Combined analysis of tumor size and histological markers

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Summary

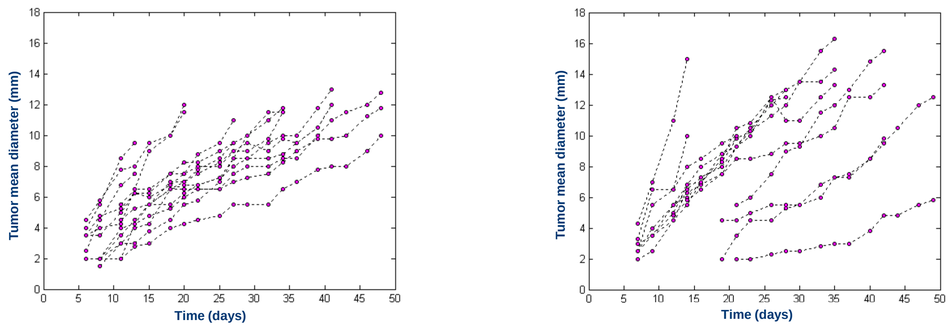
Optimizing the delivery of antiangiogenic drugs requires the development of drug-disease models of vascular tumor growth that incorporate histological data indicative of cytostatic action. We formulated a model to **simultaneously analyze longitudinal tumor size data together with histological markers** in preclinical settings.

The model is composed by four ODEs and focuses on the evolution of non-hypoxic (P), hypoxic (Q) and necrotic (N) tissue within the tumor. It integrates an unobserved variable, the carrying capacity (K), which accounts for the process of angiogenesis.
The model is shown to predict tumor size and histological markers in untreated and treated mice with the antiangiogenic **Sunitinib**. Individual predictions of the carrying capacity (K) are found to be correlated to observations on intratumoral blood vessels.

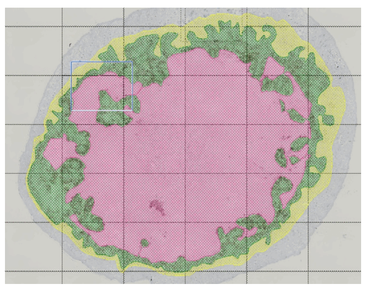
How did we obtain the data?

Human colorectal adenocarcinoma cells (HT29, HCT116) were injected into the right flank of each mouse. We collected data from each mouse every 2-3 days for up to 7 weeks:
- Two perpendicular diameter measurements were taken for each mouse. The **mean of the two diameters** and not the approximated volume was analyzed;
-Two or three mice were euthanized each week. Tumor were dissected and halved. One half was fixed in formalin then embedded in paraffin and the other was frozen.
- **Hypoxic tissue percentage** was assessed using the antibody anti-CA IX (anhydrase carbonic IX), a stable protein whose transcription is induced by HIF (hypoxia inducible factor).
- **Necrotic tissue percentage** was assessed by staining the tumor slice with Hematoxylin.
- Intratumoral blood vessel density and mean radius were assessed using the antibody anti-CD31 on the frozen tumor slices.

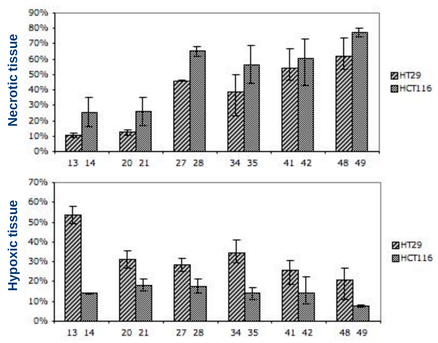
Tumor growth data



Individual tumor growth in 30 untreated mice injected with HT29 (n=15, left panel) and HCT116 (right panel) versus time after cell implantation.



A tumor slice after coloration using both IHC anti-CAIX to reveal the hypoxic tissue (green area) and Hematoxylin to highlight the necrotic tissue (pink area). Quantification was done using a microscope imaging station.

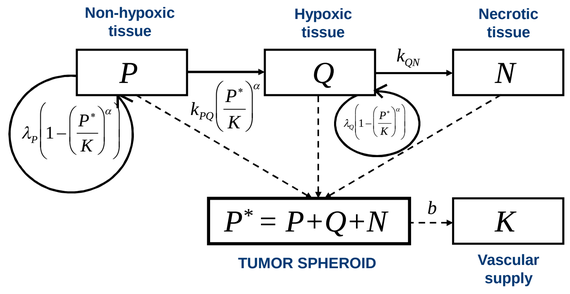


Changes in tumor biology over time for the 30 untreated mice.
Up: percentage of necrotic tissue.
Down: percentage of hypoxic tissue.

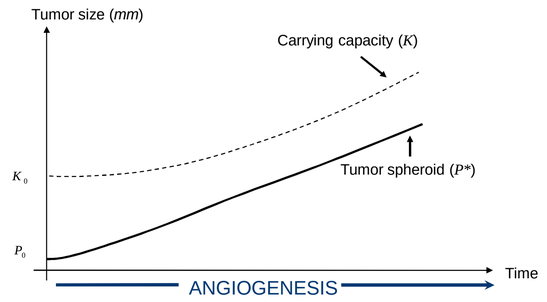
How did we model the data?

The longitudinal tumor size measurements and the histological data were analyzed with **Monolix 2.4** using **multiple-response nonlinear mixed effect models** with parameters assumed to follow a log-normal distribution. An exponential error model was used for residual variability in the three outputs.

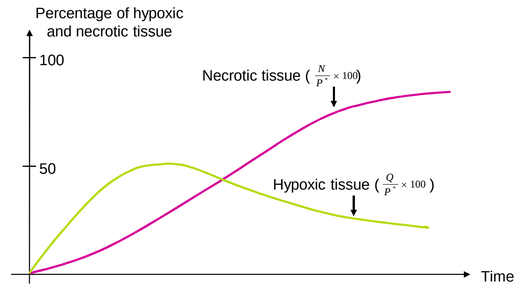
Model



$$\begin{cases} \frac{dP}{dt} = \lambda_p P\left(1-\left(\frac{P^*}{K}\right)^\alpha\right) - k_{PQ}P\left(\frac{P^*}{K}\right)^\alpha, \quad P(t=0)=P_0 \\ \frac{dQ}{dt} = k_{PQ}P\left(\frac{P^*}{K}\right)^\alpha + \lambda_Q Q\left(1-\left(\frac{P^*}{K}\right)^\alpha\right) - k_{QN}Q, \quad Q(t=0)=0 \\ \frac{dN}{dt} = k_{QN}Q, \quad N(t=0)=0 \\ \frac{dK}{dt} = bP^*, \quad K(t=0)=K_0 \\ P^* = P+Q+N \end{cases}$$

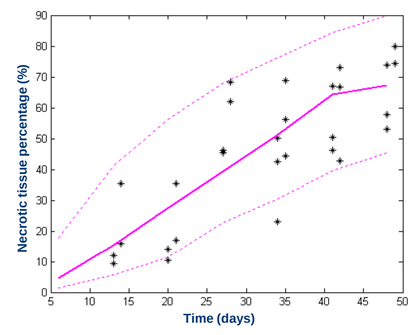


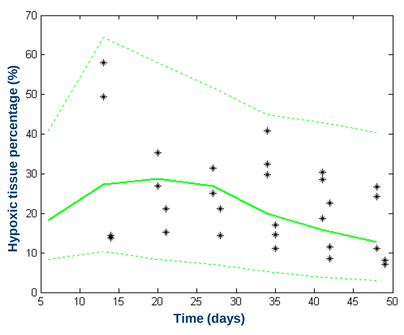
Evolution of tumor size (plain line) and carrying capacity (dotted line) versus time.



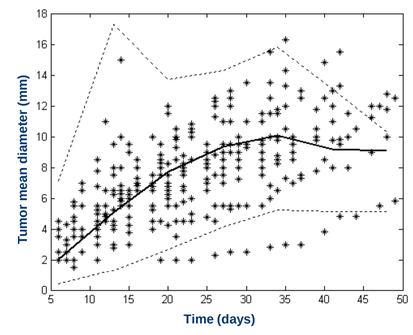
Evolution of necrotic tissue (pink line) and hypoxic tissue (green line) percentage.

Model diagnostic





Simulation of the model with 95% confidence interval are shown together with data.

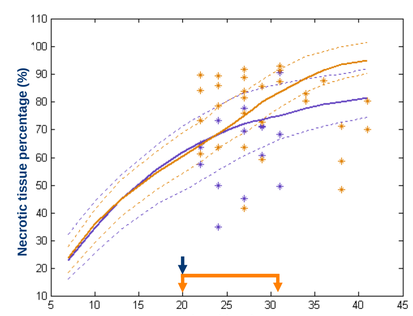


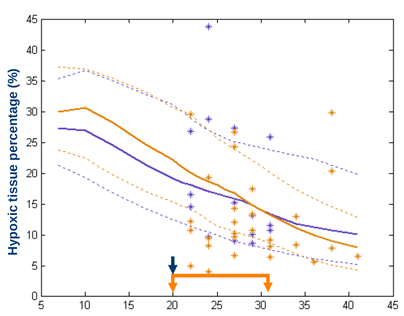
Parameters	Description	Estimate	IAV	η-shrinkage
P_0	Initial tumor size	0.32mm (25)	99 (13)	6
K_0	Initial carrying capacity	10.4 mm (9)	52 (20)	2
λ_p	Growth rate for the non-hypoxic tissue	1.24 day^{-1} (11)	62 (12)	4
k_{PQ}	Transfer rate from non-hypoxic to hypoxic tissue compartment	0.06 day^{-1} (12)	46 (35)	3
λ_Q	Growth rate for the hypoxic tissue	1.43 day^{-1} (20)	65 (18)	1
k_{QN}	Transfer rate from hypoxic to necrotic tissue compartment	0.07 day^{-1} (10)	61 (14)	
b	Rate of increase of carrying capacity	0.03 day^{-1} (30)	95 (11)	6

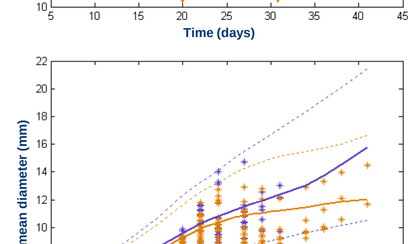
Parameter estimates. IAV, inter-animal variability expressed as percentage.

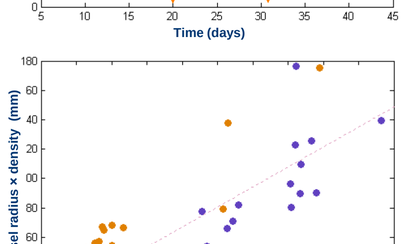
Antiangiogenic

We extended the model to integrate the action of the antiangiogenic drug **Sunitinib**. This was modeled using a kinetic-pharmacodynamic (**K-PD**) formulation and assuming the drug to induce a decline in the tumor carrying capacity (K). The resulting model was evaluated on tumor size and histological data from two groups of mice treated with Sunitinib:
- Group 1 (n=15) received a single oral dose of 40 mg/kg.
- Group 2 (n=30) received the same dose daily, for twelve days.









Individual predicted carrying capacity versus observations on intratumoral blood vessels.