drug delivery or a good tumor oxygenation [2]. It has also been proved that the start of tumor growth was directly linked to neovascular development, [3], hence, it is now accepted that inhibiting angiogenesis in tumor could increase the efficiency of standard therapies [4]. 25

However, the efficiency and secure use of anti-angiogenic drugs requires to better define their pharmacodynamic effects and consequences on tumor growth. Indeed, it will allow to determine when, how and how long these drugs should be administrated in order to optimize the therapeutic protocols.

- On the other hand, the use of mathematical models has been largely democ-30 ratized for various biological applications, either for characterization, prediction or control purposes. A literature review proposed by Mriouah et al., [5], published in 2012, showed that three main types of mathematical models have been used to: (i) simulate and understand complex phenomena involved in angiogenic
- processes [6, 7, 8], (ii) analyze interactions between tumor and vasculature sys-35 tems [9, 10] and (iii) optimize anti-angiogenic therapies [11, 12, 13, 14, 15]. This review also emphasizes that the model structures can take various forms: temporal, spatio-temporal or multi-scale. However they were all designed from physical and biological equations, and very few behavioral modeling approaches
- of angiogenesis have been tested, so far. For instance, Drexler and Kovacs have 40 used in [16, 17, 18, 19, 20] a state-space representation derived from a linearization process applied to a nonlinear model initially proposed in [11]. Nevertheless the relevance of this state-space model has never been assessed in vivo. Indeed, Mriouah et al. particularly highlight the lack of in vivo validation of existing
- models. In fact, not only very few models were confronted with real data but 45 statistical tests are barely applied to validate the model performance. Yet, without any relevant validation tests based on *in vitro* or *in vivo* data, the credibility and medical application of mathematical models remains unlikely.
- One of the main bottleneck preventing empirical modeling and practical validation is the lack of experimental data availability (in terms of quality and 50 quantity).

In such a context, the objective of the present paper is to propose an innovative approach integrating:

- an *in vivo* protocol of experiments using skinfold chambers (real time observation of *in vivo* angiogenesis processes) applied to mice.;
- image segmentation technics (access to informative biological data);
- dynamic system identification methods (data-driven modeling);

with the purpose of characterizing and comparing more accurately the antiangiogenic treatment responses.

- This paper is organized as follows. Section 2 describes material and methods performed from *in vivo* experiments to image acquisition and Section 3 presents the automatic image segmentation process by which data are extracted from images. A data-driven model structure selection is then proposed in Section 4 before being identified. Finally results are discussed in Section 5 before drawing
   conclusion and perspectives.

# 2. Material and methods

In vivo angiogenesis imaging through skinfold chambers, on nude mice, allows to visualize the creation, functionality and remodeling of blood vessels within tumor during 4 to 5 weeks [21].

70 2.1. Skinfold chamber

This chamber model is made of two titanium shields placed on either side of a skinfold on the mouse back. These shields have a central circle hole of 10 mm diameter through which each side of the skinfold is visible. The skin of one side is dissected and removed in front of one of the apertures, which is then hermetically sealed with a thin sterilized cover glass in order to visualize the skin blood vessels of the other side dermis. It is possible, by removing the cover glass then by replacing it, to implant tumor cells or to graft tumors within the visualization chamber.

The main advantages of such a system is that chambers allow repeated (almost daily) observations of both vascular network and tumor growth by *in vivo* intravital microscopy over 4 to 5 weeks [22]. An example of this model is presented in Figure 1.

#### 2.2. Animals and tumor xenografted model

The study design was approved (authorization number: CELMEA-2012-0018) by Animal Protection Bureau of the French Ministry for Fishing, Agriculture and Food and the experiments were conducted in accordance with the Guiding Principles for Research Involving Animals. Using human tumor fragments requires to work with immunodeficient mice (nude) to avoid graft rejections.

Experiments were performed on 6 to 12-weeks-old female nude mice (nu/nu) weighting between 25 and 30 grammes (n=16). They were provided by Janvier breeding (Le Genest St Isle, France).



Figure 1: Example of a xenograft and skin vascular observation from skinfold chamber placed on a nude mouse

Anesthesia was achieved by a single intraperitoneal injection of a Xylazine (8 mg/kg, Rompun 2 %, Bayer Health Care, Puteaux, France) and Ketamine (90 mg/kg, Imalgène 500, Merial, Lyon, France) mixture. To prevent postoperative pain and stress, mice were injected subcutaneously with single doses of Buprenorphine (0.05 mg/kg, Buprécare, Axience) and Meloxicam (1 mg/kg, Metacam, Boehringer Ingelheim).

The dorsal skinfold chamber is positioned and xenograft implanted respectively at days  $D_{-14}$  and  $D_{-13}$ , see Figure 2. The xenograft consists in placing a non-vascularized 1 mm thick tumor fragment (of 2 to 4 mm<sup>2</sup>) on the vascular network of the skin.We use tumor fragments derived from a human glioblastoma cell line (U87). The cells are injected subcutaneously in the flanks of nude mice to form a tumor which is then cut into small fragments that are placed on the vascular network in the skinfold chamber.

At the end of experiments, mice were killed by a lethal dose of sodium pentobarbital (Pentobarbital sodique<sup>®</sup>, CEVA Santé Animale, La Ballastière, France).

#### 2.3. Anti-angiogenic drugs and administration protocols

- Only one anti-angiogenic drug was tested: bevacizumab (Avastin<sup>®</sup>, Roche, France). It is a monoclonal antibody targeting VEGF in order to prevent the fixation of VEGF to its receptors on endothelial cells, hence hindering angiogenesis stimulation and in consequence, the angiogenesis stimulation [23, 24]. When the tumor vascular network was complete (*i.e.* when the entire visible part of the tumor is unceularized) the mice were rendemined into two batches:
- the tumor is vascularized) the mice were randomized into two batches: one treated and one control. The treated batch was daily injected intraperitoneally with bevacizumab (10 mg/kg).

## 2.4. Image acquisition

Picture acquisitions were made 3 to 6 times a week; from anesthetized mice placed on the platform of the microscope (Nikon AZ100, Champigny sur Marne, France). The vascularization of xenografts and vascular network evolution have been followed for 28 days maximum, using transmitted light with a FITC filter (Fluorescein isothiocyanate) which allows to increase contrast between vessels and skin thanks to blood hemoglobin autofluorescence. Only superficial tumor vessels were observed due to the weak microscope depth of field.

The experimental setup timeframe is illustrated in Figure 2.



Figure 2: In vivo experiments timeframe

## 3. Automatic image segmentation

A vascular network can be characterized by predicates as length between junctions, irrigation quality or cellular density. However, there is a certain lack of knowledge in comprehension of tumor vascular network features [25] 130

Ideally, angiogenesis analysis should be robust, reproducible and as nonsupervised as possible. The observed characteristics should be multiple and directly relied on preclinical and clinical results [26]. To guarantee such constraints, an automatic image segmentation becomes necessary since it can offer many advantages:

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- more reliable data (quantitative results with acceptable uncertainties);
- increased reproducibility compared to the naked eye measurements;
- data processing of large image sets in a short time;

discriminate cancerous tissues from healthy tissues.

access to barely used physical quantities due to the difficulty of manual • extraction.

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## 3.1. Image processing

3.1.1. Region of interest

It is technically difficult to detect by computer a structure that is hardly discernible to the naked eye. In our case the tumor boundaries are too tenuous (lack of contrast). Hence in this exploratory study, the tumor segmentation 145 was performed manually by biologist experts in order to define the region of interest (ROI). In the future, we plan to test multimodality imaging to better

#### 3.1.2. Vascular network detection

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The vascular network detection step deals with the definition of pixel belonging either to vessels or to tumor tissue within the previously defined ROI. The stages of the vascular network extraction are presented in Figure 3.



Figure 3: Application of Savitzky-Golay filter: estimated polynomial response (in red) compared to the 500th line of original picture (in black) in A, peaks extraction (supposed to be vessels) by subtraction of the two signals in B, result is thresholded (red curve in B) and normalized in Figure C where peaks represent blood vessels detected from original picture D

Given the sought structures type (wire-shaped and highly contrasted with respect to the background picture, *i.e.* of higher frequency), the first step was to detect the background (cancerous tissue) and to subtract it from the image.

Two first order Savitzky-Golay filters, [27], (one for horizontal and one for vertical directions) were used. This filter identifies, with the least-square method, a polynomial through a 25 pixels long sliding window. The window length is tunable: the shorter it is, the more sensitive to high frequency (including noise) will be the filter. Hence, each filter returns a background estimated in one direction, the final estimated background is defined as the maximum value, for each pixel, of the two directional backgrounds.

In order to extract vessels, the original picture and the estimated background are subtracted and thresholded. The threshold value is fixed at 3 % of the original picture maximum intensity. Finally, a calibration step is performed to keep only the wire-shaped structures, *i.e.* having:

- a minimum number of consecutive pixels by line and column;
- a minimum size, whatever the direction.

These parameters were empirically fixed and depend on the searched ele-<sup>170</sup> ments size. They have been tested on a first set of images before being validated on a second one. An example of the final blood vessel segmentation is presented in Figures 4.B<sub>1,2</sub>.

# 3.1.3. Skeletonization

This step allows to simplify the picture to ease detection and quantification algorithms application. It consists in refining every structure (blood vessels) till having unitary width lines (just like fire in a grass field). A routine implemented on Matlab was used on our images to remove pixels on the boundaries of objects while preventing structures breaks. Examples of results are presented in Figures 4.C<sub>1,2</sub>.

## 180 3.2. Quantification and data processing

Access to physical quantities on tumor-vascular network evolution is the keystone which allows system identification of angiogenesis model. These quantities, or signals, must be measured from *in vivo* experimental 2D image sequences.

The previous segmentation and skeletonization steps are necessary to quantify different predicates through time: tumor area, total endothelial cells area (blood vessels), vascular density (ratio between tumor and vessel areas), amount of junctions, amount of vascular sprouts (small length newly created vessels), vascular network total length, *etc*.

Nevertheless, as previously said, macroscopic images were taken almost everyday, it means that the sampling period is not constant (varying between 1 and 3 days). Hence, data had to be interpolated in order to be used with common system identification algorithms. This has been done by a classical linear interpolation routine on Matlab with a new sampling period of 1 day.

#### 4. Data-driven modeling and system identification

System identification consists in searching a mathematical model of a dy-195 namic system from its input/output signals. This model is characterized by a structure and parameters that need to be chosen and adjusted, in order to reproduce the input/output behavior of the studied system. The model structure can be based on a priori knowledge (from physics or biological equations, as used in [19] for example) where parameters have biological meanings or it can 200 only rely on input/output signals behavior. Unlike classic physical modeling approaches, behavioral model structures remain more parsimonious (minimally parametrized) and easy to compute or simulate [28]. In this section, we propose to use the parameters of behavioral model structures as numeric indicators of the anti-angiogenic efficiency. The comparison of the estimated values for each 205 model parameter allows us to assess more precisely the effects of new treatments with respect to reference molecules.

In our case, it is known that tumor-vascular density can be a marker for cancer evolution and, of course, for survival prognostics. In fact, this signal,



Figure 4: Example of segmentation process on a control (left) and treated tumor (right) at day +7: manual segmentation (ROI) of cancerous tissues is done in yellow on step A, vessel segmentation is performed on step B (vessels are in white), step C presents the quantification (blue and red circles) on the skeletonized vascular network (green lines)

taking into account both tumor and endothelial cells areas, appears to be one of the most informative measurable quantity in angiogenesis studies. As presented in Figure 5, it is clear that treatment by bevacizumab affects the angiogenic dynamic of tumors. Next paragraph presents the data-driven modeling process that estimates and thus quantifies this anti-angiogenic effect.



Figure 5: Mean signals and their standard deviation of tumor-vascular density for control (left) and treated mice (right)

215 4.1. Modeling process

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The effects of treatment on tumor growth and vascular network development could be considered, in a control point of view, as a system whose behavior is deviated from its natural dynamics by the effect of an external input variable. This MISO (Multiple Input Single Output) structure  $\mathcal{M}_G$ , presented in Figure 6, contains two sub-blocks:

- angiogenesis dynamics  $(\mathcal{M}_d)$ ;
- treatment dynamics  $(\mathcal{M}_t)$ .



Figure 6: Input/output representation of angiogenic phenomenon and treatment effect models, where  $u_d(t)$  and  $u_t(t)$  are respectively the grafting and treatment inputs and  $y_d(t)$  and  $y_t(t)$  are their corresponding outputs combined in  $y_G(t)$ 

Where  $y_G(t)$ , the global output, is the vascular density response measured from *in vivo* macroscopic images, as described in Section 3. This variable is composed

- of the natural growth response of the tumor angiogenesis system  $y_d(t)$  and of the treatment effect on it,  $y_t(t)$ . Each block is controlled by a specific input signal:
  - $u_d(t)$  is a step signal defining when tumor is grafted on the mouse;
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- $u_t(t)$  is the amount of anti-angiogenic agent injected to the studied mouse, approximated by a step signal beginning at day 0.

## 4.1.1. Structure selection

Continuous-time (CT) model structures were preferred to discrete-time representations for convenience reasons. Indeed, with a CT model the estimated parameters are independent of the sample time period, the method is then reproducible on different data sets, and the estimates will therefore be comparable. Moreover, some recent developments in system identification applied to the modeling of continuous-time systems from sampled data have been proposed in [29].

An Output Error (OE) equation was chosen for both systems  $\mathcal{M}_d$  and  $\mathcal{M}_t$ :

$$\mathcal{M}: y(t) = \frac{B(p)}{F(p)}u(t) \tag{1}$$

with

$$B(p) = b_0 p^{n_b} + b_1 p^{n_b - 1} + \dots + b_{n_b}$$
  

$$F(p) = p^{n_f} + f_1 p^{n_f - 1} + \dots + f_{n_f}.$$

where p is the differential operator (*i.e.*  $x(t)p^n = \frac{d^n x(t)}{dt^n}$ ). The parameter vector is defined as  $\theta = [b_0, \ldots, b_{n_b}, f_1, \ldots, f_{n_f}]$ . In such a structure, indices  $n_b$  and  $n_f$  define respectively the numerator and denominator orders.

It is assumed that the input and output signals denoted  $\{u(t_k); y(t_k)\}$ , are sampled at discrete times  $t_1, \dots, t_N$ . The measured output is described as follows:

$$y_m(t_k) = y(t_k) + e(t_k).$$
 (2)

where  $e(t_k) \sim \mathcal{N}(0, \sigma^2)$  denotes the random residuals between the observed and explained responses, and  $\sigma^2$  corresponds to the variance of this Gaussian white noise.

<sup>245</sup> The complete model structure is then given by:

$$\mathcal{M}_G : \begin{cases} y_G(t) &= \frac{B_d(p)}{F_d(p)} & u_d(t) &+ \frac{B_t(p)}{F_t(p)} & u_t(t) \\ y_{G_m}(t_k) &= y_G(t_k) &+ e(t_k) \end{cases}$$
(3)

with  $B_d(p)$ ,  $F_d(p)$ ,  $B_t(p)$  and  $F_t(p)$  four polynomials of orders  $\{n_{b_d}, n_{f_d}, n_{b_t}, n_{f_t}\} \in \mathbb{N}^+$ . Selecting a model structure consists in choosing suitable values for those four indices by looking for the best compromise between behavioral fitting (high

orders) and simplicity (low orders). Let  $\mathbb{M}$  be a set of model structures (of increasing complexity) that compete for the description of the global system:

$$\mathbb{M} = \left\{ \mathcal{M}_{G_i}, \quad i = 1, \dots, n_m \right\}$$
(4)

where  $n_m$  is the number of tested order combinations. Akaike's information criterion, [30], was selected to find the most parsimonious model structures. In fact, the AIC promotes fitting accuracy while penalizing high dimensional  $(\dim(\theta))$  structures guaranteeing a parsimoniously parametrized model. Two criteria (the first one computed with control data and the second one with treated data) were computed for each combination and the selected structure is obtained as follows:

$$\mathring{\mathcal{M}}_{G} = \arg \min_{\mathcal{M}_{G_{i}} \in \mathbb{M}} \left( \frac{AIC_{cont}(\mathcal{M}_{G_{i}}) + AIC_{treat}(\mathcal{M}_{G_{i}})}{2} \right)$$
(5)

where  $AIC_{cont}(\mathcal{M}_{G_i})$  and  $AIC_{treat}(\mathcal{M}_{G_i})$  are the two criteria computed from control and treated data. 64 structures were tested using Matlab System Identification Toolbox and selected orders are  $n_{b_d} = 0$ ,  $n_{f_d} = 2$ ,  $n_{b_t} = 1$  and  $n_{f_t} = 1$ .

Finally, the selected global model structure of tumor-vascular density development and treatment effect becomes:

$$\mathring{\mathcal{M}}_{G}: \begin{cases} y_{G}(t) &= \frac{b_{d_{0}}}{p^{2} + f_{d_{1}}p + f_{d_{2}}} & u_{d}(t) &+ \frac{b_{t_{0}}p + b_{t_{1}}}{p + f_{t_{1}}} & u_{t}(t) \\ y_{G_{m}}(t_{k}) &= y_{G}(t_{k}) &+ e(t_{k}) \end{cases}$$
(6)

## 4.2. Continuous-time system identification

Various statistical methods have been proposed to solve CT parameter estimation issues formulated in both time and frequency domains. However, only the Simplified Refined Instrumental Variable method for Continuous-time Systems (SRIVC) has been used herein. The SRIVC method has proven to be very efficient in a number of practical cases and is one of the very few methods that can be interpreted in optimal statistical terms, so providing an estimate of the parametric error covariance matrix and therefore estimate of the confidence bounds on the parameter estimates [31]. It presents the clear advantage to be simple to use since the CT parameters are directly estimated from sampled data. The conversion stage which can be delicate is therefore avoided. A SRIVC
routine is now embedded in SITB Matlab toolbox (System Identification Tool-Box 8.0, released in 2012) and allows to identify CT model from sampled data.

- This recursive algorithm is based on the instrumental variable method, [30], initialized by a first least squares estimation step. The inputs/outputs signal derivates, needed to compute the regression vector, are computed by a statevariable filter. The cut-off frequency of this filter is fixed to a sufficiently high value to take into account all the system time constants [31]. This method was
- value to take into account all the system time constants [31]. This method was used to estimate the parameters of the model (6).

## 4.3. Estimation results and model falsification

Measured and simulated outputs are presented with their corresponding inputs in Figure 7. Residuals, defined as:

$$Res(t_k) = y_{G_m}(t_k) - y_{G_{sim}}(t_k) \tag{7}$$

are also presented.



Figure 7: Measurements and estimated outputs for control and treated batches. Input signals and residuals are respectively plotted above and below

## 270 4.3.1. Fitting and stochastic analysis

Both simulated outputs fit quite well the measurements, the goodness of fit, computed as follows:

$$FIT = 100 \left( 1 - \frac{\|y_{G_{sim}}(t_k) - y_{G_m}(t_k)\|_2}{\|y_{G_m}(t_k) - \mu_{y_{G_m}}\|_2} \right)$$
(8)

with  $\mu_{y_{G_m}}$ , the mean of  $y_{G_m}(t_k)$ , was estimated between 87 and 92%.

However, in order to entirely validate the stochastic assumptions formulated in Section 4.1.1 (especially on  $e(t_k)$  whiteness), the statistical properties of the residuals have been graphically assessed by comparing:

- 1. the residual autocovariance function with a Kronecker's delta function inside a 99% confidence interval;
- 2. the residual quantiles spread with respect to one from a normal law.
- 280 Results are presented in Figure 8.



Figure 8: Residuals autocorrelation functions within a 99% confidence interval at top for control and treated data sets and quantile-quantile plots below. These curves show acceptable results in both independence and normality terms

#### 4.3.2. Parameter analysis

Parameter estimates are presented in Table 1 with their coefficients of variation  $(c_v = |\frac{\hat{\sigma}_{\theta}}{\theta}| \times 100$ , where  $\hat{\sigma}_{\theta}$  is the standard deviation of the parameter estimated by bootstrapping with  $N_{sim} = 200$ ).

# 285 4.4. Sensitivity analysis

In order to assess the impact of each parameter on the output signal, we have performed a sensitivity analysis based on the model estimated from the treatment batch data. For every simulation of the model ( $N_{sim} = 500$ ), one of the parameters was assigned a value randomly picked according to a uniform law defined on  $\left[0.9 \times \hat{\theta}, 1.1 \times \hat{\theta}\right]$  (corresponding to a 20 % variation range) while the others parameters were kept to their estimated nominal values. The simulated output signals were then centered and normalized and are presented in Figure 9.

Results show that four parameters are more sensitizing  $(b_{d_0}, f_{d_2}, b_{t_1})$  and  $f_{t_1}$  than the other ones. As presented on their corresponding figures, these parameters mainly affect the increase dynamics and steady states of the system.

Batch	Param.	Estimate	$c_v~(\%)$
Control	$b_{d_0}$	0.023	12
	$f_{d_1}$	0.23	13
	$f_{d_2}$	0.057	10
	$b_{t_0}$	0	-
	$b_{t_1}$	0	-
	$f_{t_1}$	0	-
Treated	$b_{d_0}$	0.021	18
	$f_{d_1}$	0.16	35
	$f_{d_2}$	0.051	19
	$b_{t_0}$	-0.032	45
	$b_{t_1}$	-0.068	22
	$f_{t_1}$	0.35	50

Table 1: Parameter estimates and coefficients of variation  $c_v$  for control and treated batches



Figure 9: Normalized variation ranges of output signal  $y_G(t)$  for a  $\pm 10\%$  variation on each parameters

Such features can easily be interpreted in a biological way as vascular network set up and rearrangement phases. The biological interpretation of these parameters is discussed in the next section.

## 5. Discussion

Macroparameters as static gains and time constants can be used to easily characterize the dynamics of tumor-vascular density development or treatment effect. These macroparameters are defined as follows:

- Static gains:  $K_d = \frac{b_{d_0}}{f_{d_2}}$  and  $K_t = \frac{b_{t_1}}{f_{t_1}}$
- Time constants:  $\tau_d = \frac{1}{|\lambda_d|}$  and  $\tau_t = \frac{1}{|\lambda_t|}$  with  $\lambda_i$  the roots of polynomial  $F_i(p)$  where  $i = \{d, t\}$ .

In our case, as shown in Figure 10, the tumor natural growth dynamics are equivalently estimated from control or treatment data sets (*i.e.* parameters  $b_{d_0}$ ,  $f_{d_1}$  and  $f_{d_2}$ ), which confirms the choice on the model structure.

However, these plots also highlight the regularization effect of bevacizumab. Indeed the negative static gain for the treatment effect part of the model indicates a tumor-vascular density decrease mainly due to the vascular network rearrangement caused by (i) the anti-vascular effect of the molecule (destruction of blood vessels) but also to (ii) its anti-angiogenic ability (inhibition of new sprouts creation leading to fewer neovessels). The time constants are not compared to each other in this pilot study. Nevertheless they could be useful to compare the pharmacokinetics of the competitive drugs.



Figure 10: Meta-parameter estimates and their standard deviation obtained by bootstrapping

# 6. Conclusion

The objective of this paper was to propose an innovative and integrative data-driven modeling approach that was able to estimate and compare anti-

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- angiogenic effects of therapeutic molecules on tumor-vascular density. The proposed method was confronted with experimental *in vivo* data obtained from a user-independent image segmentation process of tumors macroscopic pictures. The approach has proven its efficiency by successfully reproducing the tumor and vascular behaviors and by producing parameter estimates which can be in-
- terpreted and compared to assess the treatment effects. In short terms perspectives, it is planned to extend the previous behavioral modeling to the amount junctions and vascular sprouts as well as the total length of the vascular network.

Further experiments will be carried out to assess the model ability to discriminate different treatment doses and their consequences on vascular network and tumor growth.

Finally, this model could also be used for predictive purposes in order to foretell when the optimal normalization window occurs, during which the effects of an anti-cancer therapy (such as chemo- or radiations therapies) could be potentialized [32, 33].

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