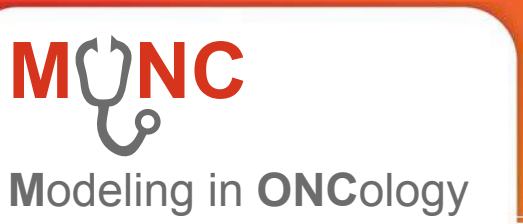
Modeling spontaneous metastasis following surgery and concomitant resistance: an in vivo-in silico approach

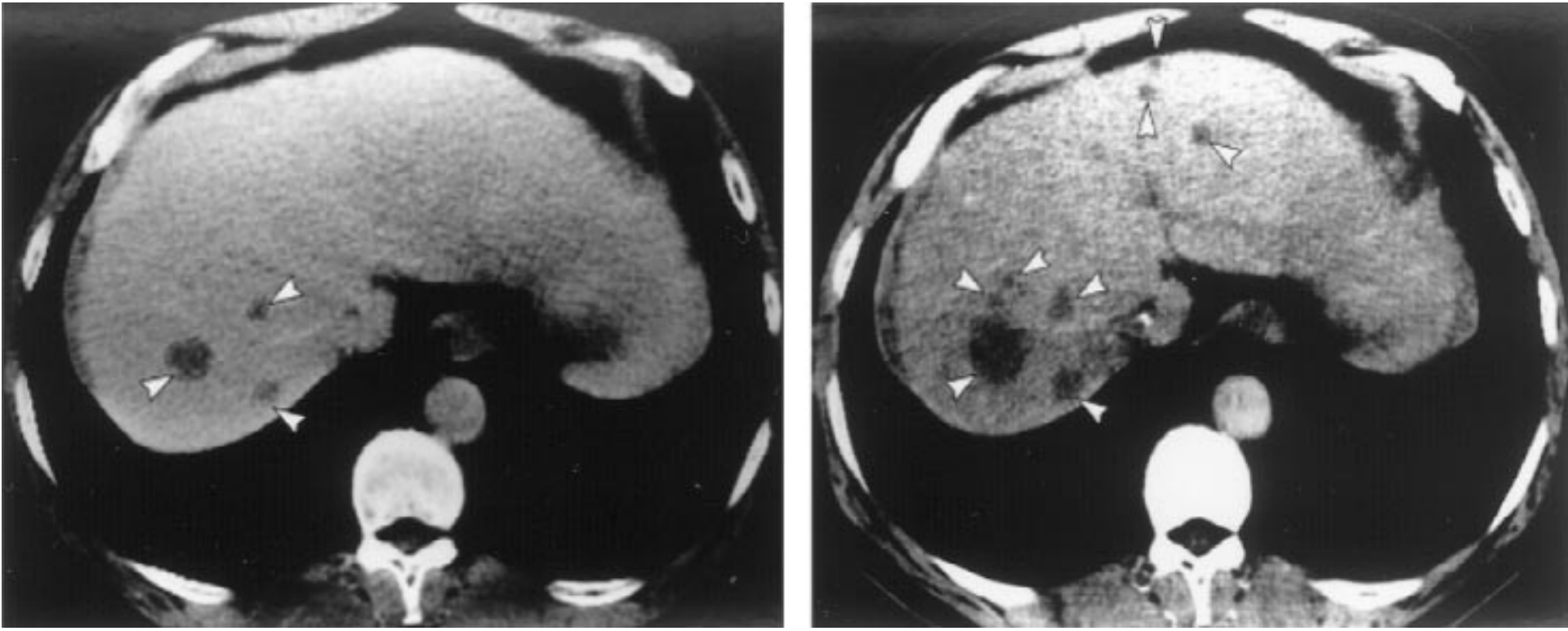
S. Benzekry

Present challenges of mathematics in oncology and biology of cancer

Marseille, Septembre 2015



Metastasis (μετά = change, στάσιζ = place)



Contrast-enhanced X-ray computed tomographies of the liver with multiple metastatic tumors. Interval : 127 days.

+ some of the metastases are **not visible**

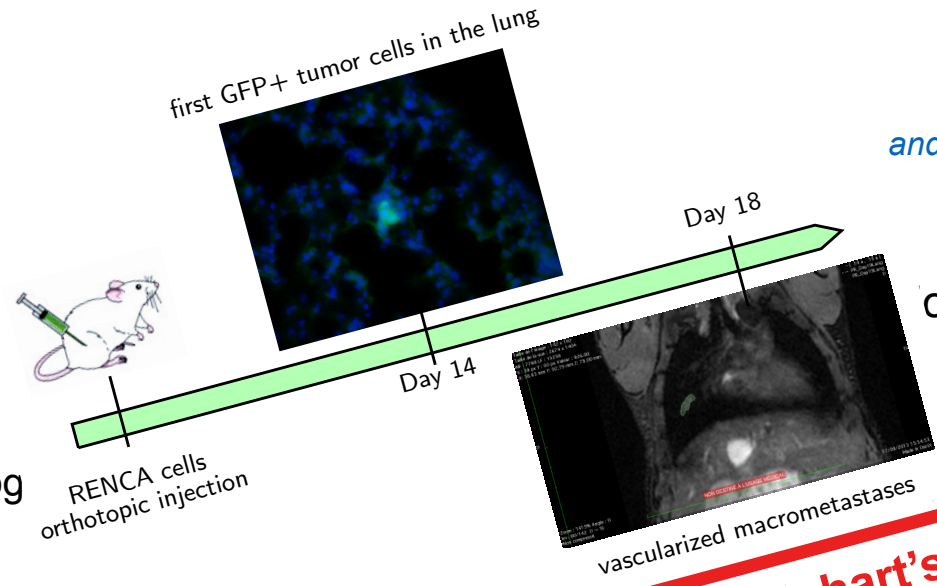
Iwata et al., J Theor Biol, 2000

Metastasis

- “Metastasis remains the cause of 90% of deaths from solid cancers" *Gupta and Massagué, Cell, 2006*
- Exciting **biological findings** amenable to dynamical/mathematical descriptions at the systemic scale in recent years:
  - Distant inhibition of angiogenesis by endogenous agents (endostatin,...) *O’Reilly, Folkman et al., Cell, 1994*
  - Self-seeding *Norton and Massagué, Nat Med, 2006*
  - Pre-metastatic niche *Kaplan et al., Nature 2005*
- **Clinical challenges**
  - What is the burden of **occult micro-metastases** at diagnosis?
  - What should be the extent of **post-surgery (“adjuvant") therapy**?
  - What is the **differential effect of therapies** on the primary tumor and the metastases? (AA therapies might accelerate mets? *Ebos et al., Cancer Cell, 2009*)
  - How to optimize the **scheduling and sequence** of anti-cancer agents?

Metastasis

- "Metastasis remains the c
  Massagué, Cell, 2006
  and
- Exciting **biological findin**
  the systemic scale in recent
  ons at
  - Distant inhibition of angiog
    al., Cell, 1994
    olkman et
  - Self-seeding *Norton and Massa*
  - Pre-metastatic niche *Kaplan et*
    ure 2005



See Etienne Baratchart's poster!

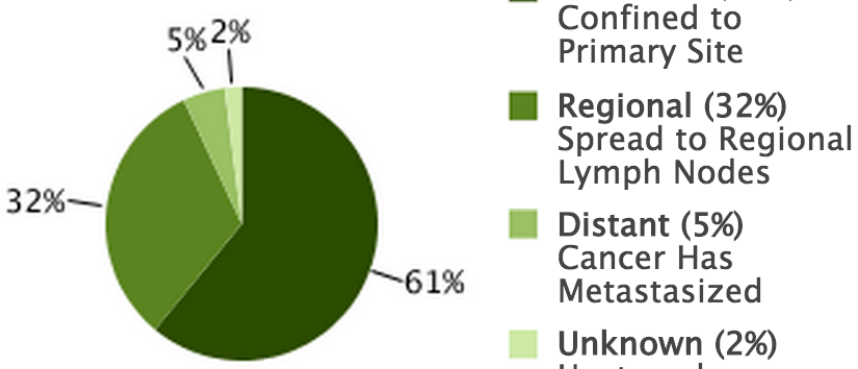
Baratchart et al. PloS Comp Biol, 2015

- **Clinical challenges**
  - What is the burden of **occult micro-metastases** at diagnosis?
  - What should be the extent of **post-surgery ("adjuvant") therapy**?
  - What is the **differential effect of therapies** on the primary tumor and the metastases? (AA therapies might accelerate mets? *Ebos et al., Cancer Cell, 2009*)
  - How to optimize the **scheduling and sequence** of anti-cancer agents?

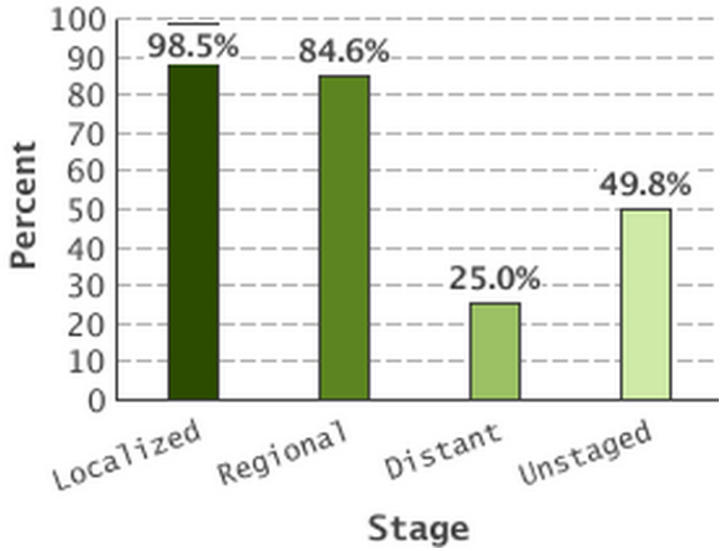
Breast cancer epidemiology

- **Most common** invasive cancer in women (14% of new cancer cases)
- Overall **5-year survival**: 89.2%
- However, about 28% will **relapse** within 15 years *Brewster et al, J Natl Cancer Inst, 2008*
- **20 year** survival is (only) 44% *Litiere et al., Lancet Oncol, 2012*

Percent of Cases by Stage



5-Year Relative Survival



Source: Surveillance, Epidemiology, and End Results (SEER) database, NCI

Outline

1. A minimally parameterized model for metastatic dynamics
    A. Open clinical questions
    B. Model
    C. Confrontation to experimental and clinical data sets
    D. Implications for assessment of the metastatic relapse risk and impact of PT size at surgery on survival
2. Concomitant resistance
    A. Biological phenomenon
    B. Data
    C. Model

Clinical questions

- For **early breast cancer** (non-metastatic)

  Q1: How to estimate the amount of **residual distant disease** at diagnosis in order to **personalize** the adjuvant (chemo)-therapy?

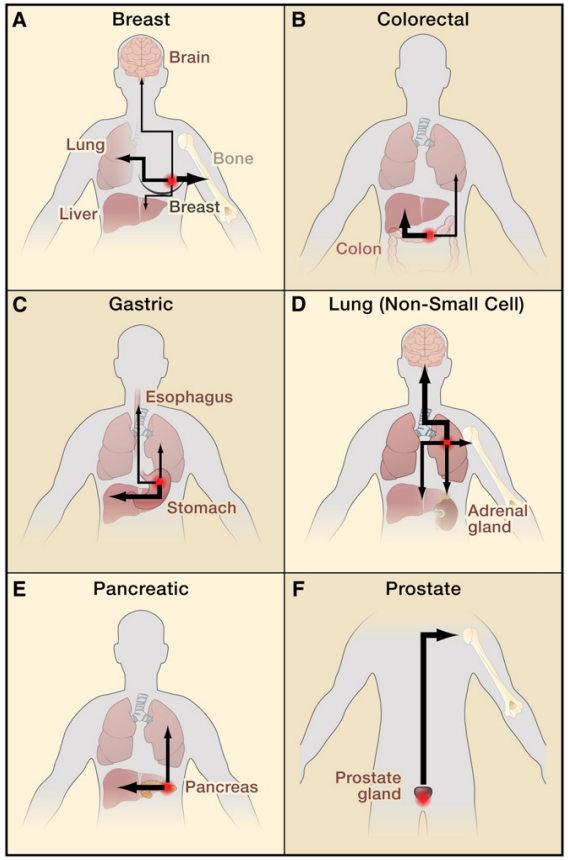
- For **metastatic breast cancer**, no consensus on the utility of surgery. Ongoing clinical trials. *Thomas et al., JAMA Surg,* ***2 dec 2015***

Q2: What is the quantitative impact of **PT resection** on the time-course of the post-surgical metastatic burden?

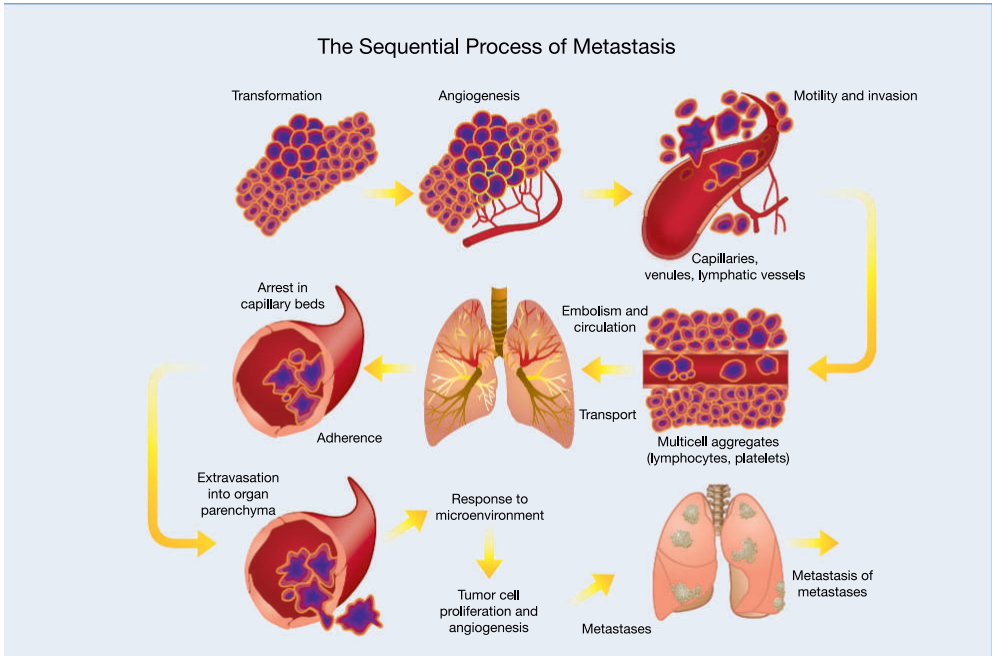
(Q3: How to **optimize the scheduling** of systemic anti-cancer agents (cytotoxic therapies, bio-therapies)?)

Metastatic biology 101

Secondary growth of disseminated cancer cells (from a **primary** location)



Metastatic process

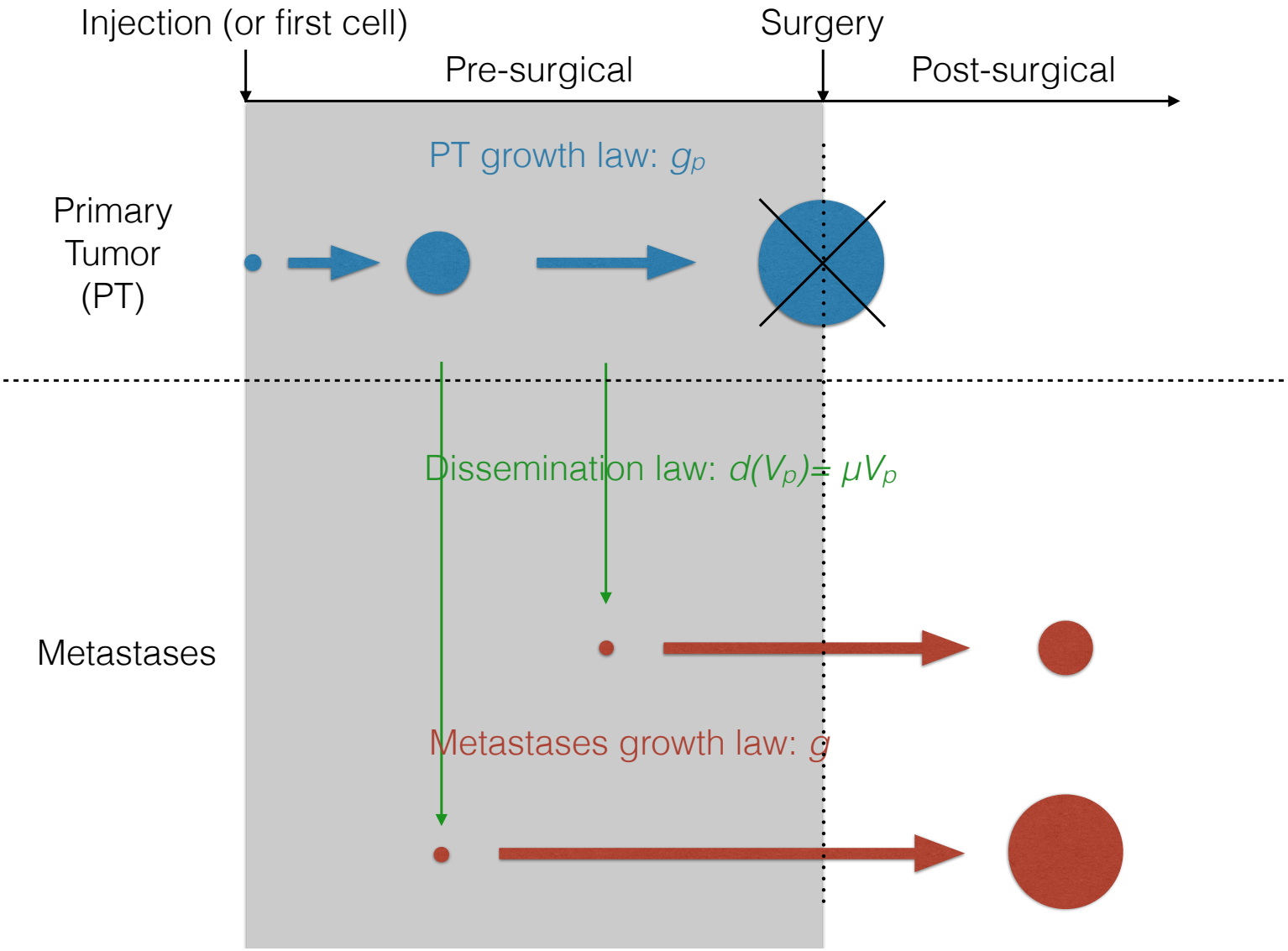


Two phases: **dissemination** and **colonization**

Talmadge and Fidler, Cancer Res, 2010

Valastyan and Weinberg, Cell, 2011

Model scheme



Mathematical formalism

- Primary tumor V_p grows with rate g_p

$$\frac{d}{dt}V_p = g_p(V_p), \quad V_p(t=0) = V_i$$

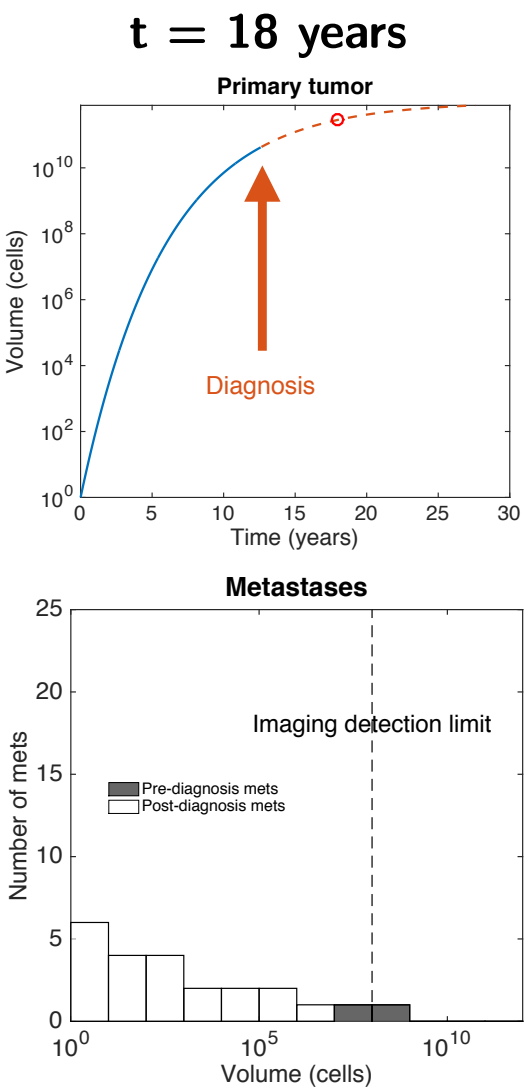
- **Population** of metastases represented by a **density *ρ(t,v)*** structured in **volume *v***

- Secondary tumors grow in size with rate $g(v)$

$$\partial_t \rho(t,v) + \partial_v(g(v)\rho(t,v)) = 0$$

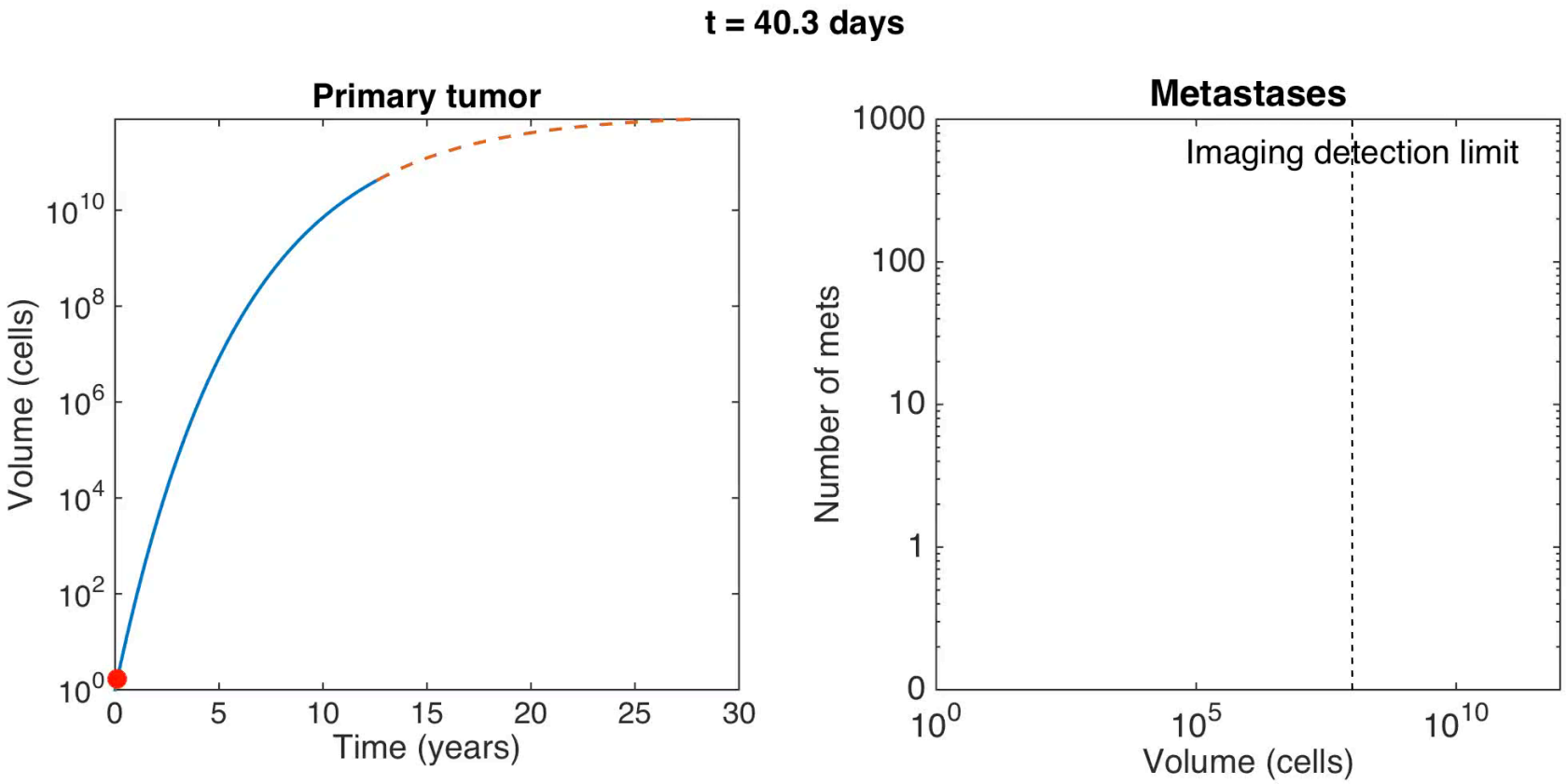
- They are spread by the PT with **dissemination rate** $d(V_p(t)) = \mu V_p(t)$

$$g(V_0)\rho(t,V_0) = d(V_p(t)) = \mu V_p(t)$$



Iwata et al., J Theor Biol, 2000

Simulation



Tumor size at diagnosis: 4.32 cm

Stochastic and discrete version of metastatic emission employed for the simulation

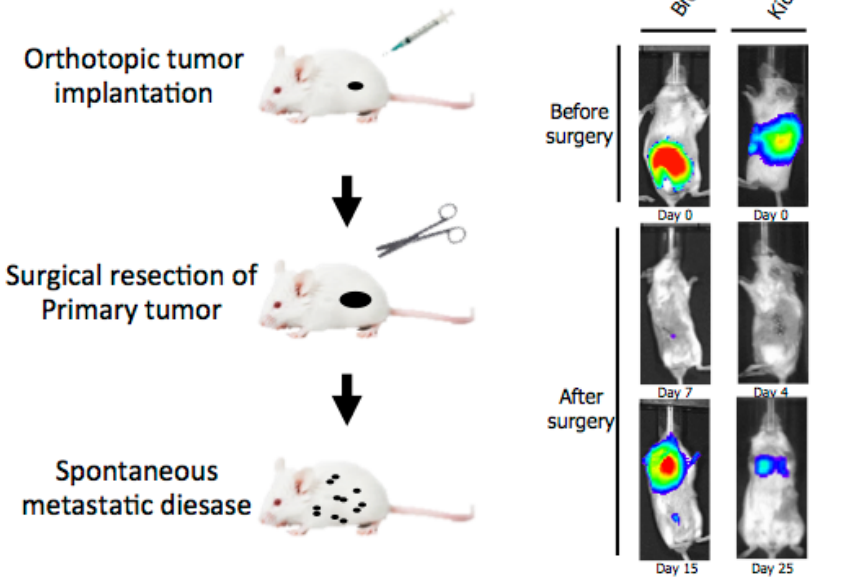
Outline

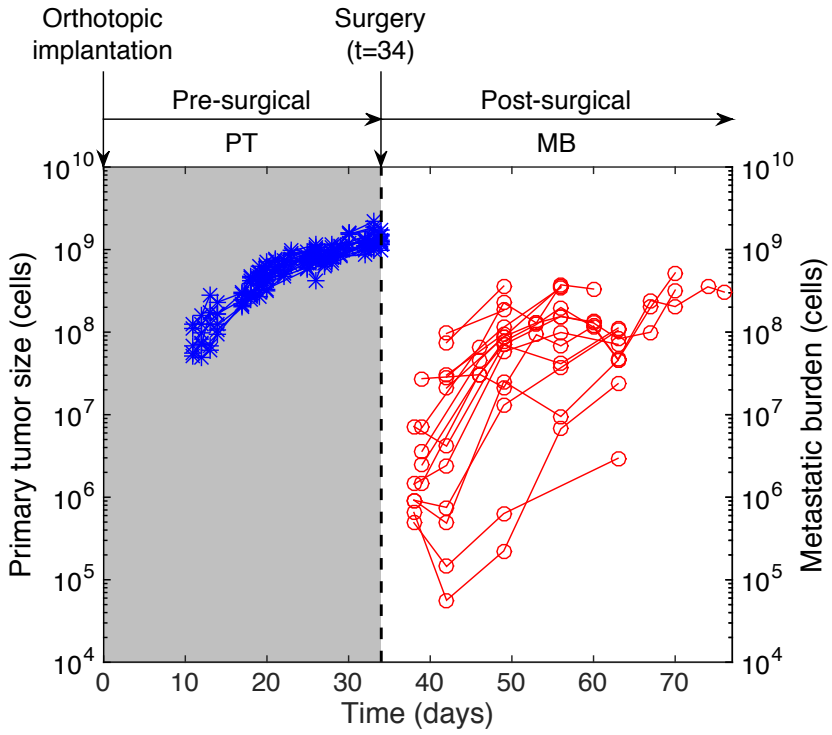
1. A minimally parameterized model for metastatic dynamics
    A. Open clinical questions
    B. Model
    *C. **Confrontation to experimental and clinical data sets***
    D. Implications for assessment of the metastatic relapse risk and impact of PT size at surgery on survival
2. Concomitant resistance
    A. Biological phenomenon
    B. Data
    C. Model

Ortho-surgical animal models of metastasis

- Metastasis is **hard to study** experimentally (intra-vital process)
- **Spontaneous** metastases

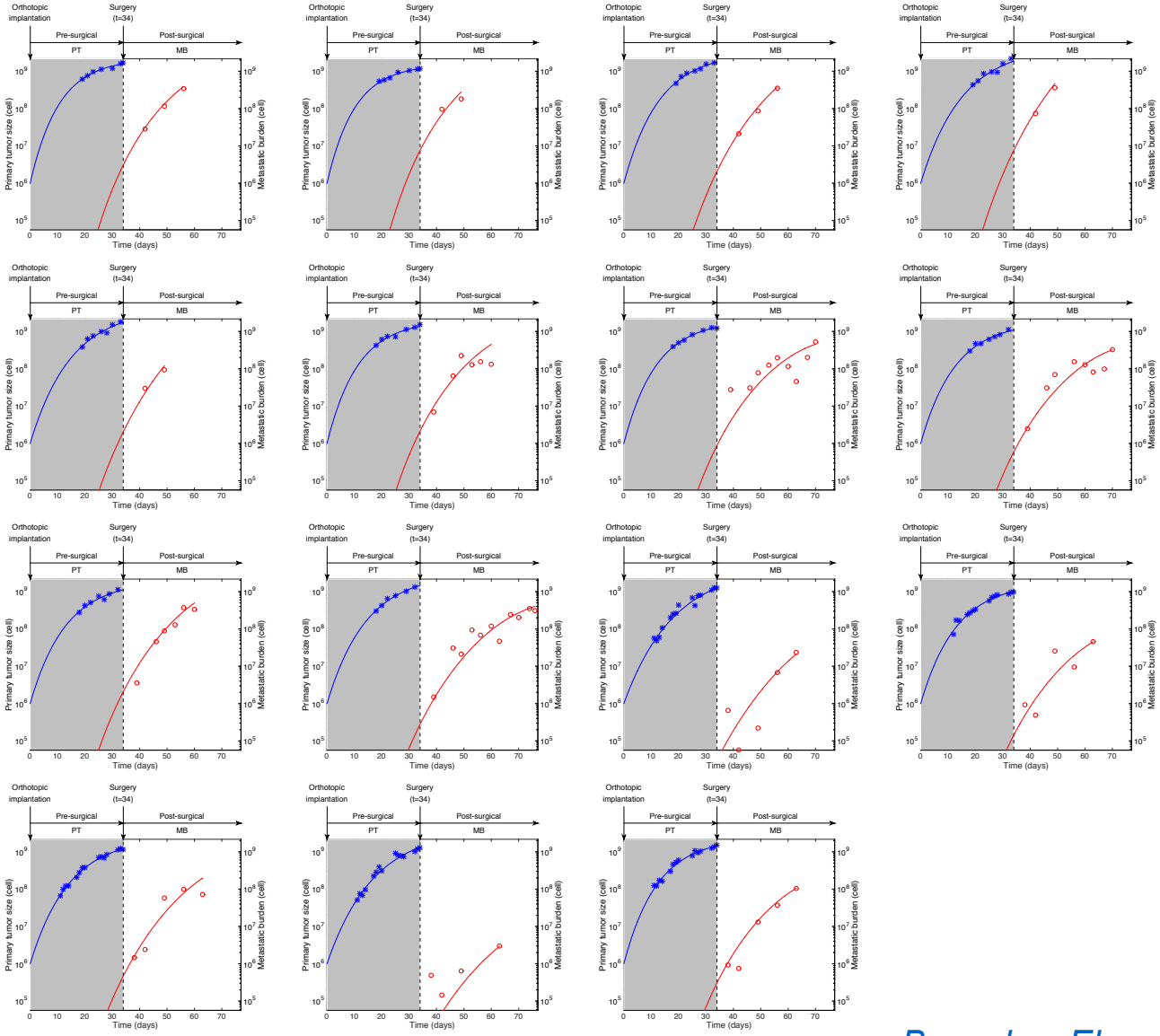
- Necessary to consider **surgery** of the primary tumor (PT) for clinical relevance
- Role of the **immune** system: 2 animal models (syngeneic and xenograft)





Benzekry, Ebos et al., Cancer Res, 2015

Individual fits



Benzekry, Ebos et al., Cancer Res, 2015

Statistical procedure: nonlinear mixed effects modeling

- Usual fitting methods consider each **time series independently**

$$y_i^j = M(t_i^j, \theta^j) + \varepsilon_i^j$$

Individual *1≤j≤N*

MLE →

$$\hat{\theta}^j = \min_{\theta^j} \sum \left(y_i^j - M(t_i, \theta^j)\right)^2$$

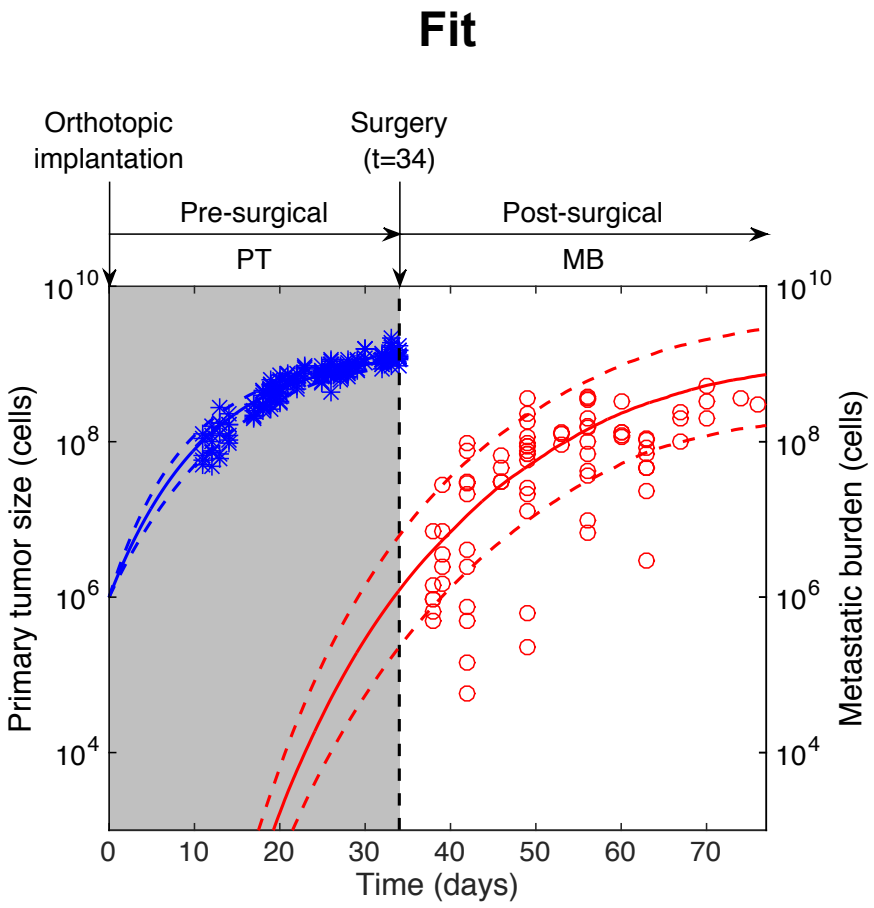
Time t_i

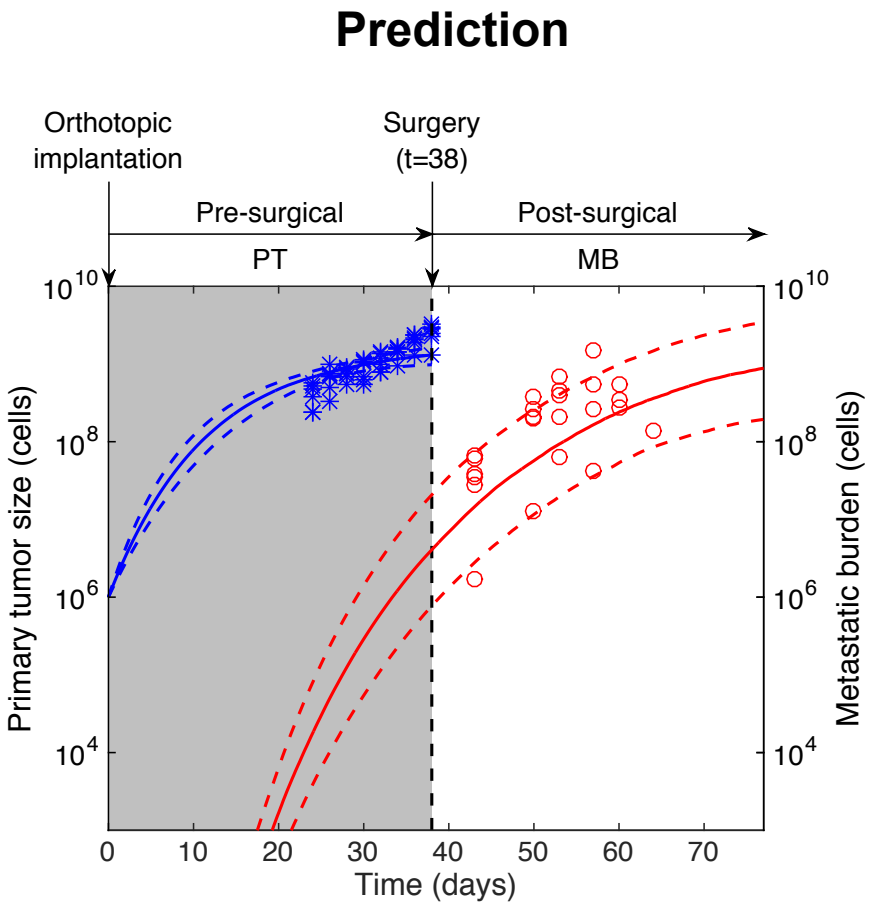
- When only sparse data are available from subjects in the same **population**, one can fit **parameters distribution** all-in-once

$$y_i^j = M(t_i^j, \theta^j) + \varepsilon_i^j, \quad \theta^1, \ldots, \theta^N \sim \mathcal{N}(\theta_\mu, \theta_\omega), \quad \theta_\mu \in \mathbb{R}^p,\ \theta_\omega \in \mathbb{R}^{p \times p}$$

- **Reduces the number of parameters from *pxN to p+p2***

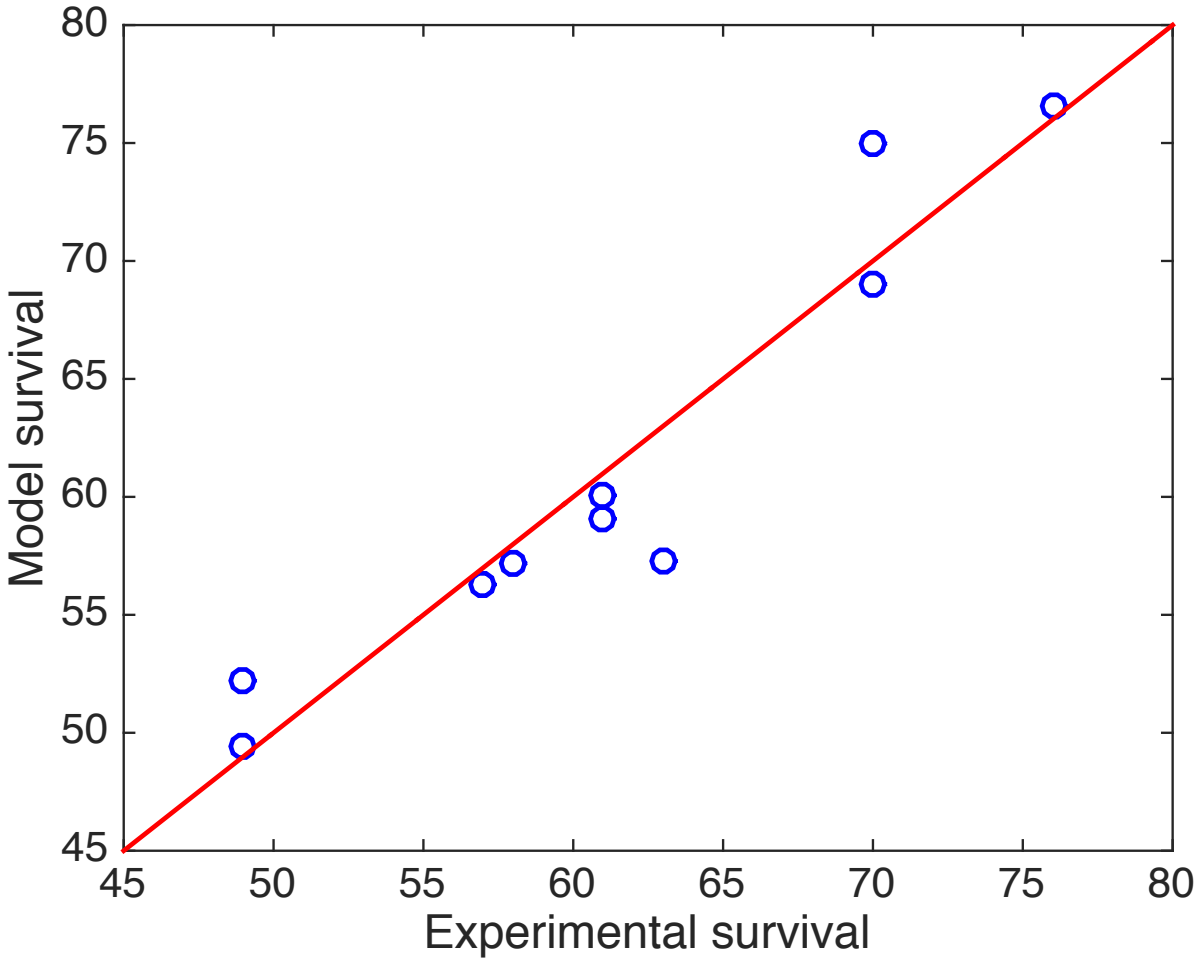
Population fit and prediction of bioluminescence data





Benzekry, Ebos et al., Cancer Res, 2015
Hartung, Mollard et al., Cancer Res, 2014

Predicted versus experimental survival



The model survival was defined as the time to reach a given lethal burden of 4×10^9 p/s, i.e.

$$\inf\{t>0;\ M(t) > 4 \times 10^9\}$$

Best model structure

- **Mechanistic assumptions**
  - Various structures tested for relationship of the PT and mets growth for optimal **trade-off between goodness-of-fit and identifiability**
  - **Same growth** between PT and mets
  - Growth model = Gomp-Exp
  - λ = in vitro proliferation rate (measured)

$$Gomp(v) = (\alpha - \beta \ln(v))\, v$$
$$g_p(v) = g(v) = \min(Gomp(v), \lambda v)$$

- **Statistical assumptions**
  - PT and mets fitted together (3 parameters)
  - **Proportional** statistical error model
  - **Lognormal** population distribution of the parameters
- Fast computation of the total metastatic burden using the FFT algorithm

  Hartung, 2015

Fits to breast cancer clinical dataset

20 year follow-up of 2648 patients

Koscielny et al., Br J Cancer, 1984

Diameter of PT (cm)	Prop. of relapse (Data)	Prop. of relapse (Model)
$1 \leq D \leq 2.5$	27.1	27.3
$2.5 < D \leq 3.5$	42.0	43.1
$3.5 < D \leq 4.5$	56.7	56.6
$4.5 < D \leq 5.5$	66.5	65.6
$5.5 < D \leq 6.5$	72.8	74.0
$6.5 < D \leq 7.5$	83.8	80.1
$7.5 < D \leq 8.5$	81.3	84.5

p = 0.0157

Pearson's χ^2 test for goodness-of-fit

- Assume **Gompertz growth** of PT, doubling time at 1 gram = 7 months and carrying capacity $K = 10^{12}$ cells
- Recover cancer **inception time** $-T_1$ from PT volume at diagnosis
- Lognormal distribution of m and fixed populational γ for **inter-individual variability**
- Probability of developing a met = probability of having one at diagnosis

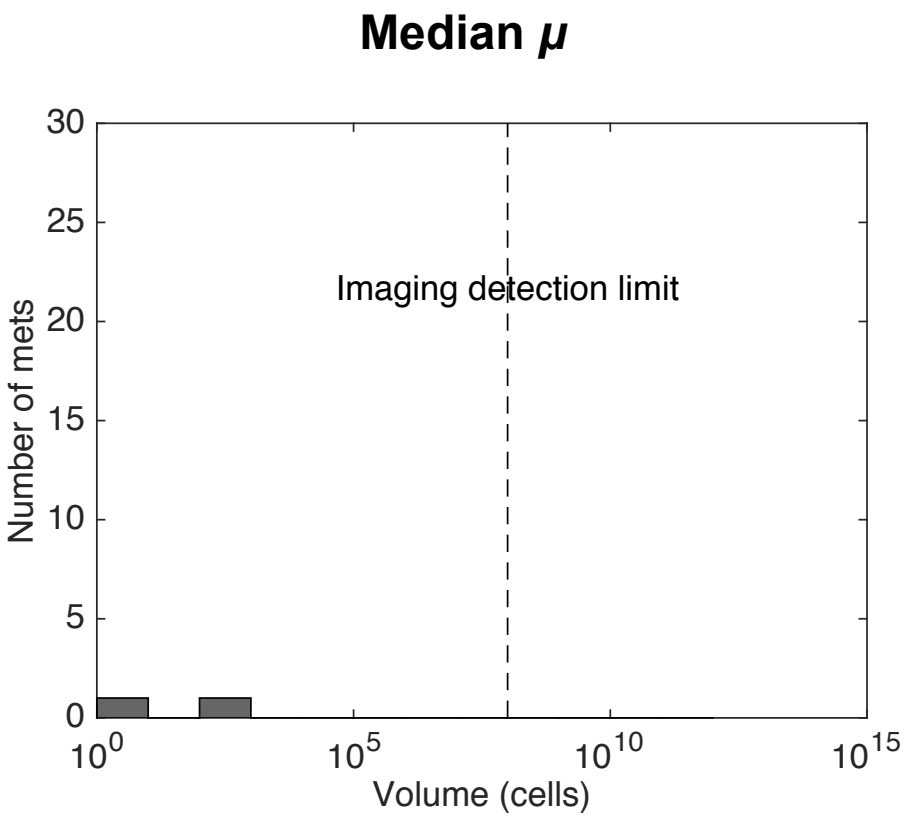
$$\mathbb{P}(\textbf{Mets}) = \mathbb{P}\left(\mu \int_0^{T_1} V_p(t) > 1\right)$$

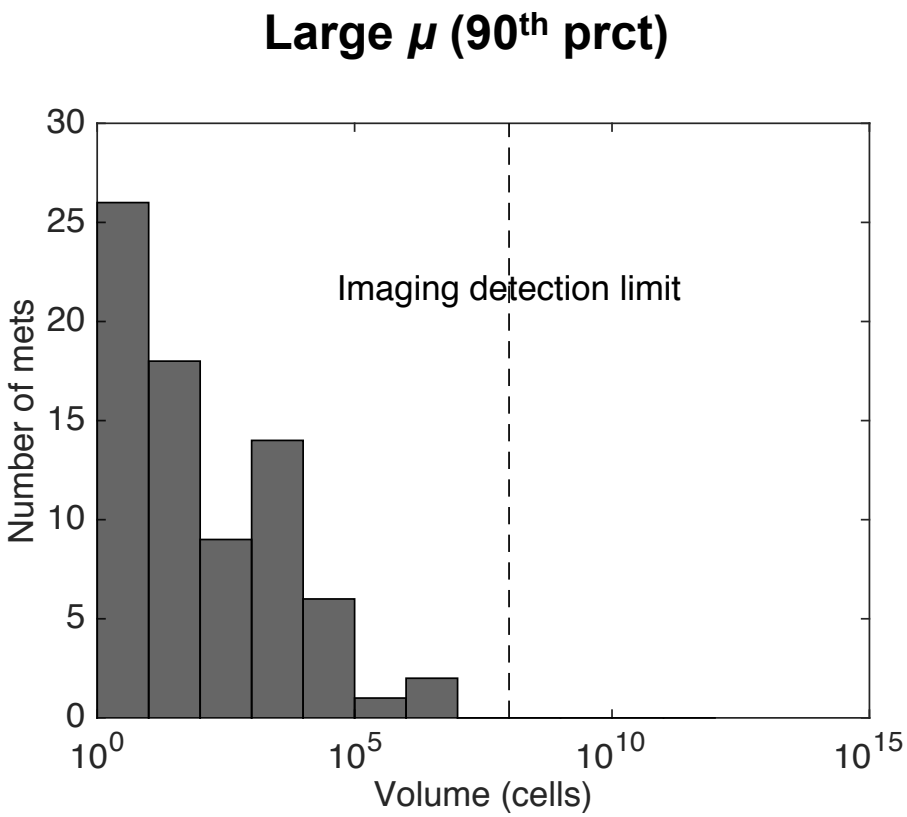
Parameters: quantification of metastatic potential

Data	Growth model	Location	Par.	Unit	Estimate (CV)	95 % CI
In vitro (Breast)	Exp.		λ	day^{-1}	0.837 (-)	(0.795 - 0.879)
Preclinical Breast	Gomp-Exp.	PT	V_i	$cell$	1.00×10^6 (-)	-
			α	day^{-1}	1.9 (5.73)	(1.84 - 1.96)
			β	day^{-1}	0.0893 (21.3)	(0.0791 - 0.101)
		Met	V_0	p/s	10 ( )	
			μ	$cell^{-1} \cdot day^{-1}$	4.43×10^{-11} (176)	$(2.70 \times 10^{-11}$ - $7.27 \times 10^{-11})$
Preclinical Kidney	Exp.	PT	V_i	p/s	1.63×10^5 (45.5)	$(9.40 \times 10^4$ - $2.83 \times 10^5)$
			α_p	day^{-1}	0.21 (60.3)	(0.151 - 0.292)
		Met	V_0	p/s	10 (-)	-
			α	day^{-1}	0.0307 (201)	(0.0133 - 0.0707)
			μ	$cell^{-1} \cdot day^{-1}$	0.0415 (397)	(0.0181 - 0.0948)
Clinical Breast	Gomp.	PT	V_i	$cell$	1 (-)	
			α	day^{-1}	0.013 (-)	
			β	day^{-1}	0.000471 (-)	
		Met	V_0	$cell$	1 (-)	
			μ	$cell^{-1} \cdot day^{-1}$	7.00×10^{-12} (1.04×10^4)	

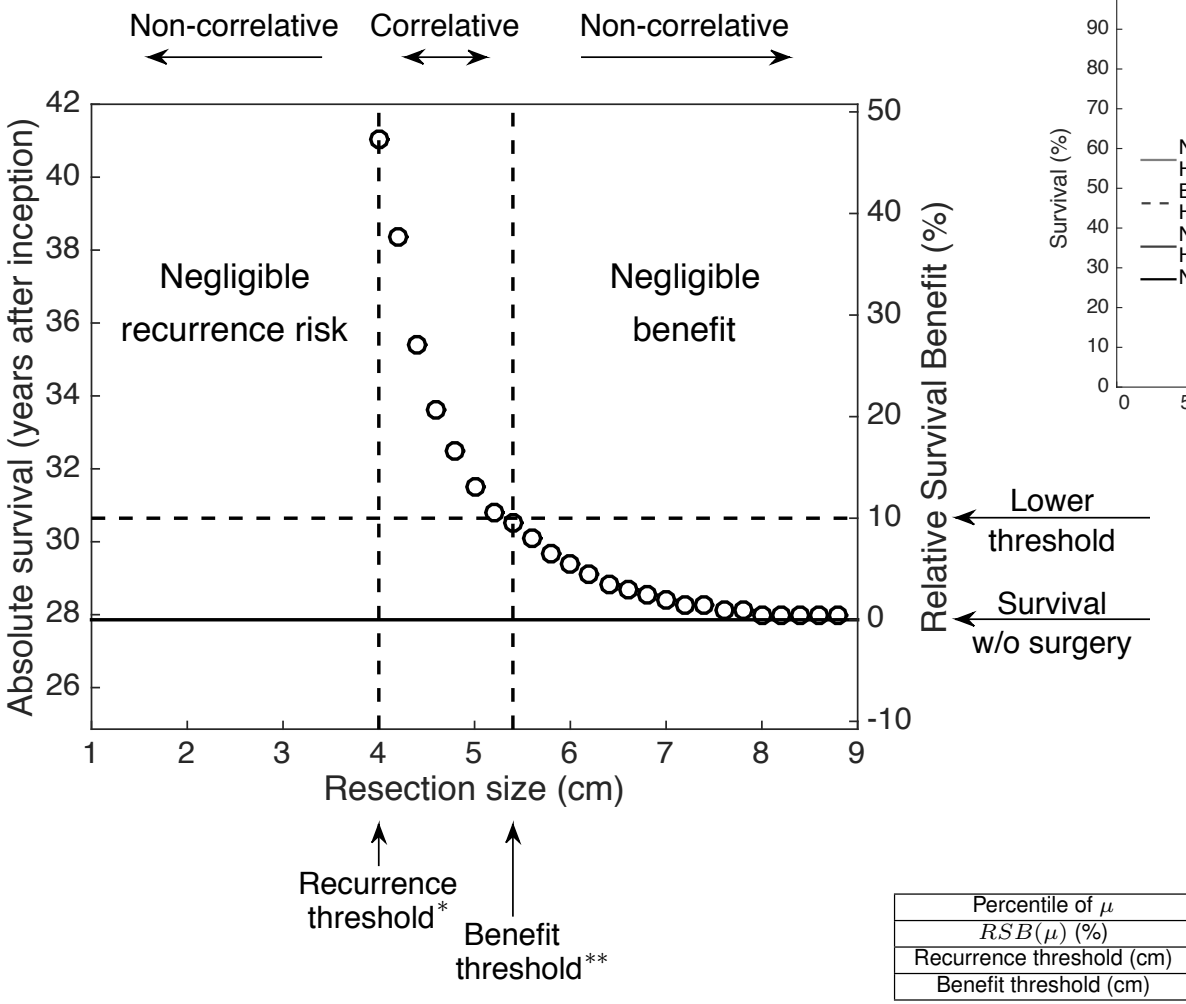
Benzekry, Ebos et al., Cancer Res, 2015

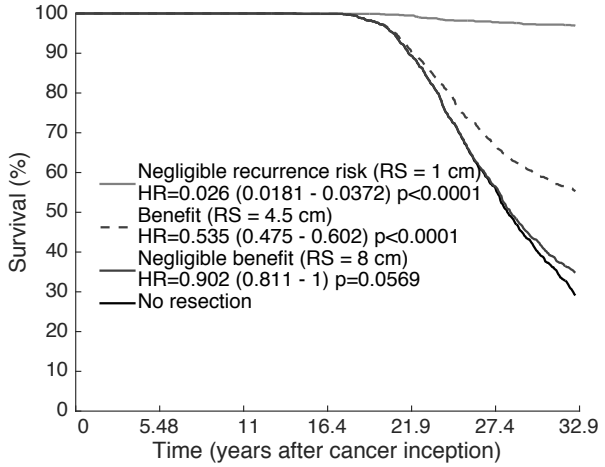
Diagnosis personalization





Nonlinear impact of PT size at surgery on survival





Percentile of μ	10	25	37.5	50	62.5	75	90
$RSB(\mu)$ (%)	∞	∞	∞	47	14	6.4	1.9
Recurrence threshold (cm)	-	6.6	5.2	4	3	2.4	1.4
Benefit threshold (cm)	-	7.8	6.4	5.4	4.6	3.6	2.6

Summary

- A biologically-based, **minimally parameterized**, mathematical model for metastatic development links pre-surgical tumor growth and post-surgical **metastatic burden dynamics**
- **Validation** against preclinical and clinical data sets
- **Same growth law** between PT and mets, **equal probability** among the PT cells of successful establishment of a distant colony and **no secondary dissemination** was a sufficient theory to explain the data
- Inter-animal/individual metastatic propensity can be reduced to variability of one **critical (patient-specific?) parameter μ**
- Nonlinear dependence of survival on primary tumor size at diagnosis suggests existence of a **threshold for efficacy of surgery** and provides a way to estimate its value

Comparison with the Marseille study *without surgery* (remember Niklas's talk)

- Quantitative comparison was **hampered by several technical aspects** (different number of cells injected, different mice strain, different bioluminescence quantification method)
- Keeping these flaws in mind, when using the **same framework** ($d(V) = \mu V^{2/3}$, different growth rates for PT and mets and a different parameterization of the Gompertz $g(v)=av\ln(b/v)$), we found:
  - A **significantly larger value of *a*** (4.91×10^{-2} day^{-1} ± 2.02×10^{-3} versus 7.9×10^{-3} day^{-1} ± 2.5 × 10−3, median ± se), possibly indicative of **post-surgery accelerated growth**
  - But also a significantly **smaller metastatic emission parameter** μ = 7.24 × 10^{-3} ± 8.5 × 10^{-3} cell$^{-2/3}$· day^{-1} versus μ = 6.31 × 10^{-1} ± 4.42 × 10^{-1} cell$^{-2/3}$· day^{-1}

2. Concomitant tumor resistance

Concomitant tumor resistance

- **Inhibition** of secondary **growth** by a primary mass
- Evidenced more than **100 years ago** *Ehrlich, 1906*
- Primary hypothesis: athrepsia (deprivation of **nutrients**)
- Other hypothesis: immune enhancement from the primary. "**Concomitant immunity**"
- 1980's: it happens in immune-deprived mice *Gorelik,, Cancer Res 1983*
- 1990's: Folkman's work on **systemic inhibition of angiogenesis (SIA)** *O'Reilly, Folkman et al., Cell, 1994*
- Others also proposed direct distant inhibition of proliferation

Post-surgery metastatic acceleration

- **Clinically** evidenced from:
  - Patients cases reports *Coffey et al., Excisional surgery for cancer cure: therapy at a cost, Lancet Oncology, 2003*
  - Bimodal relapse hazard (breast) *Retsky et al., Surgery triggers outgrowth of latent distant disease in breast cancer: an inconvenient truth?, Cancers 2010*
- Reported in numerous **animal experiments** since more than 100 years *Marie and Clunet, 1910*
- Could be due to the **surgical trauma** itself
- Experiments suggested other hypothesis, linked with **metastatic dormancy**
- **Concomitant resistance**

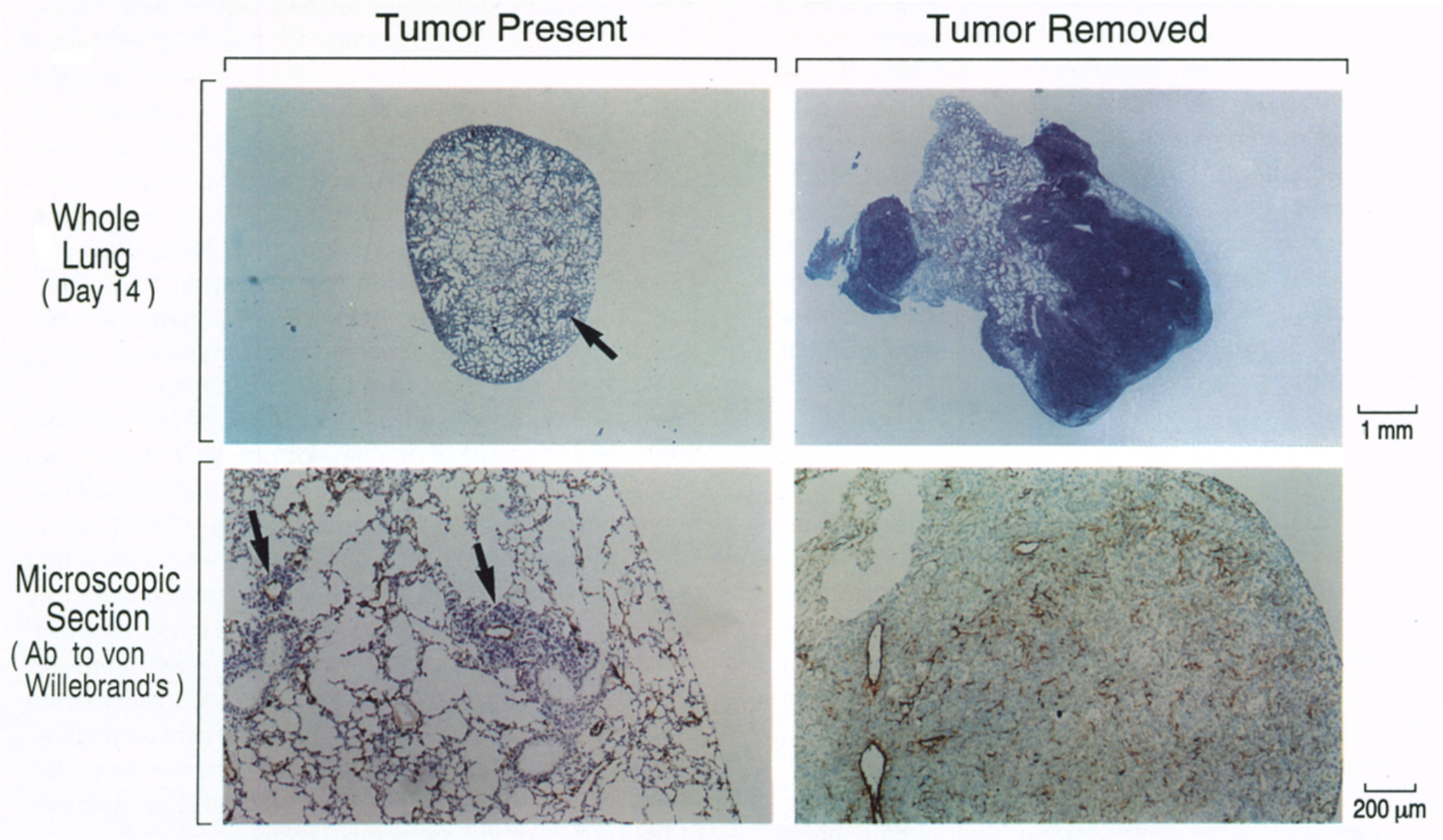


Figure 2. The Presence of a Primary Tumor Is Associated with an Inhibition of Neovascularization and Growth of Its Metastases

O'Reilly, Folkman et al., Angiostatin: A Novel Angiogenesis Inhibitor That Mediates te Suppression of Metastases by a Lewis Lung Carcinoma, *Cell 1994*

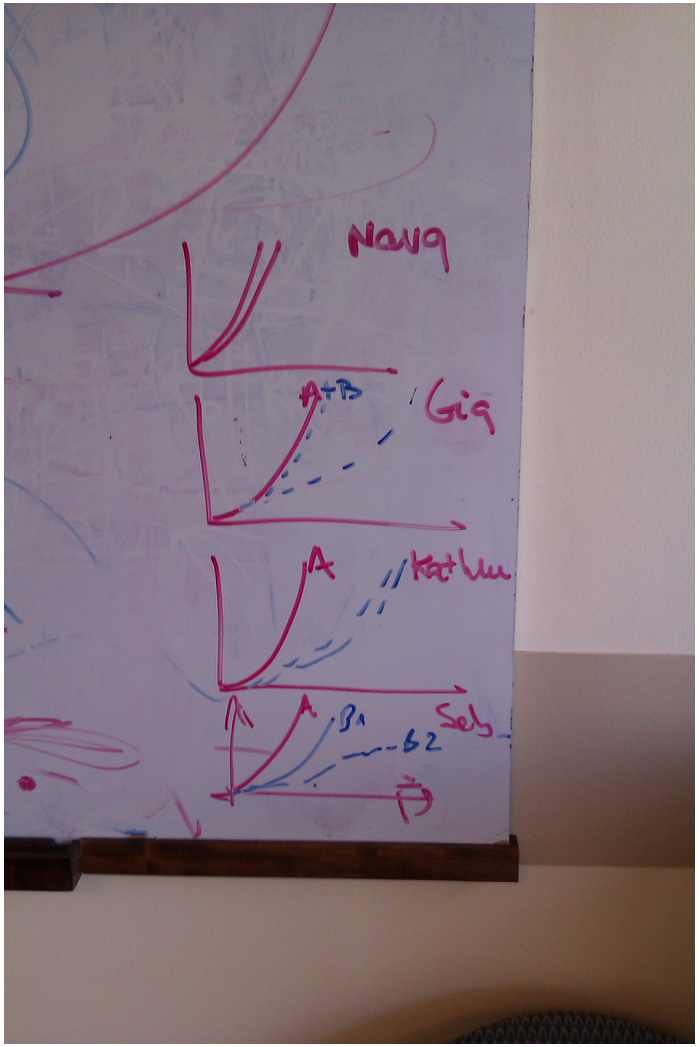
Objectives

- Are we able to give a mathematical description of the **dynamics of concomitant resistance**?
- Minimally parameterized, biologically and data-based mathematical model(s) of the process
- Test different **biological hypotheses** by confronting the (mathematical) theories to the empirical data

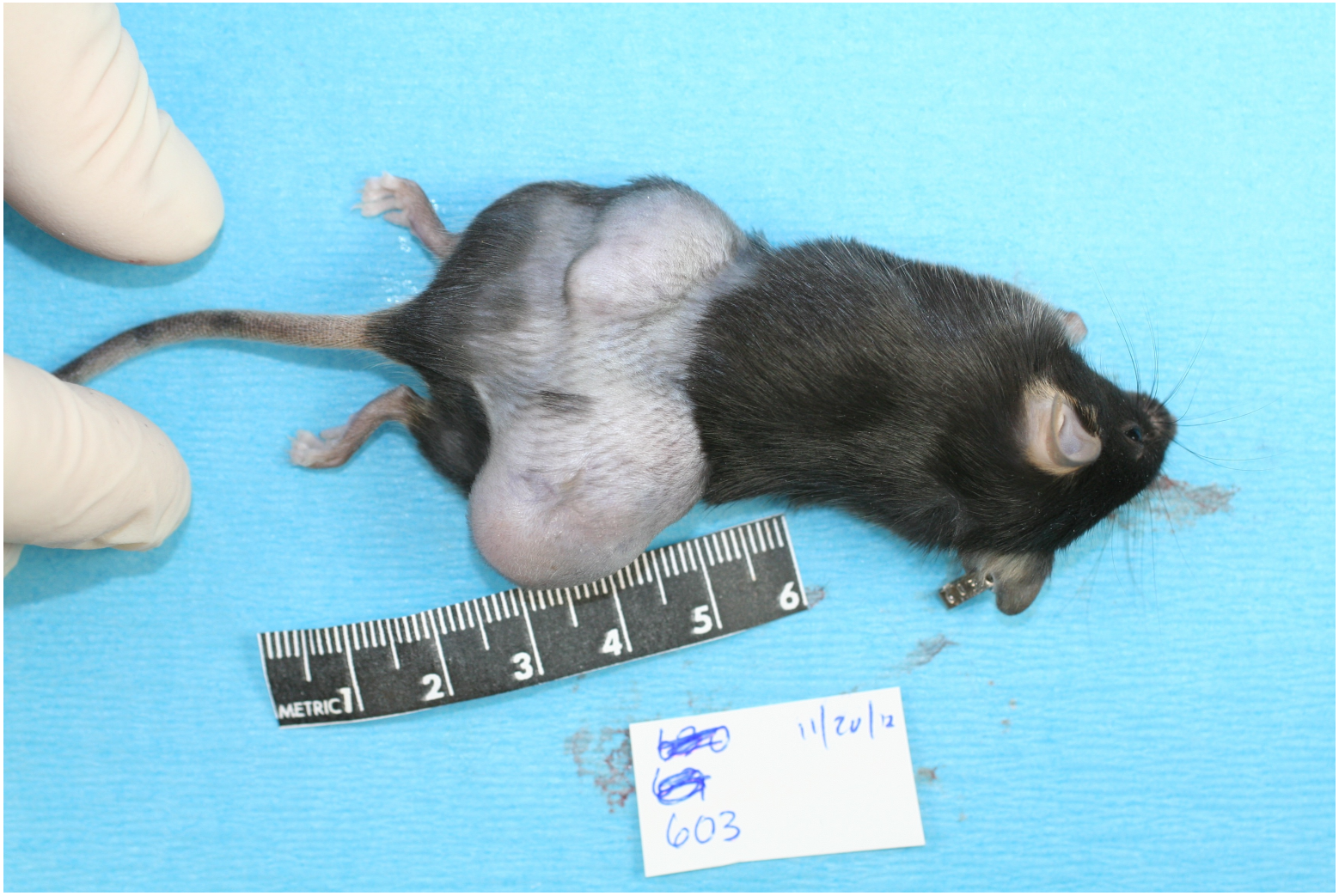
Experiment

- Injection s.c. of **two tumors** of 10^6 LLC cells in C57/BL6 mice
- Two groups
  - Control: only one tumor
  - Group S: **simultaneous** injection of cells in two different sites
- Record **tumor growth in time** at the two sites

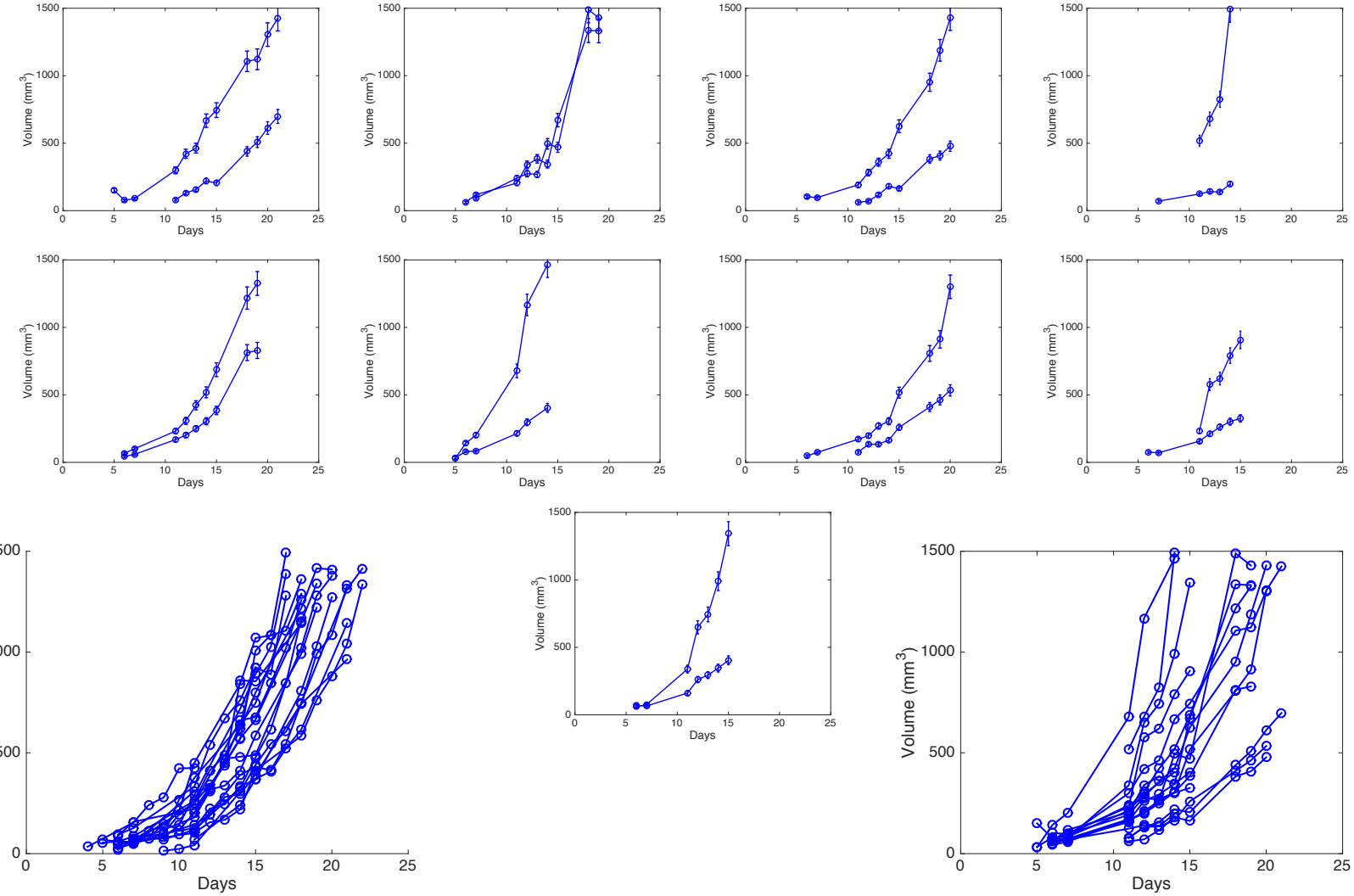
Bets



A mouse with two tumors



Something happens. One tumor has normal volume and the other is smaller

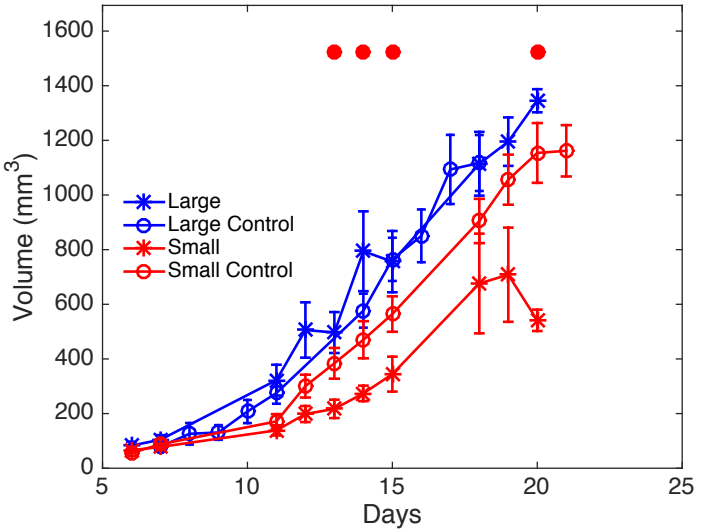


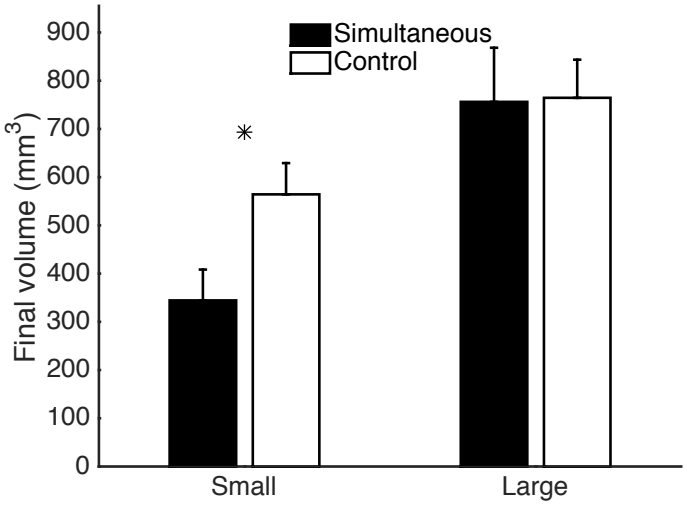
Control group (single tumors)

Double tumors

Statistical confirmation

- We want to test: is the **couple** ($L_S(t)$, $R_S(t)$) statistically different from a **couple** of two tumors growing independently?
- Generate an **artificial group of double independent tumors** by randomly dividing the control group (n=20) in 2 and pairing couples of growth curves from each subgroup
- Compare the large/small tumors of group S to the large/small tumors of the virtual control group

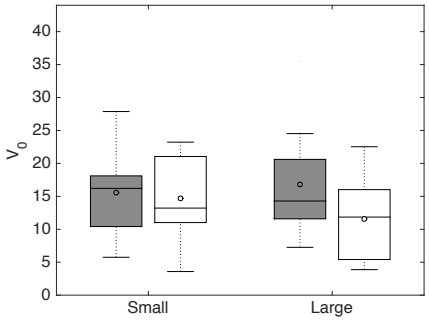


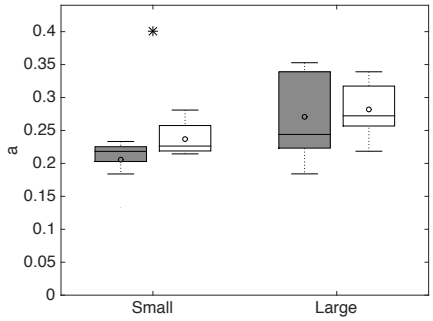


Single-tumor growth models

Exponential V_0

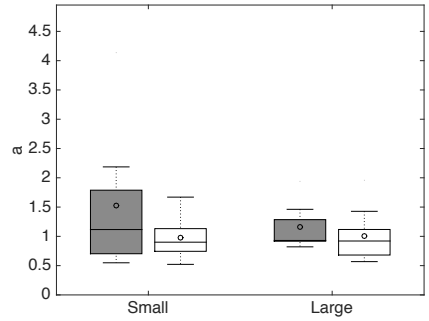
$$\begin{cases} \frac{dV}{dt} = aV \\ V(t=0) = V_0 \end{cases}$$

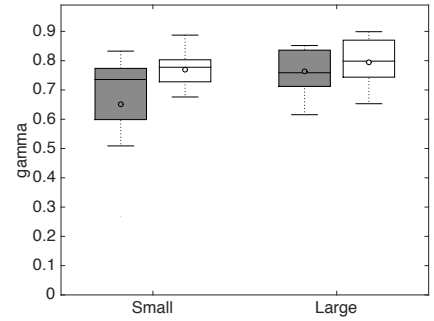




Power law

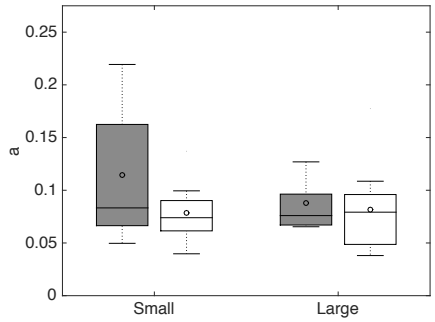
$$\begin{cases} \frac{dV}{dt} = aV^{\gamma} \\ V(t=0) = 1\ mm^3 = 10^6\ cells \end{cases}$$

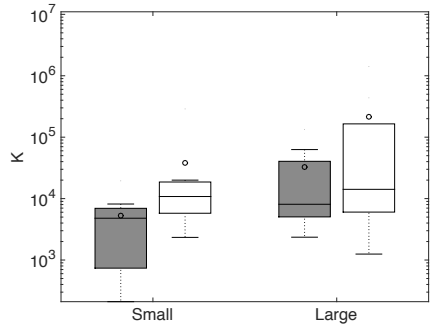


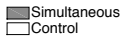


Gompertz

$$\begin{cases} \frac{dV}{dt} = aV \ln\left(\frac{K}{V}\right) \\ V(t=0) = 1\ mm^3 = 10^6\ cells \end{cases}$$







Outline

1. A minimally parameterized model for metastatic dynamics
    A. Open clinical questions
    B. Model
    C. Confrontation to experimental and clinical data sets
    D. Implications for assessment of the metastatic relapse risk and impact of PT size at surgery on survival
2. Concomitant resistance
    A. Biological phenomenon
    B. Data
    *C. **Model***

Two-tumors models

- Asymmetric inhibition

$$\begin{cases} \frac{dV_1}{dt} = aV_1 \ln\left(\frac{K}{V_1}\right), \quad V_1(t=0) = 1 \\ \frac{dV_2}{dt} = aV_2 \ln\left(\frac{K}{V_2}\right) - eI(V_1, V_2), \quad V_2(t=0) = 1 \end{cases}$$

was able to fit the data

Two-tumors models

- Asymmetric inhibition

$$\begin{cases} \frac{dV_1}{dt} = aV_1 \ln\left(\frac{K}{V_1}\right), & V_1(t=0) = 1 \\ \frac{dV_2}{dt} = aV_2 \ln\left(\frac{K}{V_2}\right) - eI(V_1, V_2), & V_2(t=0) = 1 \end{cases}$$

was able to fit the data
but **biologically unrealistic**

Two-tumors models

- Asymmetric inhibition

$$\begin{cases} \frac{dV_1}{dt} = aV_1 \ln\left(\frac{K}{V_1}\right), & V_1(t=0) = 1 \\ \frac{dV_2}{dt} = aV_2 \ln\left(\frac{K}{V_2}\right) - eI(V_1, V_2), & V_2(t=0) = 1 \end{cases}$$

REJECTED

was able to fit the data
but **biologically unrealistic**

- Symmetric direct inhibition

$$\begin{cases} \frac{dV_1}{dt} = aV_1 \ln\left(\frac{K}{V_1}\right) - eI_1(V_1, V_2), & V_1(t=0) = 1 \\ \frac{dV_2}{dt} = aV_2 \ln\left(\frac{K}{V_2}\right) - eI_2(V_1, V_2), & V_2(t=0) = V_{0,2} \end{cases}$$

  - Same growth and inhibition parameters for V_1 and V_2
  - Symmetry: $I_1(V_2,V_1)=I_2(V_1,V_2)$
  - Three possibilities for the shape of $I_1(V_1,V_2)$ shown here: V_1V_2 (1), V_2 (2), $(V_1+V_2)V_1$ (3)

Hypothesis for the origin of dissymmetry between V_1 and V_2

→ comes from the initial number of cells that « take »

Two-tumors models

- Indirect (angiogenesis-related) inhibition

$$\begin{cases} \frac{dV_1}{dt} = aV_1 \ln\left(\frac{K_1}{V_1}\right), & V_1(t=0) = 1 \\ \frac{dK_1}{dt} = bV_1 - dV_1^{2/3}K_1 - eI_1(V_1, V_2), & K_1(t=0) = K_0 \\ \frac{dV_2}{dt} = aV_2 \ln\left(\frac{K}{V_2}\right), & V_2(t=0) = V_{0,2} \\ \frac{dK_2}{dt} = bV_2 - dV_2^{2/3}K_2 - eI_2(V_1, V_2) & K_2(t=0) = K_0 \end{cases}$$

- Based on the Hahnfeldt model

  Hahnfeldt et al., Cancer Res, 1999 with

  dynamic carrying capacity *K*

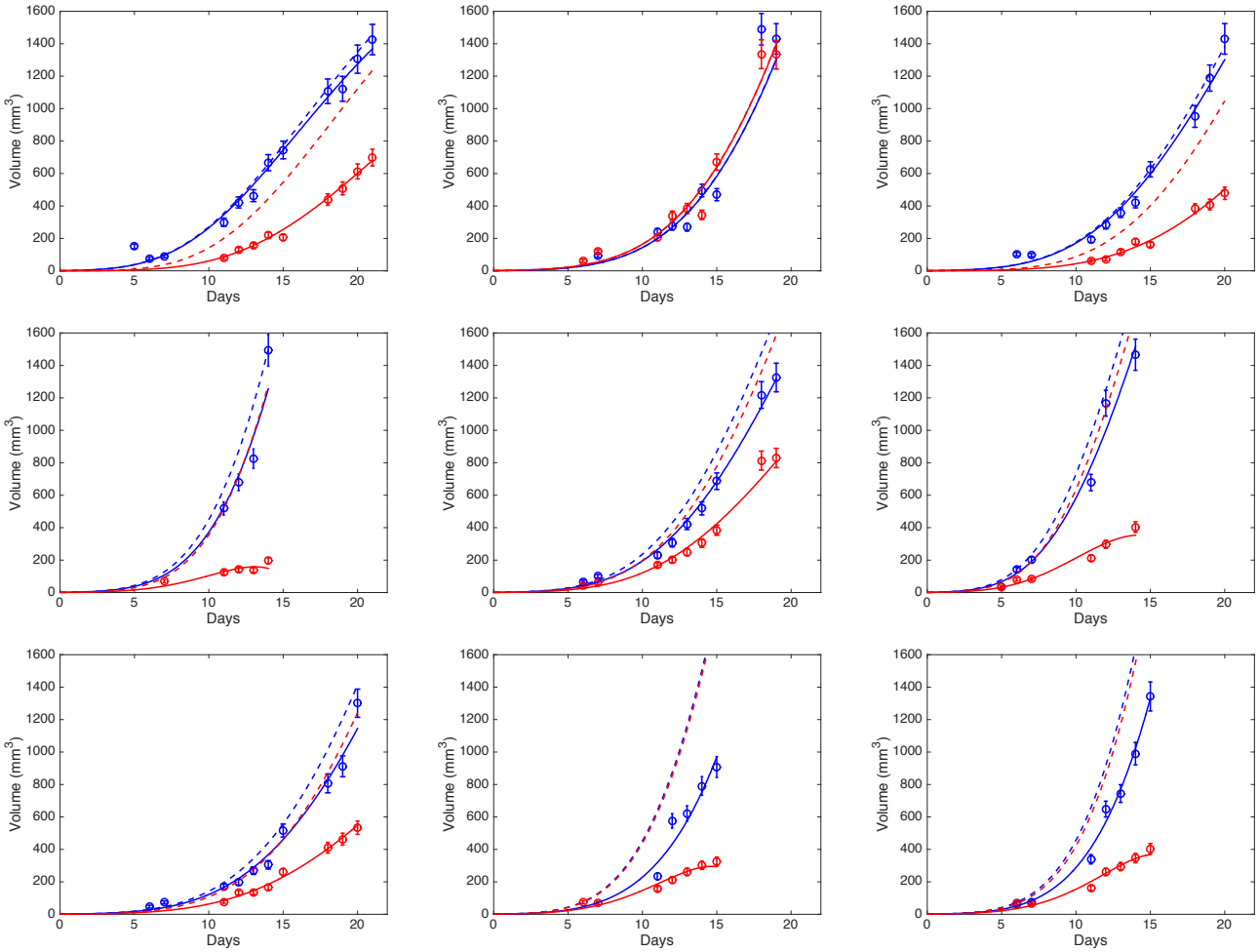
- Parameters *d* and K_0 were fixed

- Competition (athrepsia hypothesis)

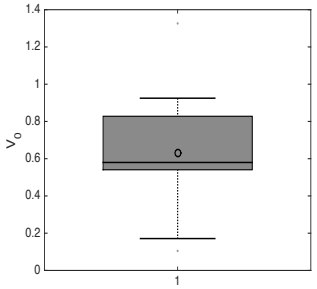
$$\begin{cases} \frac{dV_1}{dt} = aV_1 \ln\left(\frac{K}{V_1+V_2}\right), & V_1(t=0) = 1 \\ \frac{dV_2}{dt} = aV_2 \ln\left(\frac{K}{V_1+V_2}\right), & V_2(t=0) = V_{0,2} \end{cases}$$

- One parameter (degree of freedom) less than the other models

Direct inhibition (2) fit

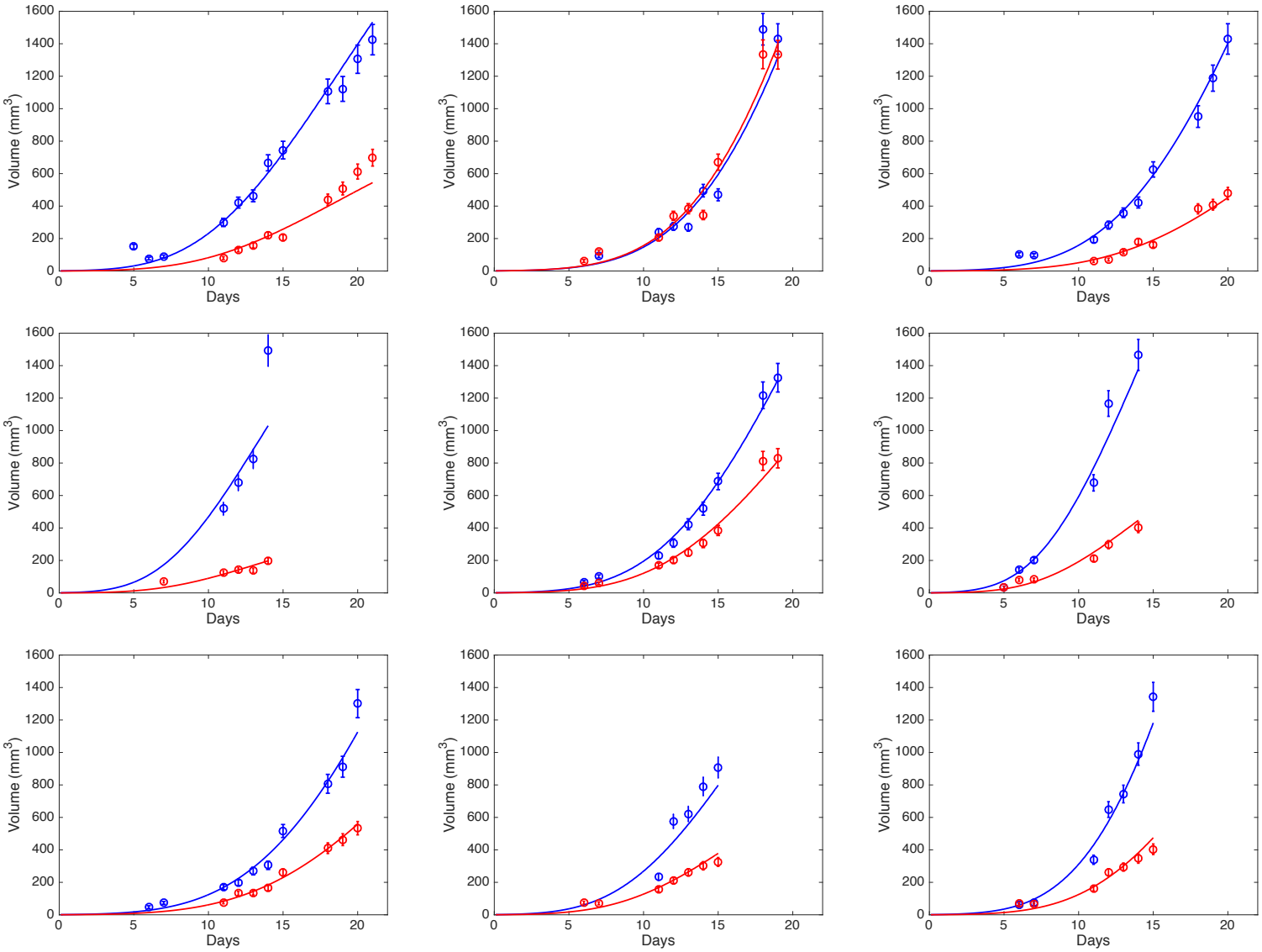


Model
Model with e=0



- Gives **satisfactory fit**
- Behavior when $e = 0$ is realistic
- Kinetic differences between V_1 and V_2 are **mostly due to inhibition** (and not to difference in $V_{0,2}$)

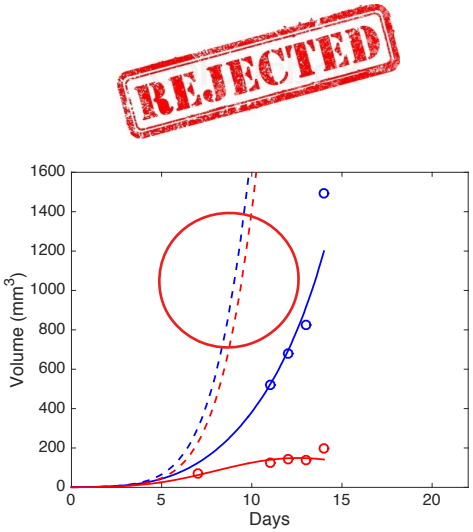
Competition model



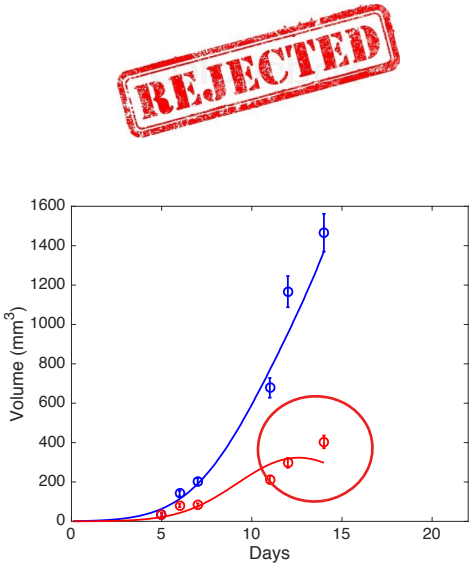
- Also gives **satisfactory fit** (and thus, possible explanatory hypothesis)

Models able to fit

- Criterias for rejection of a model:
  - Inaccurate visual goodness-of-fit
  - Yielding biologically unrealistic behavior when *e = 0*



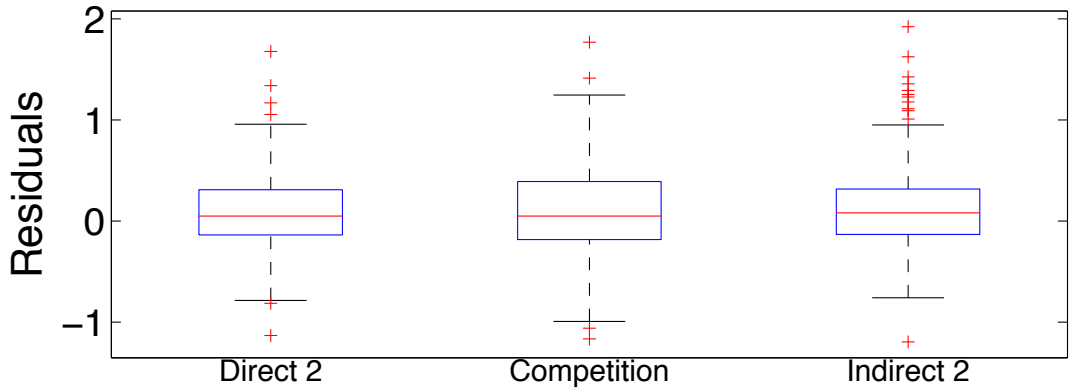
Index	1	2	3
I_1	V_2V_1	V_2	$(V_1+V_2)V_1$
I_2	V_1V_2	V_1	$(V_1+V_2)V_2$
Direct Inhibition	x	o	x
Indirect Inhibition	x	o	x
Competition	o		



Goodness-of-fit metrics

Model	SSE	AIC	RMSE	R2	p > 0.05	#
Direct 2	**0.183(0.102 - 0.388)[1]**	**-17.6(-31.2 - -6.08)[1]**	**0.428(0.324 - 0.63)[1]**	**0.973(0.934 - 0.991)[1]**	100	4
Competition	0.241(0.102 - 0.398)[2]	-15.8(-33 - -3.96)[2]	0.492(0.326 - 0.635)[2]	0.956(0.871 - 0.99)[3]	100	3
Indirect 2	0.273(0.151 - 0.506)[3]	-10.9(-24.1 - -1.58)[3]	0.523(0.393 - 0.715)[3]	0.967(0.934 - 0.986)[2]	100	4

SSE = Sum of Squared Errors, AIC = Akaike Information Criterion, RMSE= Root Mean Squared Errors



Parameter values/identifiability

Model	Par.	Unit	Median value (CV)	NSE (%)	95% CI
Direct 2	a	-	0.0957 (21.9)	11.3	
	K	-	1.02e+04 (90.2)	46.5	
	$V_{0,2}$	-	0.58 (64.4)	8.9	
	e	-	0.048 (91.5)	2.35	**(0.044, 0.052)**
Competition	a	-	0.0988 (28.8)	11.2	
	K	-	8.52e+03 (82.2)	42.1	
	$V_{0,2}$	-	0.402 (63.1)	12.5	
Indirect 2	a	-	0.206 (35.8)	7.81	
	b	-	18.7 (32.1)	13.2	
	$V_{0,2}$	-	0.685 (45.3)	11.8	
	e	-	4.07 (57.8)	1.36	**(3.96, 4.18)**

NSE = Normalized Standard error

CV = Coefficient of Variation

Summary

- In mice bearing two tumors implanted simultaneously, **tumor growth is suppressed in one of the two tumors**
- New quantitative and **identifiable** mathematical models of tumor-tumor growth interactions were developed and able to match the data.
- Possible explanation of dissymmetry: difference in number of cells that take
- Based only on tumor growth kinetics we could not clearly discriminate between **three possible theories**: competition, direct or indirect (angiogenesis) inhibition
- But we could discriminate the shape of the inhibition term: $I_1(V_1,V_2) = V_2$

Perspective: integrate this model for tumor-tumor interactions into the **organism-level** for the dynamics of the metastatic population

Acknowledgements

- Preclinical data of ortho-surgical animal models of metastases

John Ebos's laboratory, Roswell Park Cancer Institute, Buffalo, NY, USA

- Two-tumors study

Center of Cancer and Systems Biology, Boston, MA, USA

Claire Lamont, P. Hahnfeldt, L. Hlatky

Thank you for your attention!!