A tumor-in-host DEB-based approach for modeling cachexia and bevacizumab resistance

Elena M. Tosca, Maurizio Rocchetti, Enrico Pesenti, Paolo Magni

Received: date/Accepted: date

E. M. Tosca, P. Magni
Dipartimento di Ingegneria Industriale e dell’Informazione, Università degli Studi di Pavia, I-27100 Pavia, Italy

M. Rocchetti
Consultant, Milano, Italy

E. Pesenti
Accelera srl, Nerviano (MI), Italy

Running title:
A tumor-in-host DEB-based approach for modeling cachexia and bevacizumab resistance

Corresponding author’s:
Paolo Magni
Dipartimento di Ingegneria Industriale e dell’Informazione, Università degli Studi di Pavia, I-27100 Pavia, Italy
Phone: 0382-985511
Email: paolo.magni@unipv.it

Conflict of interest statement:
E. Pesenti is an Accelera srl employee.
ABSTRACT

Adequate energy intake and homoeostasis are fundamental for the appropriate growth and maintenance of an organism; the presence of a tumor can break this equilibrium. Tumor energy requests can lead to extreme weight loss in animals and cachexia in cancer patients. Angiogenesis inhibitors, acting on tumor vascularization, counteract this tumor-host energy imbalance with significant results in preclinical models and more limited results in the clinic. Current pharmacokinetic-pharmacodynamic models mainly focus on the anti-angiogenic effects on tumor growth but do not provide information about host conditions. A model that can predict energetic conditions that provide significant tumor growth inhibition with acceptable host body weight reduction is therefore needed. We developed a new tumor-in-host Dynamic Energy Budget (DEB)-based model to account for the cytostatic activity of anti-angiogenic treatments. Drug effect was implemented as an inhibition of the energy fraction subtracted from the host by the tumor. The model was tested on seven xenograft experiments involving bevacizumab and three different tumor cell lines. The model successfully predicted tumor and host body growth data, providing a quantitative measurement of drug potency and tumor-related cachexia. The inclusion of a hypoxia-triggered resistance mechanism enabled investigation of the decreased efficacy frequently observed with prolonged bevacizumab treatments. In conclusion, the tumor-in-host DEB-based approach has been extended to account for the effect of bevacizumab. The resistance model predicts the response to different administration protocols and, for the first time, the impact of tumor-related cachexia in different cell lines. Finally, the physiological base of the model strongly suggests its use in translational human research.

Keywords anti-angiogenic therapy · bevacizumab · pharmacokinetic-pharmacodynamic modeling · tumor-in-host growth · DEB theory

Significance:

A mathematical model describes tumor growth in animal models taking into consideration the energy balance involving both the growth of tumor and the physiological functions of the host.
INTRODUCTION

Angiogenesis, the development of new capillary blood vessels, plays a key role in the growth and progression of solid tumors (1). Indeed, like normal tissues, tumor cells need an adequate supply of oxygen, nutrients and an effective way to remove waste products (2). Tumors can directly cause the development of this blood supply or induce nearby normal cells to produce pro-angiogenic molecules. This dense vascular network ensures to tumor cells the amount of energy needed to proliferate. In a large number of cases, especially in advanced stages of cancer, the homeostatic control of energy and protein balance is so compromised in favor of tumor to result in a dramatic loss of host body weight, attributable to the decreases of both skeletal muscle (biomass) and adipose tissue (energy reserve). In particular, depletion of skeletal muscle is a key component of cancer-associated cachexia and it is responsible for increased chemotherapy toxicity, complications from cancer surgery, poor quality of life and mortality (3, 4).

On February 2004, the U.S. Food and Drug Administration (FDA) approved the first antiangiogenic agent, bevacizumab (Avastin), a monoclonal antibody targeting the vascular endothelial growth factor (VEGF), for the treatment of advanced colorectal cancer (5, 6, 7). Since then, bevacizumab, together with an array of other angiogenesis inhibitors, was tested in various clinical trials getting approval for the treatment of multiple cancers, alone or in combination with other cytotoxic/chemotherapy drugs (8, 9). Despite the demonstrable efficacy of anti-angiogenesis targeted therapies in preclinical models (10, 11) and the increasing number of successful translations to clinic (12, 13, 14, 15, 16), the most effective approach to the anti-angiogenic cancer treatment is still under debate and additional efforts and investigations are necessary (17, 18, 19).

Several models, employing a number of different techniques, have been developed to describe tumor growth and its inhibition following the administration of anti-angiogenic agents given alone or in combination with cytotoxic drugs (20, 21, 22, 23, 24). Despite many of them have been successfully applied to analyze preclinical xenograft experiments involving antiangiogenic therapies, they suffer of some relevant limitations. Indeed, these models, completely focused on anticancer drug activity on tumor growth, always describe tumor as an independent entity and neglect its interaction with the host organism. In this way, important effects, like cachexia that could also severely limit drug efficacy, are not included in modeling efforts. Furthermore, the goal of anti-angiogenic treatments is to reduce the energy supply to the tumor (25, 26) by promoting the restoration of energetic balance between tumor and host, with the primary consequence of modulating tumor growth and secondary of improving cachexia condition.

For all these reasons, the mathematical modeling of tumor-related cachexia during an antiangiogenic treatment in preclinical setting is still an open issue. A tumor-in-host modeling approach, developed on the basis of the Dynamic Energy Budget (DEB) theory (27, 28), could cope with this. Based on physiological mechanisms that all living organisms have in common, the DEB-theory provides a general framework to describe the major aspects of metabolism (energy and mass budgets) of the host organism. Tumor-in-host modeling approaches have been already successfully exploited to describe the effects of cytotoxic treatments (29, 30). Aim of the present work was to extend their applicability, developing a new DEB-TGI model able to account for the cytostatic effect of anti-angiogenic drugs on both tumor and host body weight growth. Specific modeling efforts were also focused on the description and evaluation of tumor-related anorexia/cachexia. Moreover, the tumor hypoxia condition and the arising of a hypoxia-resistance mechanism were described and integrated within the model. In this way, the model accounts for the decreased drug efficacy observed during prolonged treatments, allowing adequately predictions also in response to these schedules frequently adopted in clinics.
The proposed model was assessed on several preclinical xenograft experiments, involving different tumor cell lines treated with bevacizumab at different schedules. In addition to the typical tumor growth predictions and estimates of drug potency already provided by the other PK-PD models, it resulted able to predict also the host body weight dynamics in control and treated animals, providing, for the first time, quantitative measurements of the expected degrees of cachexia in the different cell lines and schedules.

The results here presented encourage to investigate the applicability of the tumor-in-host DEB approach as translational tool in oncology.

MATERIALS AND METHODS

Experimental methods

Compound

Bevacizumab (Avastin), a humanized anti-VEGF monoclonal antibody, was used for the in vivo anti-tumor assessments.

Animals, tumor cell lines and in vivo tumor growth experiments

14 female CD1 athymic Nu/Nu mice (6 weeks of age, 22-31g of weight), and 111 male Balb athymic Nu/Nu mice (5-6 weeks of age, 21-36g of weight) were obtained from Harlan, S. Pietro al Natisone, Italy.

DU145 cell line was obtained from American Type Culture Collection (ATCC), HT29 cell line from European Collection of Cell Cultures (ECACC) and MX1 cell line from Cell Lines Service (CLS). All cell lines were authenticated by short tandem repeat (STR) analysis (AmpFISTR Identifier PCR Amplification Kit, Applied Biosystems) and checked for mycoplasma presence (Mycoplasma Detection Kit, Lonza LT07) every 2 months. They were implanted in animals within 10 in vitro passages.

In all the experiments, tumor fragments were implanted subcutaneously into the left flank of animals. After about five days since inoculation, mice bearing a palpable tumor (100-200mm$^3$) were selected and randomized into untreated (control) and treated groups. From 0 to 4 days after randomization (i.e., from day 5 to 9 of the experiment), the anticancer treatment started. Mice were clinically evaluated daily and tumors were measured usually every two or three days using callipers. Tumor masses were calculated as:

$$\text{Tumor weight (g)} = \frac{\text{length (cm) \times width}^2\text{(cm}^2\text{)}}{2}$$

assuming unit density (1g/cm$^3$). All the procedures adopted for housing and handling of animals were in strict compliance with the Italian and European guidelines for Laboratory Animal Welfare. Studies were approved by Accelera Animal Care and Use Committee and Italian Ministry of Health.

Treatments
Exp a: 33 tumor-bearing Balb Nu/Nu mice, implanted with DU145 tumor cells, were randomized in 3 groups (11 animals for group). Vehicle or bevacizumab at the dose level of 10 or 20mg/kg were given intraperitoneally (i.p.), q4dx6 in days 5,9,13,17,21,25.

Exp b: 16 tumor-bearing Balb Nu/Nu mice, implanted with DU145 tumor cells, were randomized in 2 groups (8 animals for group). Vehicle or bevacizumab at the dose level of 20mg/kg were given i.p., q4dx6 in days 9,13,17,21,25,29.

Exp c: 16 tumor-bearing Balb Nu/Nu mice, implanted with DU145 tumor cells, were randomized in 2 groups (8 animals for group). Vehicle or bevacizumab at the dose level of 20mg/kg were given i.p., q4dx6 in days 6,10,13,17,20,24.

Exp d: 16 tumor-bearing Balb Nu/Nu mice, implanted with DU145 tumor cells, were randomized in 2 groups (8 animals for group). Vehicle or bevacizumab at the dose level of 20mg/kg were given i.p., q4dx4 in days 8,12,16,20.

Exp e: 16 tumor-bearing Balb Nu/Nu mice, implanted with DU145 tumor cells, were randomized in 2 groups (8 animals for group). Vehicle or bevacizumab at the dose level of 20mg/kg were given i.p., q4dx3 in days 9,13,17.

Exp f: 14 tumor-bearing Balb Nu/Nu mice, implanted with HT29 tumor cells, were randomized in 2 groups (7 animals for group). Vehicle or bevacizumab at the dose level of 20mg/kg were given i.p., q4dx4 in days 8,12,16,20.

Exp g: 14 tumor-bearing CD1 Nu/Nu mice, implanted with MX1 tumor cells, were randomized in 2 groups (7 animals for group). Vehicle or bevacizumab at the dose level of 20mg/kg were given i.p., q4dx4 in days 7,11,15,19.

In total, 7 different experiments involving 125 xenograft mice and 3 different tumor cell lines were considered. Exp a, Exp b, Exp f and Exp g were already partially analyzed in (20), whereas the others are originally data.

Pharmacokinetic model

The collection of drug plasma concentration samples from the same animals used for determining the antitumor activity could improve the PK-PD model parameter estimates, however it may perturb the animal status and the response to the anticancer treatments. Therefore, a common practice, adopted in this kind of experiments to avoid possible artefacts, is to evaluate the pharmacokinetics of the compound under study in satellite groups of animals or literature data. Here, the one-compartment model with first-order absorption and elimination proposed in (31) and already used in (20) to analyze part of the experiments here considered, was adopted to generate average plasma profile of bevacizumab. Parameters were fixed to the mean values (ka=2.69 1/day, ke=0.115 1/day and V/F=0.119 l/kg).

PK-PD model structure

Unperturbed tumor-free DEB-based model (tumor-free animals)

Following the standard hypothesis of DEB modelling approach (32), host body is described by two components: the energy reserve, $e(t)$, and the structural biomass, $V(t)$. The first pool represents the energy mainly stored in the adipose tissue, coming from assimilation (i.e., feeding and nutrient absorption) and essential to carry out all the physiological processes, among which maintenance and growth of structural biomass. The second pool represents the structural part of the host body, like lean mass including skeletal muscles. The dynamics...
of these two components follow from energetic balances between assimilation process, energetic consumption, and maintenance/growth costs.

The actual host body weight, \( W(t) \), results from the contribution of both structural biomass and reserve:

\[
W(t) = W_V(t) + W_e(t) = d_V (1 + \xi e(t)) V(t),
\]

where \( \xi \) is a dimensionless scaling parameter.

The complete formulation of the unperturbed tumor-free host growth model is reported in the Supplementary Material S1.

Unperturbed tumor-in-host DEB-based model (untreated tumor-bearing animals)

Tumor cells, as all the other cells, have to obtain nutrients (energy) from the host to cover the maintenance and growth costs \( (m_u \text{ and } g_u) \) to survive and proliferate. As reported in Van Leuween (33), the model assumes that the energy distribution between tumor and host structural biomass is determined by the partition fraction \( k_u(t) \), quantifying at each instant the energy available to tumor cells (Fig.1):

\[
k_u(t) = \frac{\mu_u V_u(t)}{V(t) + \mu_u V_u(t)}
\]

From Eq. 3, it follows that the amount of energy assigned to tumor or to host biomass depends on their corresponding volumes \( (V_u(t) \text{ and } V(t)) \), whilst the parameter \( \mu_u \), called coefficient of gluttony of tumor cells, determines their relative importance. If \( \mu_u = 1 \), the energy amount exploited by a certain volume of tumor cells is equal to that exploited by the same volume of normal cells; whereas, if \( \mu_u > 1 \), tumor cells are more successful in extracting energy in comparison to normal cells.

As tumor exploits host resources normally destined to physiological processes, the growth rate of the organism decreases until when host has to degrade its structural biomass to survive and, at the same time, to satisfy tumor energy demand (tumor-related cachexia). Both tumor and host benefit of the energy regained from degradation of host tissues. Differently from (33), in absence of a drug treatment, the degradation rate increases until a time instant, \( t_{\delta V_{\text{max}}} \), in which a maximum threshold, \( \delta V_{\text{max}} \), is reached. Tumor starts growing exponentially while host consumes its structural biomass until \( t_{\delta V_{\text{max}}} \), when it slowdowns the growth reaching eventually a plateau due to the exhaustion of the available energy.

To characterize the tumor-related anorexia, it was hypothesized that the presence of tumor masses induces animal sufferings leading to symptoms, like lack of appetite or limited assimilation, get aggravated with tumor progression. Then, the assimilation process is governed by the time-dependent coefficient \( \rho(t) \) accounting for the caloric reduction linked to tumor progression:

\[
\rho(t) = \rho_b \left( 1 - \frac{V_u(t)}{IV_{u,50} + V_u(t)} \right)
\]

where \( \rho_b \) represents the food-supply coefficient in absence of tumor.

The differential equations describing the unperturbed tumor-in-host DEB-based model coincide with Eq. 6, described in the next section for the treated group, in absence of treatment (i.e., \( c(t) = 0 \)). Their complete mathematical derivations, in presence or absence of degradation of structural biomass, is reported in the Supplementary Material S1. In Fig.1 the main processes are summarized.

Tumor-in-host DEB-TGI anti-angiogenic model (treated tumor-bearing animals)
A tumor-in-host DEB-based approach for modeling cachexia and bevacizumab resistance

In case of an anti-angiogenic treatment, the reduction of tumor vascularization leads to a modification of the energy partition between tumor and host, with the specific aim of inhibiting the energy flow to tumor. As the energy distribution is driven by the fraction $k_u(t)$, drug effect was implemented as an inhibitory $I_{\text{max}}$ function on $k_u(t)$:

$$k_u(t) = \frac{\mu_u V_u(t)}{V(t) + \mu_u V_u(t)} \left(1 - \frac{I_{\text{max}} c(t)}{I_{C_{50}} + c(t)}\right)$$  \hspace{1cm} (5)$$

where $I_{C_{50}}$ represents the drug concentration exerting the 50% of the maximal inhibitory effect, $I_{\text{max}}$. As expected, in absence of treatment, the partition fraction reported in Eq.5 coincides with that of the untreated animals, Eq.3.

Bevacizumab only binds human-VEGFs produced by inoculated tumor cells and does not interact with murine cells (6, 31). Therefore, none direct pharmacological effect on the host organism was included, in accordance with experimental mice net body weight data and literature information (34).

The tumor-in-host DEB-TGI anti-angiogenic model is described by the following system of differential equations, where $W(t)$ and $W_u(t)$ represent the net body weight of host organism and the tumor weight, respectively. The main model parameters are summarized in Table 1, while the definition of all the PK-PD model parameters and variables are reported in the Supplementary Material S1.

\[
\begin{align*}
\frac{d e(t)}{dt} &= \frac{\nu}{V(t)^{2/3}} \left(\rho(t) \left(\frac{V_{\infty}}{V_u(t) + V(t)}\right)^{7/3} - e(t)\right) \\
\frac{d V(t)}{dt} &= F_V(e, V, V_u) \\
\frac{d V_u(t)}{dt} &= F_{V_u}(e, V, V_u) \\
\rho(t) &= \rho_b \left(1 - \frac{V_u(t)}{I V_{50} + V_u(t)}\right) \\
k_u(t) &= \frac{\mu_u V_u(t)}{V(t) + \mu_u V_u(t)} \left(1 - \frac{I_{\text{max}} c(t)}{I_{C_{50}} + c(t)}\right) \\
W(t) &= d_f \left(1 + \xi e(t)\right) V(t) \\
W_u(t) &= d_{V_u} V_u(t)
\end{align*}
\]  \hspace{1cm} (6)

with $e(t_0) = e_0$, $V(t_0) = V_0$, $V_u(t_0) = V_{0U}$; and function $F_V(e, V, V_u)$ and $F_{V_u}(e, V, V_u)$ defined as:

\[
F_V(e, V, V_u) = \begin{cases} \\
\frac{\{V[e(t)^{7/3} - gm(\mu_u V_u(t) + V(t))]\}}{g \mu_u V_u(t) + V(t) + e(t)^{7/3}} & \text{Structural biomass growing} \\
\{V[e(t)^{7/3} - gm(\mu_u V_u(t) + V(t))]\} & \text{Structural biomass degradation} < \delta_{\text{max}} \\
\{V[e(t)^{7/3} - gm(\mu_u V_u(t) + V(t))]\} & \text{Structural biomass degradation at max rate} \\
\end{cases} \hspace{1cm} (7a)
\]

\[
F_{V_u}(e, V, V_u) = \begin{cases} \\
\{e(t) V(t)^{7/3} + m V(t)\} - m_u & \text{Structural biomass growing} \\
\{e(t) V(t)^{7/3} + m V(t)\} & \text{Structural biomass degradation} < \delta_{\text{max}} \\
\{e(t) V(t)^{7/3} + m V(t)\} & \text{Structural biomass degradation at max rate} \\
\end{cases} \hspace{1cm} (8a)
\]

\[
\begin{align*}
\frac{V_{\infty}}{V(t) + \mu_u V_u(t)} V_u(t) & - m_u V_u(t) \\
\frac{\mu_u V_u(t)}{V(t) + \mu_u V_u(t)} V_u(t) & - m_u V_u(t) \\
\frac{\mu_u V_u(t)}{V(t) + \mu_u V_u(t)} V_u(t) & - m_u V_u(t)
\end{align*}
\]  \hspace{1cm} (7b)

\[
\begin{align*}
\frac{V_{\infty}}{V(t) + \mu_u V_u(t)} V_u(t) & - m_u V_u(t) \\
\frac{\mu_u V_u(t)}{V(t) + \mu_u V_u(t)} V_u(t) & - m_u V_u(t) \\
\frac{\mu_u V_u(t)}{V(t) + \mu_u V_u(t)} V_u(t) & - m_u V_u(t)
\end{align*}
\]  \hspace{1cm} (8b)

\[
\begin{align*}
\frac{V_{\infty}}{V(t) + \mu_u V_u(t)} V_u(t) & - m_u V_u(t) \\
\frac{\mu_u V_u(t)}{V(t) + \mu_u V_u(t)} V_u(t) & - m_u V_u(t) \\
\frac{\mu_u V_u(t)}{V(t) + \mu_u V_u(t)} V_u(t) & - m_u V_u(t)
\end{align*}
\]  \hspace{1cm} (8c)
where the three situations (structural biomass growing, structural biomass degradation-rate $< \delta v_{\text{Max}}$, and structural biomass degradation at maximum rate) are formally defined as reported in the Supplementary Material S1.

Data analysis

For each experiment, tumor and mice net body weight data of control and treated groups were analyzed simultaneously. First the model was assessed on average data and then a non-linear mixed effect approach (35) was adopted, allowing to describe both the typical behavior and the inter-individual variability.

Plasma concentration-time profiles of bevacizumab were simulated at the correspondent dose schedules by using the PK model (see Fig. S1 in Supplementary Material S2).

Following the DEB theory, the model is characterized by four sets of parameters, one related to the host organism, one to the tumor, one to the cachexia and one to the drug activity (Table 1). The host-related parameters can be derived from healthy (tumor-free) mice growth data (29, 30) and depend on species, strain and sex. In particular, for the studies involving Bulb Nu/Nu mice parameters were fixed to the values reported in (29), whereas, for Exp g, involving CD1 Nu/Nu mice, they were estimated as part of the present work (see Fig. S2 and Table S1 in Supplementary Material S3).

In accordance to the general way to describe xenograft experiments, the inoculation day was considered as the initial time instant, $t_0$. Thus, the initial condition for energy reserve, $e_0$, was computed by the tumor-free model, considering the age of mice at the beginning of each experiment (see Table S2 in Supplementary Material S3). $W_0$ and $V_{u0}$ were estimated during model identification, while the initial value for the structural biomass, $V_0$, was derived from $V_0 = W_0/(1 + e_0 \xi)$. The thermodynamic efficiency coefficient, $\omega$, was fixed to 0.75 and the density of tumor volume, $d_{Vu}$, to 1 g/cm$^3$.

Tumor-related ($\mu_u$, $g_u$, $m_u$), cachexia-related ($\delta v_{\text{max}}$, $IV_{u50}$) and drug-related parameters ($I_{\text{max}}$, $IC_{50}$) were identified, for each experiment, on mice and tumor weight data of control and treated arms.

After a preliminary model evaluation, $I_{\text{max}}$ was fixed to 1 because its estimation did not provide any relevant improvement.

Inter-individual variability was considered for the initial conditions of structural biomass and volume of inoculated tumor cells ($W_0$ and $V_{u0}$), for the half maximal inhibitory concentration ($IC_{50}$) and for the food-supply coefficient $\rho_b$. Individual parameters $P_i$ were in general supposed to be log-normally distributed: $P_i = \theta \exp(\eta_i)$ where $\theta$ is the typical population value and $\eta$ a normally distributed random effect with zero mean and variance $\sigma^2(P)$. However, because $\rho_b$ takes value in $[0, 1]$, it was preliminary re-parametrized as $\rho_b = 1/(1 + R_b)$, with $R_b$ in $[0, +\infty]$, and then $R_b$ was supposed to be log-normally distributed.

Among the different tested models (i.e. additive, proportional, combined), a residual error proportional to the square root of the net mice body or tumor weight predicted value ($\sqrt{f}$), i.e. $y = f + b f e$, where $y$ is the measurement, $b$ a coefficient and $e$ a standardized random variable normally distributed, was chosen based on goodness-of-fit plots and model selection criteria.

Analysis was performed in Monolix (version 2016R1, http://lixoft.com/products/monolix/). Goodness-of-fit (GOF) plots were produced in R (Version 3.4.4).

RESULTS

For each experiment, individual tumor and mice net body weight data of control and treated groups were analyzed through a non-linear mixed effect approach. Parameter
estimates are reported in Table 2 and Table S3. Model parameters were identified with good precision except for few cases in Exp f that is characterized by a low number of animals and sampling times.

Tumor and net body weight profiles of Exp d, Exp f and Exp g, involving three different tumor cell lines (i.e., DU145, HT29 and MX1) treated with the same schedule (20mg/kg, q4dx4), are shown in panels A of Fig.2, as representative examples. The time course of the energy partition $k_u(t)$ (panels B of Fig.2) and of the assimilation coefficient $\rho(t)$ (panels C of Fig.2) are reported for both control and treated groups. Results for the remaining experiments, performed on DU145 cell line treated with different schedules, are included in the Supplementary Material S4 together with diagnostic plots (Fig. S3-Fig.S31). In each experiment, the model was able to grasp the typical and the individual dynamics of tumor and mice net body weight in both control and treated groups. The average root mean square error (RMSE) between typical model predictions and average observed data is 0.05g for tumor weight and 0.21g for host body weight, with individual RMSEs ranging respectively from 0.03g to 0.62g and from 0.18g to 2.4g. The GOF plots visually confirm that the proposed population DEB-TGI model adequately described the collected data; weighted residuals are randomly distributed around zero, indicating the absence of model bias and, finally, VPCs confirm that also the inter-individual variability is well captured.

Overall, results, obtained in a variety of cell lines and administration schedules, highlighted model capability to adequately describe experimental data. Tumor growth and its modulation after the anti-angiogenic treatment were well fitted. In addition, thanks to the set of energy balance rules on which the model is based, also the dynamics of mice body weight was well described in both the control and bevacizumab treated groups. In particular, the model is able to grasp the host body growth slowdown due to tumor progression in control animals (tumor-related cachexia) and the positive indirect anti-cachectic effect (lower body weight decrease) observed in treated animals due to bevacizumab activity on the tumor growth in the different cell lines.

The availability of different experiments involving the same tumor cell line DU145 (Exp a - Exp e) allowed to compare the parameter estimates. The tumor-related and cachexia-related parameters resulted consistent among these studies (Table 2), confirming the link between estimates and tumor line. Differently, the $IC_{50}$ value increases with the duration of the anti-angiogenic treatment, indicating a decreasing activity of the bevacizumab in case of prolonged treatments (see also Fig.S32).

For further exploring this observation, Exp b, Exp d and Exp e, in which bevacizumab was administered for a different treatment period (q4dx6, q4dx4 and q4dx3, respectively) but at the same dose level (20mg/kg) and starting from the same day, were reanalyzed. A simultaneous fitting was performed, considering a different potency parameter ($IC_{50}$) for each experiment and including a categorical covariate “Experiment” on the typical value of $W_0$, $V_0$ and log-normally distributed random effects on parameters $W_0$, $V_0$, $R_b$ to account for the inter-experiment and inter-individual variability. The obtained results ($IC_{50}$ 2.44, 1.55 and 0.94 µM in Exp b, Exp d and Exp e, respectively) are in good agreement with the separated fittings and confirmed the previous observations (see Fig.S33-Fig.S35 and Table S4 in Supplementary Material S4).

On this basis, it was considered the presence of a possible tumor reaction to a prolonged hypoxia condition caused by the persistent inhibition of the energy flow to the tumor mass due to the bevacizumab exposure. On this hypothesis, a model was developed supposing that a prolonged tumor hypoxia over a critical threshold $\bar{H}$ could trigger a VEGF-independent tumor re-vascularization, enhancing the energy available to the tumor with a
subsequent decrease of the bevacizumab effectiveness. Consequently, the partition coefficient \( k_u \) (Eq.5) was modified as:

\[
k_u(t) = \frac{\mu U V_u(t)}{V(t) + \mu U V_u(t)} \left( 1 - \frac{I_{max,Inh}(t)c(t)}{IC_{50} + c(t)} \right)
\]

(9)

where

\[
I_{Max,Inhib} = \begin{cases} I_{max} & \text{if } H(t) \leq \bar{H} \\ I_{max} e^{k_H(H-H(t))} & \text{if } H(t) > \bar{H} \end{cases}
\]

(10)

and the expected hypoxia condition imputable to the VEGF-blockade, \( H(t) \), is defined as:

\[
H(t) = \frac{\int_{0}^{t} \left( \frac{\mu U V_u(s)}{\mu U V_u(s) + V(s)} \right) \left( I_{max} c(s) \right) ds}{\int_{0}^{t} \left( \frac{\mu U V_u(s)}{\mu U V_u(s) + V(s)} \right) ds}
\]

(11)

that is the ratio between the energy not absorbed by the tumor due to drug effect (in the hypothesis of a VEGF-dependent tumor angiogenesis) and the energy absorbable in absence of anti-angiogenic therapy. When \( H(t) \) exceeds a critical threshold \( \bar{H} \), the maximum effect of the VEGF-blockade induced by bevacizumab is reduced.

The hypoxia-triggered resistance model was evaluated on Exp b, Exp d and Exp e. Observed and predicted tumor and host body weight data are shown in Fig.3, parameter estimates and model diagnostics are reported in the Supplementary Material S4 (see Fig.S36-Fig.S39 and Table S5). The obtained results show that, with the integration of a hypoxia-mediated resistance mechanism, the model is able to capture the decreased efficacy affecting prolonged therapies.

**DISCUSSION**

Based on the DEB-theory, we proposed a new tumor-in-host growth inhibition model able to describe and predict the dynamics of both tumor and host net body weight in control and treated xenograft mice following the administration of an angiogenesis inhibitor. The new model was successfully tested on a set of seven studies, originally designed and performed within different drug development projects for the in vivo anti-tumor assessment of bevacizumab given alone and in combination with new molecules under development, on three different tumor cell lines (i.e., DU145, HT29 and MX1). In that context, the goal of these studies were mainly the efficacy assessment of the combination and the selection of the more promising ones. In this paper, control and single agent (bevacizumab) arms were re-used to develop and test the new DEB-TGI model.

Differently from other standard PK-PD models completely focused on the evaluation of the antitumor efficacy (20, 21, 22, 24), the present approach, thanks to its physiological hypothesis, provides quantitative measurements of various outcomes such as host body weight, energy-intake and assimilation (anorexia), tumor-host energy distribution and structural biomass degradation (cachexia), Fig.2.

The energy intake reduction directly linked to tumor progression (tumor-related anorexia) was included in the model (3, 4). This effect was accounted for by Eq.4 parametrized in terms of \( IV_{eso} \), which value, providing a quantitative measure of tumor-related anorexia, could be extremely useful to compare its severity among different tumor cell lines, anticancer drugs or anti-cachectic compounds. For example, considering all the cell lines involved in this work, the experiments on DU145 cell line showed a relatively
similar IV_{50} higher than 10cm$^3$ (10.1-12.8cm$^3$ or even higher for Exp b) corresponding to a 10% reduction of the energy supply in presence of a tumor mass of around 1.5g. Interestingly, a different behavior was observed in the other two cell lines, MX1 and HT29. In the first case, no significant host body weight decreases followed tumor growth and, accordingly, the estimate of IV_{50} was so high to result unidentifiable. On the opposite, for HT29, IV_{50} was about 3cm$^3$, providing a strong energy reduction (about 35%) for analogous tumor masses (1.5g). The strong body weight losses observed in the control animals of Exp f (more than 15% of BW in a three-week period) confirmed the severity of this effect and suggested a possible higher anorexic impact of a gastric line, as HT29, in comparison to other lines (3, 4). This result is in agreement with the greater incidence and impact of cachexia/anorexia observed in gastric cancer patients with respect to other tumor types (36, 37, 38, 39).

In addition to the inhibition of host assimilation due to tumor progression, this mechanistic model allows to determine the time course of the energy fraction exploited from the host by the tumor (Fig.2, panels B). It is described by $k_u(t)$ as a function of the tumor and host biomass volumes and the parameter $\mu_u$ (Eq.3). Considering animals of 32-36g of BW, corresponding approximatively to a biomass volume of 30cm$^3$, a tumor mass of 1.5g and $\mu_u$ values in the range 5.62-7.93, as in the considered case studies, the model indicates that about the 25-35% of the available energy is delivered to the tumor compared to the 5% expected in case of $\mu_u=1$. For this reason, the gluttony parameter $\mu_u$ can be considered as a measurement of tumor aggressiveness.

The overall reduction of the energy available for the host growth and maintenance processes due to tumor progression and its impact on host energy intake is determined by the contribution of both tumor-related anorexia and tumor-host energy distribution. Its impact on the mice body weight may be quite relevant, with an observed body weight loss in the analyzed experiments up to 14% in control animals, depending on tumor line. A model prediction of the expected energy reduction can be obtained by the product of the assimilation coefficient, $\rho(t)$, and the fraction $1-k_u(t)$. In the Supplementary Material S5, as an example, a table for each of the three cell lines (DU145, HT29 and MX1) summarizes the expected energy reduction for tumor masses in the range 0-4 g and a structural biomass of 30cm$^3$ (see Fig. S40-Fig.S42).

The inclusion of information about food consumption, presence of adverse events effecting nutrient assimilation (diarrhea or vomiting) and host body composition may improve the model characterization of tumor-related anorexia/cachexia. However, their evaluation was not provided by the protocol of the considered experiments, because the required complex set-up (e.g., individual metabolic cages or NMR analyses for the body host composition) may interfere with the assessment of the treatment antitumor efficacy.

In case of treatment, the anti-angiogenic activity of bevacizumab should limit the energy delivery to tumor inhibiting the capability of the tumor vascularization and, thus, rebalancing the energetic distribution between tumor and host. Coherently, Eq.3 for $k_u(t)$ has been modified adding an $l_{max}$ inhibitory function, parametrized in terms of $IC_{50}$ (Eq.5) that provides a measurement of the drug potency on the considered tumor cell lines. Interestingly, the ranking obtained for Exp b, Exp f and Exp g, involving DU145, HT29 and MX1 tumor lines, is in agreement with previous results (20).

Further considerations about the drug potency can be obtained considering the biomass degradation process. In particular, the time $t_{\delta V_{max}}$ at which this process reaches its maximum rate, $\delta V_{max}$ represents a meaningful index of cachexia severity. A higher impact of tumor progression on host conditions causes a greater biomass degradation and, thus, an early achievement of the maximum rate, $\delta V_{max}$. Because an anti-angiogenic treatment counteracts tumor-related cachexia, the biomass degradation is slowed down in treated...
animals that reach the maximum degradation rate later than controls. Hence, the time delay, $\Delta t_{\delta V_{\text{max}}}$, between control and treated groups may be considered an additional quantitative measure of drug activity on energy distribution (Fig. 2).

A description of tumor hypoxia (imputable only to VEGF-blockade) was integrated within the model (Eq. 11). It was hypothesized that a prolonged tumor hypoxia over a critical threshold $\bar{H}$ triggers a VEGF-independent tumor re-vascularization and, consequently, more energy is available to the tumor mass, with a decrease of the bevacizumab effectiveness (Eqs. 9-10).

Results obtained in Exp $b$, Exp $d$ and Exp $e$ show that, with the integration of a hypoxia-mediated resistance mechanism, the model is able to capture the decreased efficacy affecting prolonged therapies. In agreement with the literature data, in which a response phase of 10-14 days was documented (40), the hypoxia condition remains in present study below the threshold $\bar{H}$ ($\bar{H}_{\text{Max}} = 1$) for a period of about 11 days.

This limitation is reported also in the clinical setting where a reduced efficacy due to resistance mechanisms is frequently observed during prolonged anti-angiogenic administrations (7, 41). Current experimental evidence suggests that tumors can adapt to the presence of VEGF-target agents acquiring different mechanisms to functionally evade the therapeutic VEGF-blockade (40, 42). In particular, activation of alternative pro-angiogenic signaling, independent from VEGF, was revealed in xenograft models (43): after an initial response phase (10-14 days), tumors started re-growing and the typically dense tumor vascular network was restored. Notably, the VEGF-blockade seems persist during all the study and tumors showed regions of acute hypoxia, a known inducer of angiogenic responses in a wide variety of cancer types (44).

Since the proposed DEB-TGI anti-angiogenic model always showed excellent fitting capabilities, its predictive power was also explored. To this purpose, Exp $a$, in which bevacizumab was administered at 10mg/kg or 20mg/kg, was considered. Model parameters were first estimated on data of the placebo and lowest dose arm and, subsequently, used to predict tumor and host dynamics in the higher dose arm that was considered as external dataset. The agreement between predictions and observations, considering both typical ($r^2=0.94$ for mice body weight and $r^2=0.98$ for tumor weight) and individual profiles (panels A and B in Fig.4), confirmed the predictive capabilities of the model and its potential use to predict tumor and host responses to new treatment schedules.

To further assess the predictive performances of the hypoxia-triggered resistance model on new experiments, data related to patient-derived colorectal cancer (CRC) xenograft mice treated with bevacizumab at long-term intermittent and continuous schedules were taken from the literature (45). In this experiment bevacizumab was administered (5mg/kg) twice-a-week for a treatment period of 30 days, 50 days, 30 days followed by 20 days without treatment, 70 days or 70 days-treatment with a break period between day 30 and 50. The hypoxia-triggered resistance model and its parameter values derived on DU-145 cell line were used to predict the CRC tumor response to bevacizumab treatments, whereas the tumor-related parameters were estimated by using the experimental tumor weight data reported in (45) (host body weights were not available). As documented by the agreement between model simulations and observations (Panels C in Fig.4), the DEB-TGI model integrated with the hypoxia-resistance mechanism was able to adequately predict tumor response to the different administration schedules ($r^2=0.97$), showing its good potentiality as predictive tool of bevacizumab anticancer effects.

Once established, the hypoxia-triggered resistance model can be used also to explore the expected tumor and host responses to different administration protocols accounting for the resistance effect. In particular, continuous or intermittent schedules, whose convenience is
still under debate in the literature, can be evaluated. As an example, considering the DU145 line, tumor-in-host response to 20mg/kg bolus q4dx6 (Exp b) was compared to the response to 20mg/kg bolus q4dx3 for two cycles 28-day spaced and to q4dx2 for three cycles 16-day spaced. Simulations are reported in Fig.5. In panel A, a slight positive indication towards the use of intermittent schedules seems to be present. In panel B, the comparison of the tumor profiles predicted by the model with or without hypoxia provides a clearer view of the negative effect of hypoxia in case of continuous repeated administration.

CONCLUSIONS

Tumor-in-host DEB-based models, thanks to their physiological approach, could be particularly useful to support the drug development process and the translation human research of anticancer agents. Nevertheless, up to now, their complexity and the high number of parameters have limited their application to preclinical experimental data. Only recently, a tumor-in-host model, based on a reduced version of the DEB theory and able to predict the dynamics of host body and tumor weights following cytotoxic treatment in xenograft mice, has been presented (29).

Aim of the present work was to deeper exploit the potentialities of the DEB approach in characterizing tumor-host energetic interactions. Specific modeling efforts have been made on the description of tumor-related cachexia/anorexia and cytostatic activity of anti-angiogenic agents on tumor. Results, obtained for a set of xenograft experiments on bevacizumab, highlighted the good model capability in describing tumor growth as well as host body growth in control and treated animals. In addition, for the first time, a quantitative measurement of the cachectic effect of tumor growth for different cell lines and drug schedules was provided. Finally, a hypoxia-triggered resistance model allowed to describe the decreased efficacy of bevacizumab observed after prolonged treatments.

For all these reasons, this paper wants to promote the introduction of DEB-based modeling approaches in the cancer research area. The physiological properties of the model and its predictive capabilities strongly recommend its use for translational purposes during the anti-cancer drug development process and its application for a better characterization of cancer-associated cachexia. In particular, the DEB theory already provides general rules for scaling parameters from one species to another one. Exploiting these rules and the body weight growth data of tumor-free individuals and making some additional hypothesis, it should be possible to translate tumor-in-host models from preclinical to clinical setting.
REFERENCES


34. Avastin: Epar scientific discussion. European medicin agency.


36. Hongli Li, Yan Li, Yuanyuan Liu, Dingzhi Huang, Ming Bai, Shaohua Ge, Ting Deng, Rubing Han, Rui Liu, Xia Wang, et al. The incidence and impact of weight loss with cachexia in gastric cancer patients., 2015.


### Table 1: Structural parameters of tumor-in-host DEB-TGI anti-angiogenic model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dimension</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Host-related parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\nu$</td>
<td>$L/T$</td>
<td>Energy conductance</td>
</tr>
<tr>
<td>$\rho_b$</td>
<td>-</td>
<td>Tumor-free food-supply coefficient</td>
</tr>
<tr>
<td>$V_{1,\infty}$</td>
<td>$L^3$</td>
<td>Maximum structural biomass volume</td>
</tr>
<tr>
<td>$g$</td>
<td>-</td>
<td>Growth energy-investment ratio</td>
</tr>
<tr>
<td>$m$</td>
<td>$1/T$</td>
<td>Maintenance-growth rate ratio</td>
</tr>
<tr>
<td>$\xi$</td>
<td>-</td>
<td>Energy reserve weight scaling factor</td>
</tr>
<tr>
<td>$d_v$</td>
<td>$W/L^3$</td>
<td>Specific weight of structural biomass</td>
</tr>
<tr>
<td><strong>Tumor-related parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\mu_u$</td>
<td>-</td>
<td>Coefficient of gluttony</td>
</tr>
<tr>
<td>$g_u$</td>
<td>-</td>
<td>Tumor growth energy-investment ratio</td>
</tr>
<tr>
<td>$m_u$</td>
<td>$1/T$</td>
<td>Tumor maintenance-growth rate ratio</td>
</tr>
<tr>
<td>$d_{Vu}$</td>
<td>$W/L^3$</td>
<td>Specific weight of tumor mass</td>
</tr>
<tr>
<td><strong>Cachexia-related parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\omega$</td>
<td>-</td>
<td>Biomass thermodynamic extraction efficiency coefficient</td>
</tr>
<tr>
<td>$\delta_{V_{\text{Max}}}$</td>
<td>$L^3/T$</td>
<td>Maximum structural biomass degradation rate</td>
</tr>
<tr>
<td>$lV_{50}$</td>
<td>$L^3$</td>
<td>Half maximal inhibitory tumor volume</td>
</tr>
<tr>
<td><strong>Drug-related parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$IC_{50}$</td>
<td>$CONC$</td>
<td>Half maximal inhibitory concentration</td>
</tr>
<tr>
<td>$I_{\text{max}}$</td>
<td>-</td>
<td>Maximum inhibitory effect</td>
</tr>
</tbody>
</table>
Table 2: Model parameter estimates.

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Exp a</th>
<th>Exp b</th>
<th>Exp c</th>
<th>Exp d</th>
<th>Exp e</th>
<th>Exp f</th>
<th>Exp g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mice strain</strong></td>
<td>Bulb</td>
<td>Bulb</td>
<td>Bulb</td>
<td>Bulb</td>
<td>Bulb</td>
<td>Bulb</td>
<td>CD1</td>
</tr>
<tr>
<td><strong>Tumor line</strong></td>
<td>DU145</td>
<td>DU145</td>
<td>DU145</td>
<td>DU145</td>
<td>DU145</td>
<td>HT29</td>
<td>MX1</td>
</tr>
<tr>
<td><strong>Protocol</strong></td>
<td>q4dx6</td>
<td>q4dx6</td>
<td>q4dx6</td>
<td>q4dx4</td>
<td>q4dx3</td>
<td>q4dx</td>
<td>q4dx</td>
</tr>
<tr>
<td><strong>Starting day</strong></td>
<td>5</td>
<td>9</td>
<td>6</td>
<td>8</td>
<td>9</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td><strong>Dose level</strong></td>
<td>10mg/kg</td>
<td>20mg/kg</td>
<td>20mg/kg</td>
<td>20mg/kg</td>
<td>20mg/kg</td>
<td>20mg/kg</td>
<td>20mg/kg</td>
</tr>
</tbody>
</table>

**Typical values (RSE%)**

<table>
<thead>
<tr>
<th>Host-related parameters</th>
<th>W_s [g]</th>
<th>26</th>
<th>23.2</th>
<th>28.1</th>
<th>27.7</th>
<th>27</th>
<th>34.2</th>
<th>27.1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(2%)</td>
<td>(3%)</td>
<td>(2%)</td>
<td>(2%)</td>
<td>(2%)</td>
<td>(3%)</td>
<td>(3%)</td>
<td></td>
</tr>
<tr>
<td>ρ_b [-]</td>
<td>0.997</td>
<td>0.986</td>
<td>0.993</td>
<td>0.997</td>
<td>0.986</td>
<td>0.892</td>
<td>0.954</td>
<td></td>
</tr>
</tbody>
</table>

**Tumor-related parameters**

| | V_s [cm³] | 0.01 | 0.015 | 0.017 | 0.023 | 0.022 | 0.0405 | 0.055 |
|-----------------|---------|------|-------|------|-------|--------|--------|
|                 | (14%)   | (15%)| (16%) | (11%)| (13%) | (16%)  | (12%)  |
| μ_s [-]         | 7.58    | 7.09 | 7.93  | 7.93 | 6.5   | 5.62   | 5.71   |
|                 | (3%)    | (3%) | (4%)  | (3%) | (5%)  | (94%)  | (4%)   |
| g_s [-]         | 13.34   | 13.83 | 13.58 | 13.79 | 12.37 | 12.16  | 14.36  |
|                 | (<1%) | (<1%)| (1%)  | (1%) | (6%)  | (99%)  | (2%)   |
| m_s [1/day]     | 0.014   | 0.017 | 0.015 | 0.016 | 0.0132| 0.021  | 0.012  |
|                 | (7%)   | (<1%)| (4%)  | (3%) | (11%) | (99%)  | (<1%)  |

**Cachexia-related parameters**

<table>
<thead>
<tr>
<th>I_V_cach** [cm³]</th>
<th>10.1</th>
<th>-</th>
<th>11</th>
<th>12.1</th>
<th>12.8</th>
<th>2.96</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(2%)</td>
<td></td>
<td>(10%)</td>
<td>(5%)</td>
<td>(17%)</td>
<td>(64%)</td>
<td></td>
</tr>
<tr>
<td>δ_V_cach [cm³/day]</td>
<td>0.016</td>
<td>0.016</td>
<td>0.013</td>
<td>0.011</td>
<td>0.016</td>
<td>0.083</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>(1%)</td>
<td>(2%)</td>
<td>(2%)</td>
<td>(3%)</td>
<td>(6%)</td>
<td>(35%)</td>
<td>(105%)</td>
</tr>
</tbody>
</table>

**Drug-related parameters**

<table>
<thead>
<tr>
<th>I_C50 [µM]</th>
<th>3.29</th>
<th>2.22</th>
<th>2.76</th>
<th>1.31</th>
<th>0.966</th>
<th>4.12</th>
<th>1.74</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(19%)</td>
<td>(9%)</td>
<td>(23%)</td>
<td>(17%)</td>
<td>(13%)</td>
<td>(34%)</td>
<td>(21%)</td>
</tr>
</tbody>
</table>

**Inter-individual variability (RSE%)**

<table>
<thead>
<tr>
<th>sd (W_s)</th>
<th>0.097</th>
<th>0.095</th>
<th>0.091</th>
<th>0.089</th>
<th>0.061</th>
<th>0.081</th>
<th>0.117</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(16%)</td>
<td>(20%)</td>
<td>(19%)</td>
<td>(19%)</td>
<td>(23%)</td>
<td>(21%)</td>
<td>(20%)</td>
</tr>
<tr>
<td>sd (ρ_b)*</td>
<td>0.004</td>
<td>0.152</td>
<td>0.769</td>
<td>0.464</td>
<td>0.55</td>
<td>0.183</td>
<td>0.175</td>
</tr>
<tr>
<td></td>
<td>(20%)</td>
<td>(21%)</td>
<td>(22%)</td>
<td>(33%)</td>
<td>(22%)</td>
<td>(23%)</td>
<td>(29%)</td>
</tr>
<tr>
<td>sd (V_s)</td>
<td>0.433</td>
<td>0.419</td>
<td>0.435</td>
<td>0.212</td>
<td>0.36</td>
<td>0.328</td>
<td>0.289</td>
</tr>
<tr>
<td></td>
<td>(20%)</td>
<td>(21%)</td>
<td>(22%)</td>
<td>(33%)</td>
<td>(22%)</td>
<td>(23%)</td>
<td>(29%)</td>
</tr>
<tr>
<td>sd (IC50)</td>
<td>0.515</td>
<td>0.21</td>
<td>0.597</td>
<td>0.452</td>
<td>0.342</td>
<td>0.406</td>
<td>0.462</td>
</tr>
<tr>
<td></td>
<td>(27%)</td>
<td>(30%)</td>
<td>(27%)</td>
<td>(26%)</td>
<td>(29%)</td>
<td>(52%)</td>
<td>(28%)</td>
</tr>
</tbody>
</table>

**Residual variability (RSE%)**

<table>
<thead>
<tr>
<th>b (W)</th>
<th>0.199</th>
<th>0.179</th>
<th>0.186</th>
<th>0.166</th>
<th>0.235</th>
<th>0.256</th>
<th>0.182</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(6%)</td>
<td>(7%)</td>
<td>(7%)</td>
<td>(6%)</td>
<td>(7%)</td>
<td>(7%)</td>
<td>(6%)</td>
</tr>
<tr>
<td>b (W_s)</td>
<td>0.223</td>
<td>0.152</td>
<td>0.179</td>
<td>0.221</td>
<td>0.173</td>
<td>0.115</td>
<td>0.158</td>
</tr>
<tr>
<td></td>
<td>(6%)</td>
<td>(7%)</td>
<td>(7%)</td>
<td>(6%)</td>
<td>(7%)</td>
<td>(7%)</td>
<td>(6%)</td>
</tr>
</tbody>
</table>

Individual parameters are given by $P = \theta \exp(\eta i)$ where $\theta$ is the typical parameter value and $\eta$ a random effect with standard deviation $sd(\theta)$. *Values for $\rho_b$ and $sd(\rho_b)$ were approximations from the estimates of $\rho_b$ and $sd(\rho_b)$ reported in Table S3 in the Supplementary Material S4. **In Exp b and Exp f the estimates of $I_{V_cach}$ were so high that the tumor inhibitory effect on $\rho$ can be considered negligible. In these cases, $\rho$ was set equal to $\rho_b$. 

---

*Values for $\rho_b$ and $sd(\rho_b)$ were approximations from the estimates of $\rho_b$ and $sd(\rho_b)$ reported in Table S3 in the Supplementary Material S4. **In Exp b and Exp f the estimates of $I_{V_cach}$ were so high that the tumor inhibitory effect on $\rho$ can be considered negligible. In these cases, $\rho$ was set equal to $\rho_b$. 

---

Downloaded from cancerres.aacrjournals.org on April 29, 2021. © 2019 American Association for Cancer Research.
LIST OF FIGURE LEGENDS

Fig. 1: Energy fluxes in treated tumor-bearing animals. Energy is taken up from food and delivered to the reserves. Energy required by the somatic processes is obtained from reserves and assigned to host or to tumor through the partition fraction $k_u(t)$ on the basis of the gluttony coefficient $\mu_u$. In case of high energy request, reserves may be not sufficient and host starts to degrade its structural biomass (tumor-related cachexia). In case of anti-angiogenic treatments, the energy flow to the tumor is reduced ($k_u$, eq.5). The presence of tumor mass itself may reduce the host energy intake (tumor-related anorexia), inhibiting the coefficient $\rho(t)$.

Fig. 2: (A) Tumor and mice net body weight profiles obtained by using typical parameter values together with the average observed data (dots) for control (solid line) and treated (dashed line) arms. Vertical bars represent the standard deviation of the experimental measurements. RMSE is 0.15, 0.17 and 0.20g for host body weight and 0.04, 0.03 and 0.04g for tumor weight in Exp d, f and g, respectively. (B) Time course of the energy fraction $k_u$. Vertical lines mark $t_{\text{delta}}$; time delay, $\Delta T$, between treated and control arms is also assessed and reported. (C) Time course of the assimilation coefficient $\rho$.

Fig. 3: Hypoxia-resistance model profiles of tumor and mice net body weight obtained by using typical values of model parameters together with the average observed data (dots). Solid lines show control groups, dashed lines treated groups. Vertical bars represent the standard deviation of the experimental data. RMSE is 0.12g and 0.03g for mice body weight and tumor weight, respectively.

Fig. 4: (A) Model predicted profiles for the 20mg/kg bevacizumab arm of Exp a obtained using typical parameter values identified on 10mg/kg bevacizumab arm of Exp a together with experimental data (dots). Vertical bars represent the standard deviation of the experimental data. (B) External VPC plots (500 replicates of the dataset): dashed lines show the 10th, 50th and 90th percentiles of observed data, shaded areas represent the 90% confidence interval for the corresponding model predicted percentile, empty dots are individual observed data. (C) Observed and model predicted tumor weights related to patient-derived CRC xenograft mice treated with placebo (black) or 5mg/kg bevacizumab for 30 days, 50 days, 30 days followed by 20 days-break, 70 days or 70 days with a 20 days-break between day 30 and 50. Data from (45).

Fig. 5: (A) Tumor and mice net body weight predicted by the hypoxia-resistance model after the administration of 20mg/kg bolus q4dx6 (solid grey line), q4dx3 for two cycles 28-day spaced (dotted grey line), or q4dx2 for three cycles 16-day spaced (dashed grey line). Dots showed average data collected in the control and q4dx6 treated arms of Exp b. (B) Tumor and mice net body weight predicted by the model with (solid grey line) or without (dashed grey line) the hypoxia-resistance mechanism following the administration of 20mg/kg bolus q4dx6; black line showing control group. Dots showed average data.
Figure 1

**Tumor-related Anorexia**

Energy intake inhibition - eq(4)

\[ \rho(t) = \rho_b \left( 1 - \frac{V_u(t)}{V_u(t) + IV_{a,50}} \right) \]

**Anti-angiogenic Drug Action – Treated animals**

Energy partition function: drug inhibition - eq(5)

\[ k_u(t) = \left( \frac{\mu_u V_u(t)}{\mu_u V_u(t) + V(t)} \right) \left( 1 - \frac{I_{max} C_{Angio}(t)}{C_{Angio}(t) + IC_{50}} \right) \]

**Control animals**

Energy partition function - eq(3)

\[ k_u(t) = \left( \frac{\mu_u V_u(t)}{\mu_u V_u(t) + V(t)} \right) \]

**1 - k_u(t)**

Tumor mass \( V_u(t) \)

**Host structural biomass** \( V(t) \)

**Somatic processes energy consumption**

**Energy from biomass degradation**

**Tumor-related Cachexia**

Structural biomass degradation eqs (6)-(8)
Figure 2

Exp d

A

B

C

Exp f

A

B

C

Exp g

A

B

C
Figure 3
Figure 4

A

Mice body weight (g) vs. Time (day)

B

Mice body weight (g) vs. Time (day)

C

Mice body weight (g) vs. Time (days)
A tumor-in-host DEB-based approach for modeling cachexia and bevacizumab resistance

Elena Maria Tosca, Maurizio Rocchetti, Enrico Pesenti, et al.

Cancer Res  Published OnlineFirst December 9, 2019.

Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-19-0811

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2019/12/06/0008-5472.CAN-19-0811.DC1

Author Manuscript
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/early/2019/12/06/0008-5472.CAN-19-0811.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.