A mathematical model separates quantitatively the cytostatic and cytotoxic effects of a HER2 tyrosine kinase inhibitor

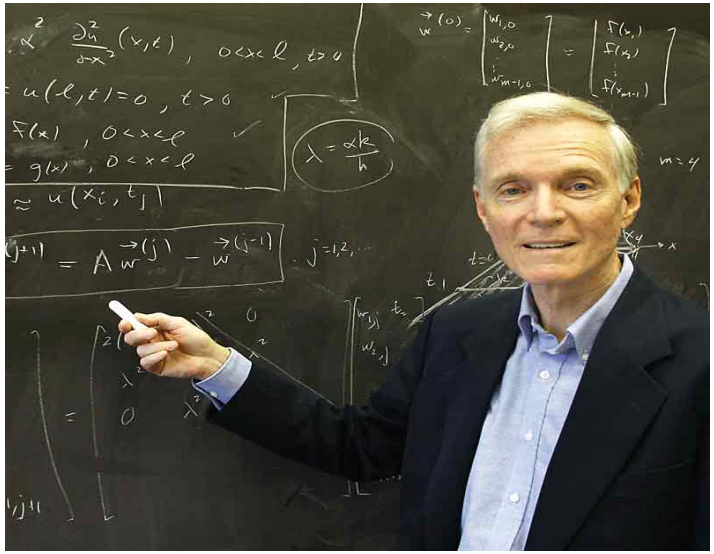
Peter Hinow

Institute for Mathematics and its Applications
University of Minnnesota
Minneapolis, MN 55455
USA
hinow@ima.umn.edu

Winter Workshop on Pharmacokinetics-Pharmacodynamics of Anticancer Drugs, Paris, December 18th-19th, 2008

Collaborators

Emily Wang,
now at City of Hope

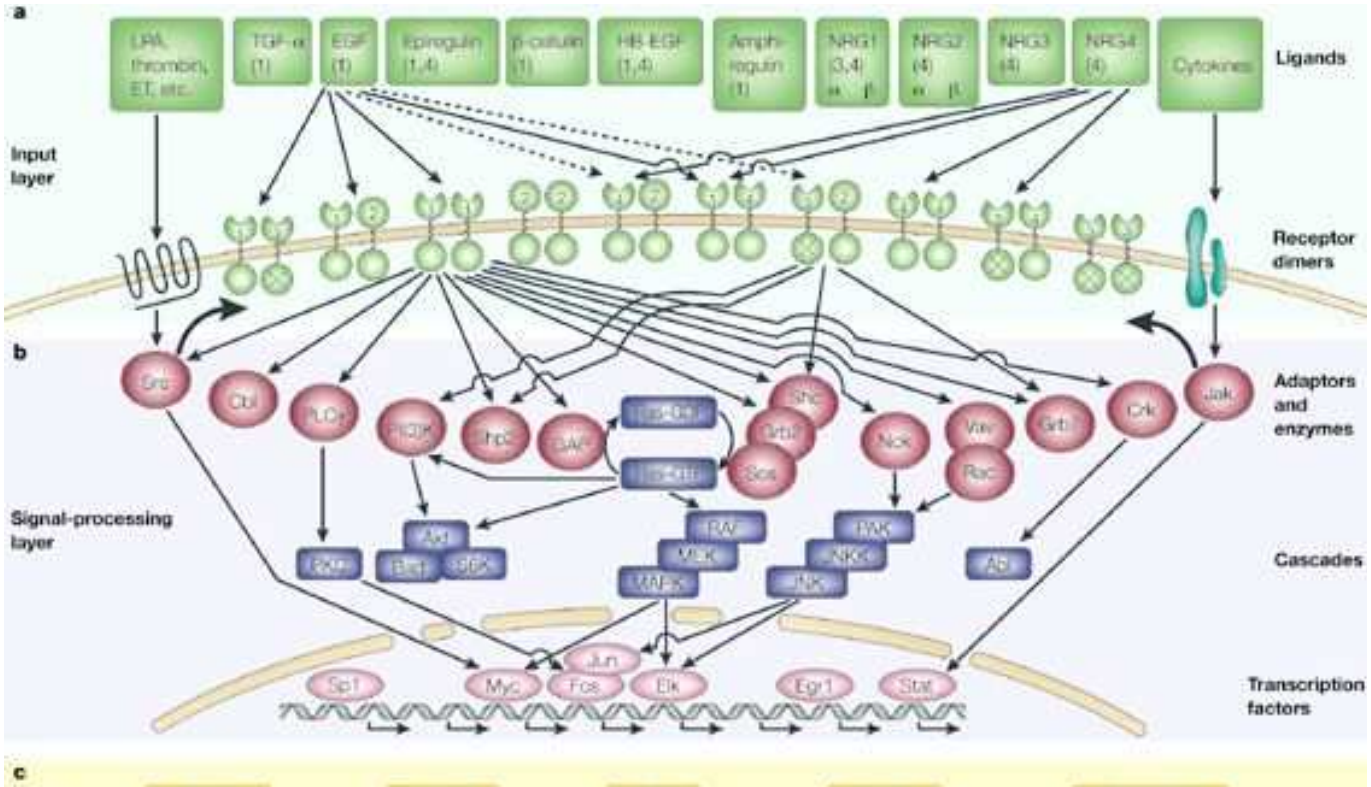


Glenn Webb, Vanderbilt University

Outline of talk

- Introduction - the role of HER2 and lapatinib
- Experimental methods
- Construction of the mathematical model and parametrization
- Results
- Conclusions

Biology of the HER2 (ErbB2) receptor I



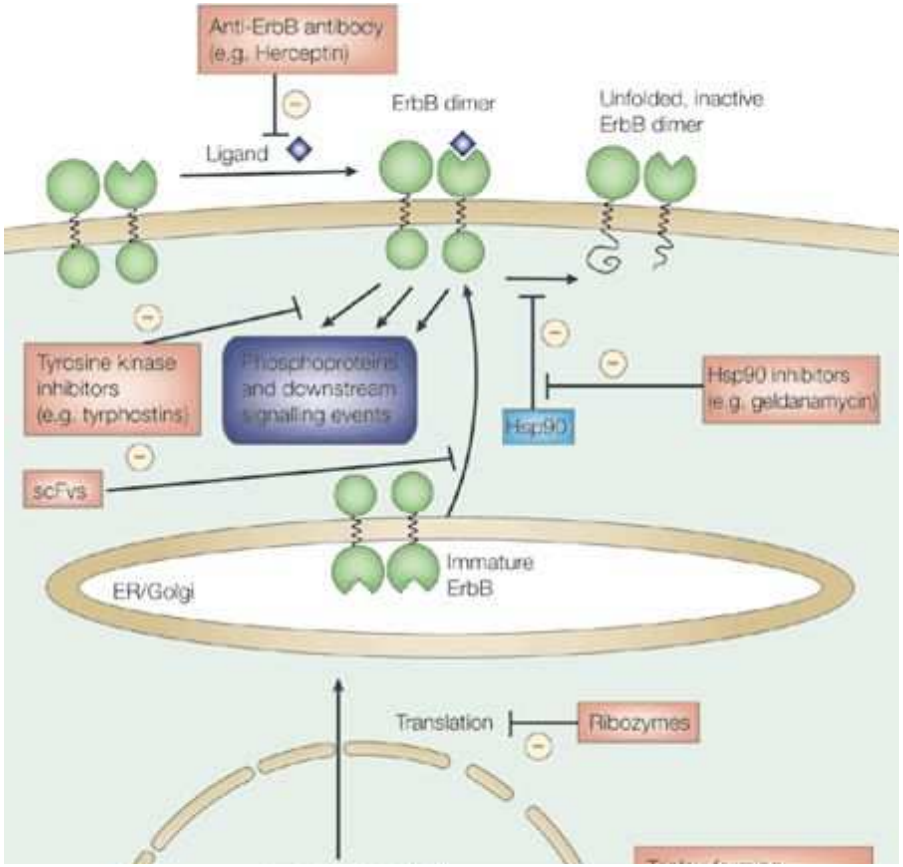
Yarden & Sliwkowski, *Nat. Rev. Mol. Cell Biol.* **2**:127

Receptor tyrosine kinases play a crucial role in growth and differentiation of both normal and malignant mammary epithelial cells.

Biology of the HER2 (ErbB2) receptor II

- HER2 is a potent signal amplifier via heterodimerizing with other HE receptors.
- HER2 is overexpressed in 20-30 % of breast cancers.
- Overexpression of HER2 is associated with shorter survival of cancer patients (3 years vs. 6-7 years).

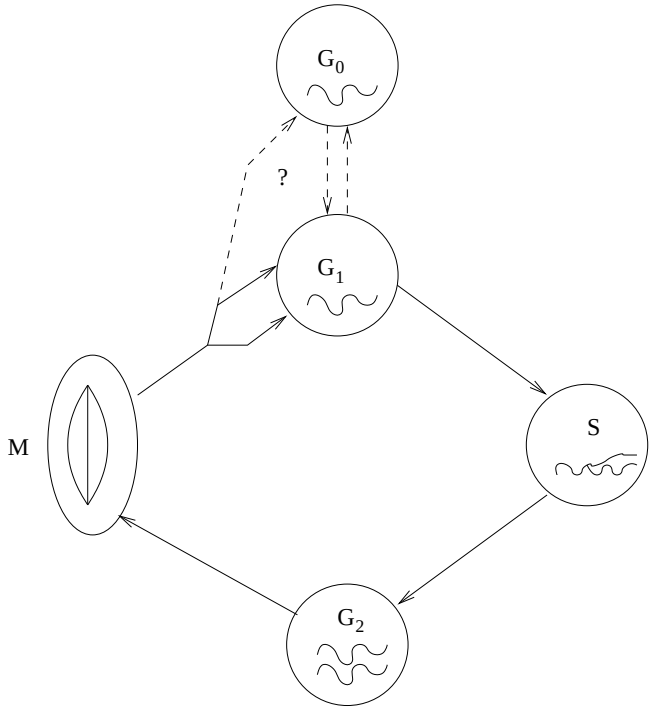
The role of lapatinib



Yarden & Sliwkowski, *Nat. Rev. Mol. Cell Biol.* **2**:127

Lapatinib binds to the ATP binding site and blocks the receptor's catalytic activity.

Cell cycle and drug action



Drugs can

- slow progression of cells through specific phases of the cell cycle (*cytostatic* effects), and
- kill cells in specific phases of the cell cycle (*cytotoxic* effects).

Goals of our study

We wanted to

- separate quantitatively cytostatic and cytotoxic effects of lapatinib,
- investigate the cell cycle specificity of the cytostatic action, and
- determine temporal dynamics and dose-dependence of drug effects.

Related works

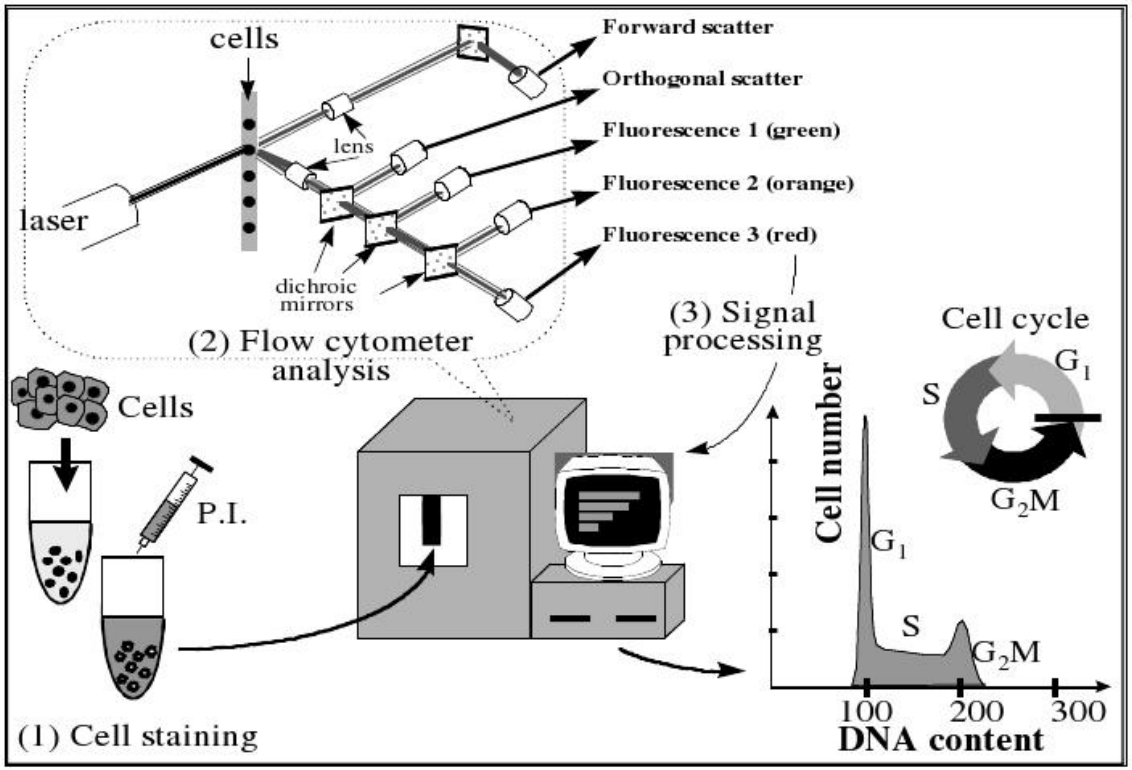
A very thin list . . .

- Ubezio et al. (1998, 2004, 2006) investigate phase-specific cytotoxic and cytostatic effects of cisplatin, melphalan and topotecan – discrete partition of cell cycle into compartments, discrete progression of time
- Świerniak and Kimmel (and others, 2003) propose compartmental ODE models and apply methods from otpimal control theory
- Kheifetz, Kogan and Agur (*M3AS* **16**:1155) predict the effect of periodic treatments with cycle-specific cytotoxic drugs using properties of positive compact operators (linear PDE model)

Experimental procedures

- MCF10A/HER2 cells are grown in well plates over 6 days and exposed to constant concentrations of drug.
- The cell numbers are counted using a Coulter counter.
- The cell cycle distribution is analyzed using flow cytometry.
- Cells are stained for markers of proliferation and apoptosis (immunoflurescence assay).

Flow cytometry



Ubezio, *Discrete Contin. Dyn. Syst. Ser. B* **4**:323

The cell population can be sorted according to the DNA content of each cell.

The mathematical model

- We introduce structured populations of proliferating and nonproliferating cells.
- Nonproliferating cells became necessary as we observed a saturation of the initially exponential growth after 5 days.
- Cells are characterized by their position in the cell cycle, a variable we call the *maturity* of a cell. It can be interpreted for example as cell size or DNA content.

Variables of the model

Let $t \geq 0$ denote time since the begin of experiment and $a \in [0, a_m]$ denote maturity (where a_m is the maximal maturity). In the absence of cytostatic effects a coincides with the time since the last mitosis.

Let $p(a, t)$ and $n(a, t)$ denote the densities of proliferating and nonproliferating cells, respectively.

Variables of the model

The total number of cells is

$$M(t) = \int_0^{a_m} (p(a,t) + n(a,t))\,\mathrm{d}a.$$

Proliferating cells become nonproliferating as the total cell number exceeds a critical size. Nonproliferating cells have a maturity, the point at which they exited the cell cycle. Their number is

$$N(t) = \int_0^{a_m} n(a,t)\,\mathrm{d}a.$$

Model equations for an exponentially growing population

The linear model is given by

$$\underbrace{\frac{\partial}{\partial t}p(a,t) + \frac{\partial}{\partial a}p(a,t)}_{\textit{aging of cells}} = \underbrace{-\beta(a)p(a,t)}_{\textit{loss through mitosis}},$$

$$p(0,t) = \underbrace{2\int_0^{a_m} \beta(a)p(a,t)\,\mathrm{d}a}_{\textit{binary renewal}},$$

$$p(a,0) = p_0(a).$$

Mitosis occurs at a rate β that depends on maturity.

Model equations for untreated cells

The model with nonproliferating cells

$$\begin{aligned}
\frac{\partial}{\partial t}p(a,t) + \frac{\partial}{\partial a}p(a,t) &= -(\beta(a) + \tilde{\mu}(a, M(t)))p(a,t), \\
\frac{\partial}{\partial t}n(a,t) &= \tilde{\mu}(a, M(t))p(a,t), \\
p(0,t) &= 2\int_0^{a_m} \beta(a)p(a,t)\,\mathrm{d}a, \\
p(a,0) &= p_0(a), \\
n(a,0) &= 0.
\end{aligned}$$

The function $\tilde{\mu}$ realizes the transition from the proliferating to the nonproliferating class, depending on the total cell number $M(t)$.

Model equations for treated cells

$$\left(\frac{\partial}{\partial t} + \frac{\partial}{\partial a}(1 - \delta(a,t))\right) p(a,t) = -(\beta(a) + \tilde{\mu}(a, M(t)) + \epsilon(t))p(a,t),$$

$$\frac{\partial}{\partial t} n(a,t) = \tilde{\mu}(a, M(t))p(a,t) - \epsilon(t)n(a,t),$$

$$(1 - \delta(0,t))p(0,t) = 2\int_0^{a_m} \beta(a)p(a,t)\,\mathrm{d}a,$$

$$p(a,0) = p_0(a),$$

$$n(a,0) = 0.$$

The effects of the drug are

- decreased maturation velocity $1 - \delta(a,t)$, dependent on maturity a
- additional mortality $\epsilon(t)$.

Characteristic equations

The characteristic curves are given by the ordinary differential equation

$$\frac{da}{dt} = 1 - \delta(a, t),$$

with $0 \leq \delta(a, t) \leq 1$.

In the absence of cytostatic effects, we have $\delta = 0$ and $a - t = const$.

What are the outputs of the model?

Apart from the total population $M(t)$ the model predicts the fractions of cells in any of the stages of the cell cycle.

$$G_1(t) = \int_0^{a_{G_1}} (p(a,t) + n(a,t))\,\mathrm{d}a \Big/ M(t),$$

$$S(t) = \int_{a_{G_1}}^{a_S} (p(a,t) + n(a,t))\,\mathrm{d}a \Big/ M(t),$$

$$G_2(t) = \int_{a_S}^{a_m} (p(a,t) + n(a,t))\,\mathrm{d}a \Big/ M(t),$$

Here a_{G_1} and a_S are suitably chosen boundaries between the age compartments.

Parameters to choose

Fixed for all scenarios are

- the maturity space $[0, a_m]$ and boundaries between phases a_{G_1} and a_S,
- the birth rate $\beta(a)$, and
- the crowding function $\tilde{\mu}$ and threshold M_0.

Depending on drug dose we choose

- delay δ, and
- death rate ϵ.

Choice of the age space

Let

$$\begin{aligned} a_{G_1} &= 7, \\ a_S &= 11, \\ a_m &= 30. \end{aligned}$$

If no cytostatic effects are present, cells age as time progresses. Then these values are *hours after mitosis*. The control scenario supports our choices.

Choice of the proliferation rate

The distribution of intermitotic times ϕ is a shifted Γ-distribution $\Gamma(a-15;2,2)$ with mean $19\,h$ (Dibrov et al. *Math. Biosci.* **66**:167–185).

The corresponding age-dependent proliferation rate is given by

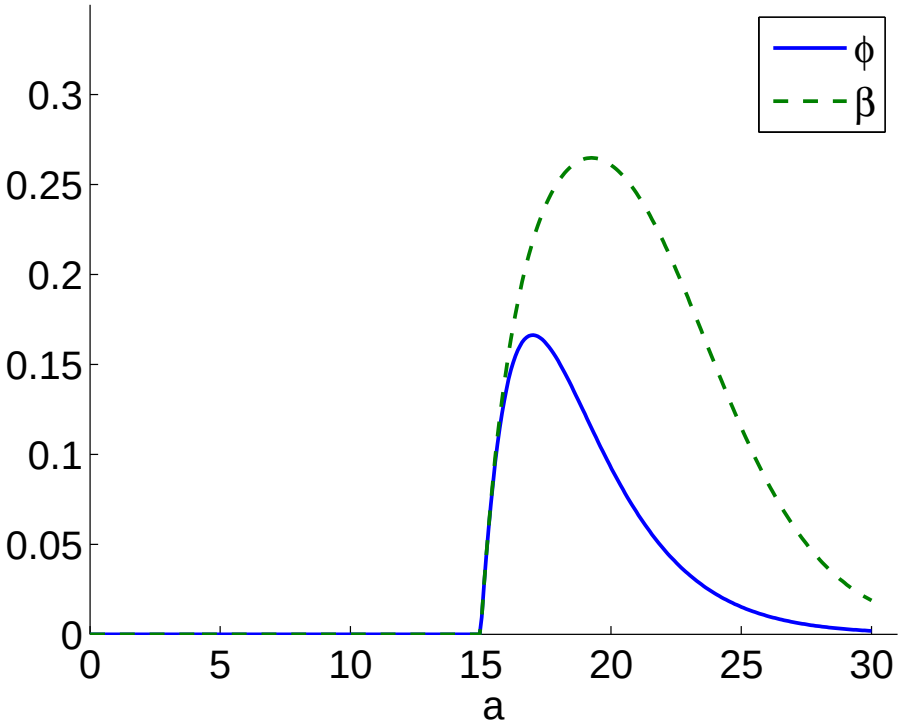
$$\beta(a) = \frac{\phi(a)}{\alpha(a)},$$

where

$$\alpha(a) = \int_a^\infty \phi(s)\,\mathrm{d}s$$

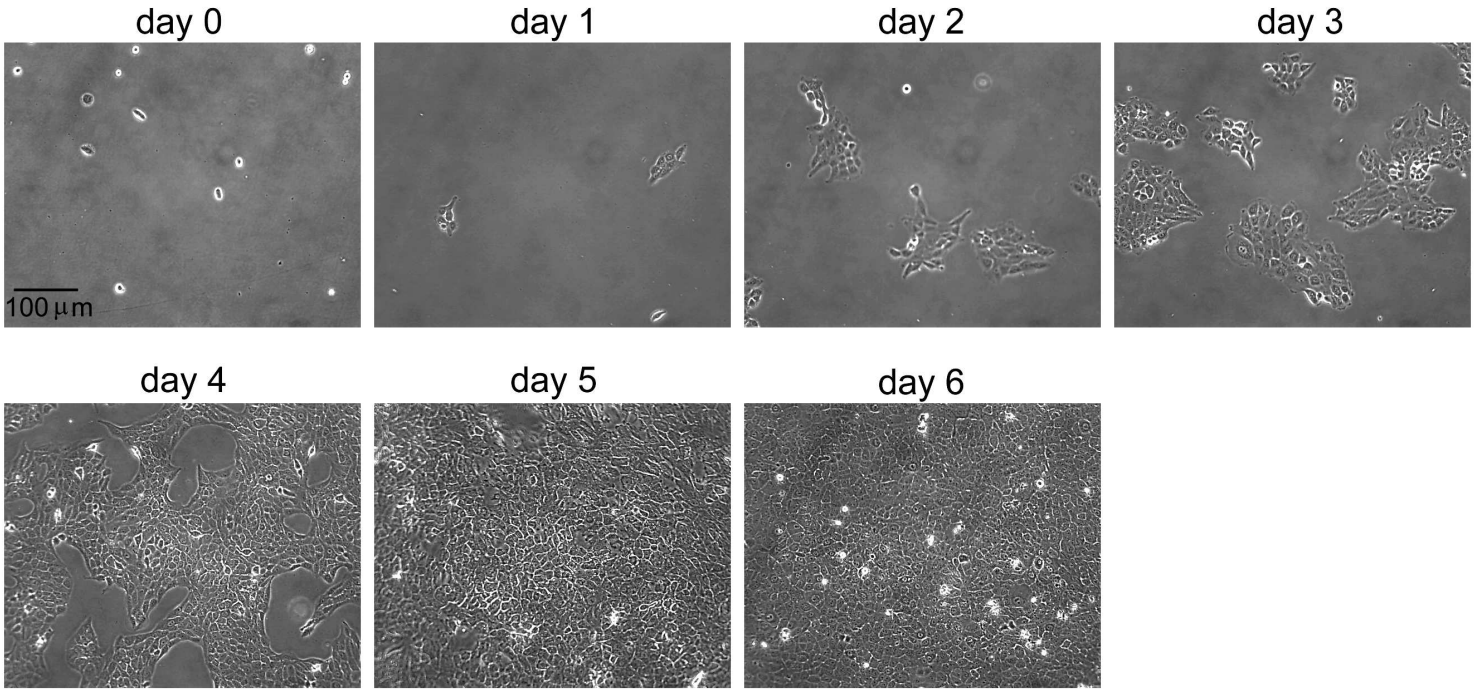
is the fraction of cells that reach age a without division.

Choice of the proliferation rate



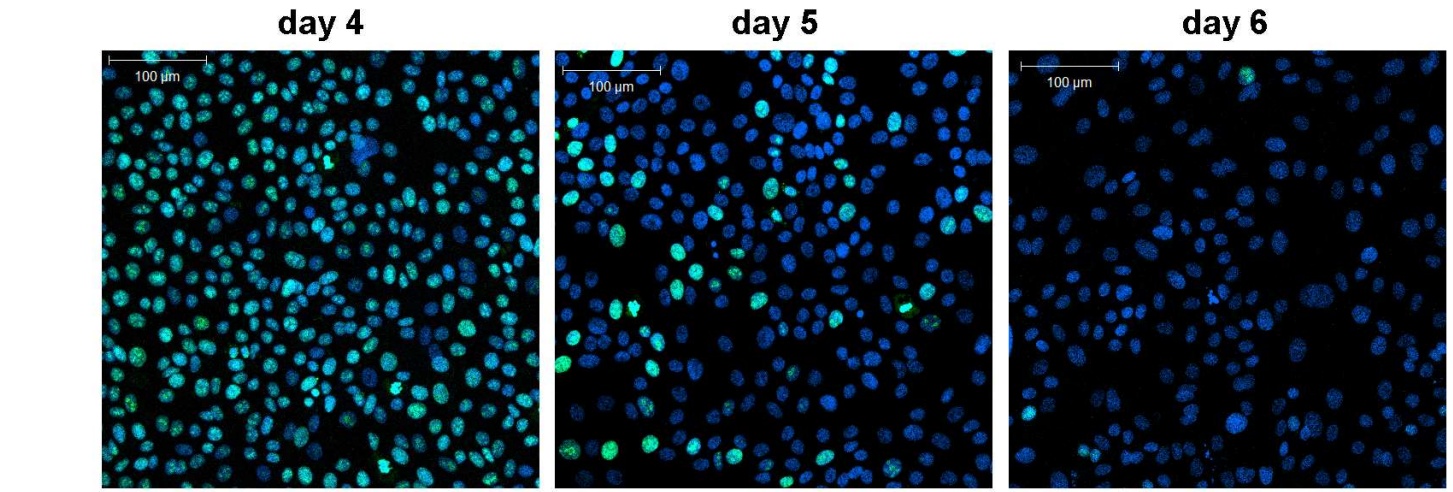
blue: distribution of intermitotic times, red: corresponding proliferation rate

Control scenario



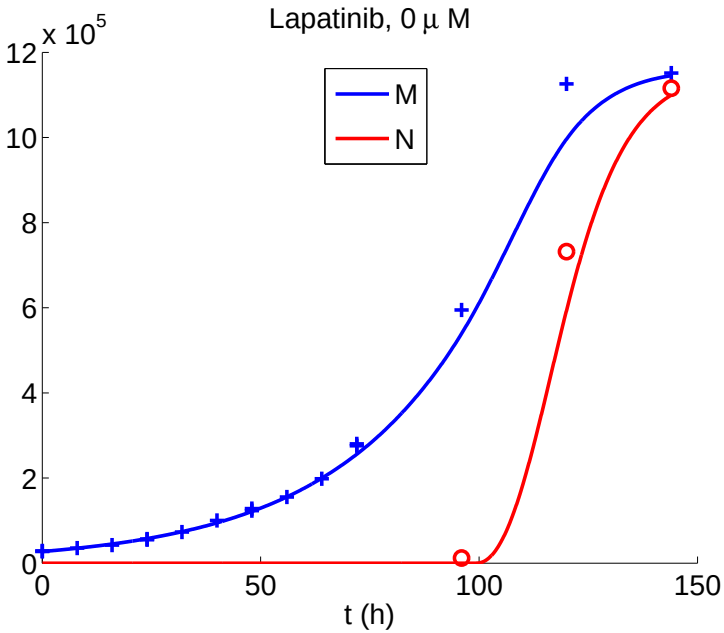
Phase contrast images of untreated cells on different days. Cells are growing in monolayer culture until they reach contact inhibition.

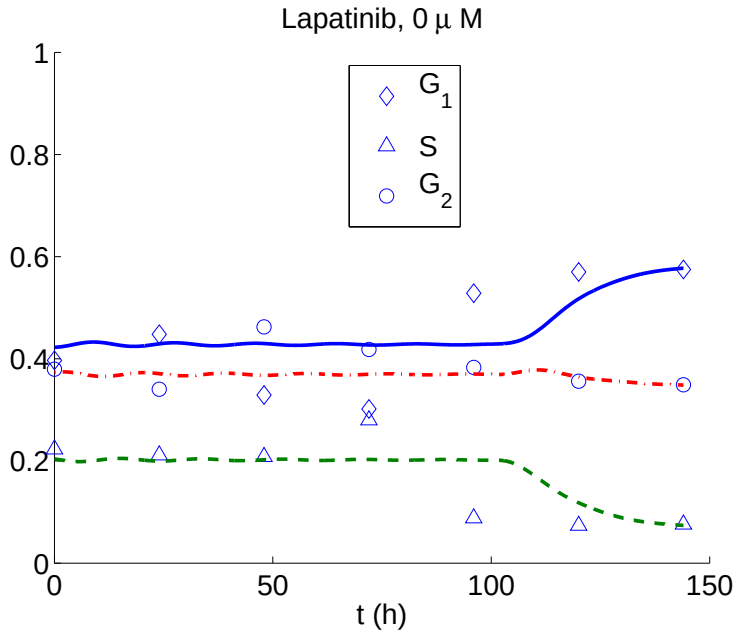
Control scenario



Staining of untreated cells. Blue – all nuclei, green – marker of proliferation Ki-67.

Control scenario

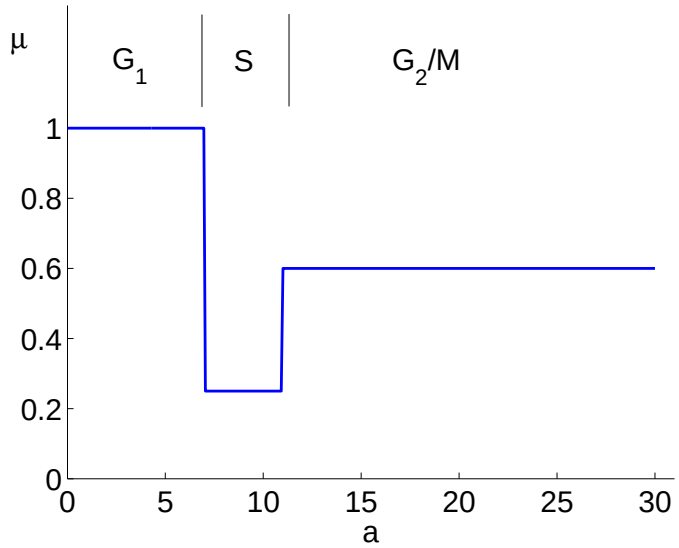




As the number of cells exceeds $M_0 = 6 \cdot 10^5$ we see a delayed growth and a change in the steady-state cell cycle distribution (discrete symbols – experimental data, continuous curves – model predictions).

Choice of transition to nonproliferating class

$$\tilde{\mu}(a, M) = \mu(a) \begin{cases} c(M - M_0) & \text{if } M \geq M_0 \\ 0 & \text{otherwise} \end{cases} .$$



A cell that has entered S-phase will finish it and therefore is less prone to entering nonproliferation.

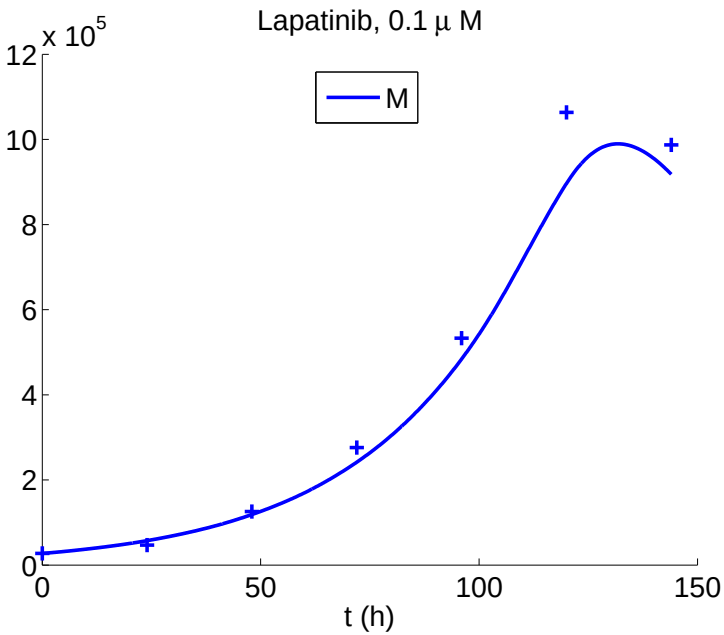
Cell-cycle specificity of delay effect

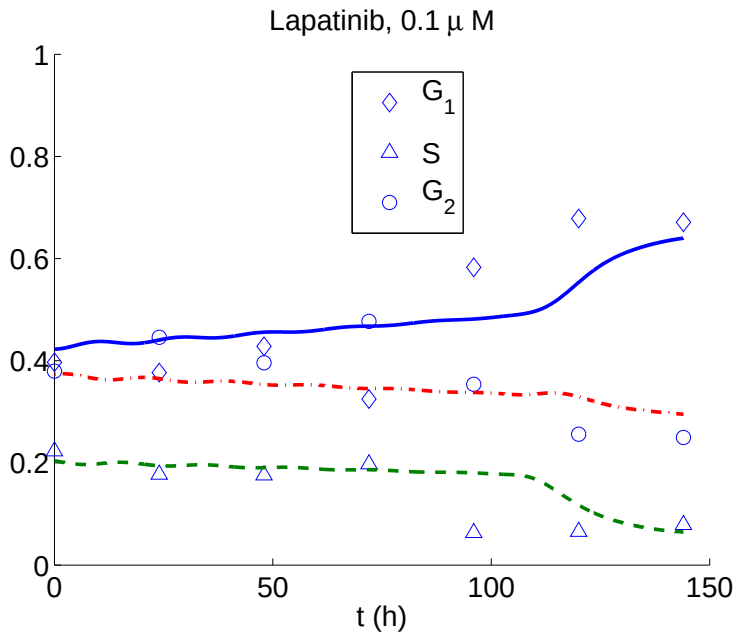
We want to test the hypothesis that lapatinib affects chiefly cells in G_1 phase. Moreover, the cytostatic effects increase with time,

$$\delta(a,t) = \delta_{G_1} \frac{t}{T} \begin{cases} 1 & \text{if } 0 \leq a \leq a_{G_1} \\ 0 & \text{otherwise.} \end{cases}$$

A sudden onset of cytostatic effects would cause oscillations in the percentages that are not seen in the experimental data.

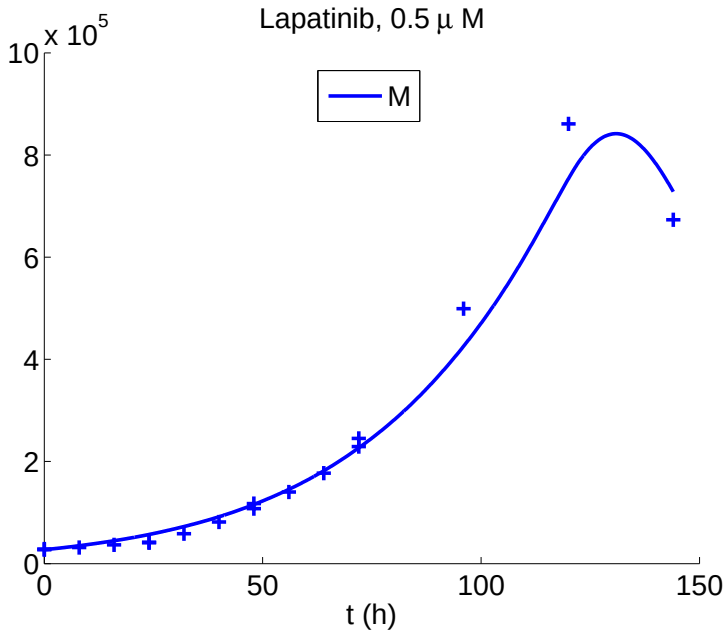
0.1 μM lapatinib

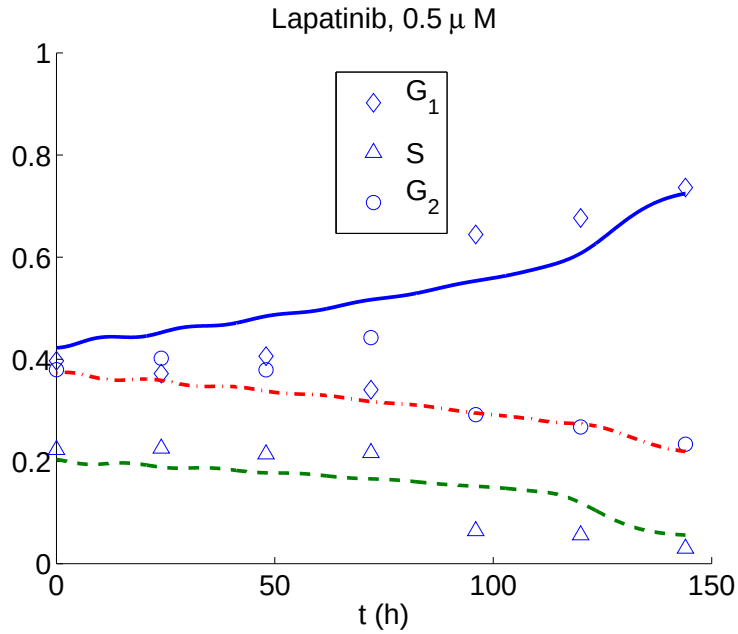




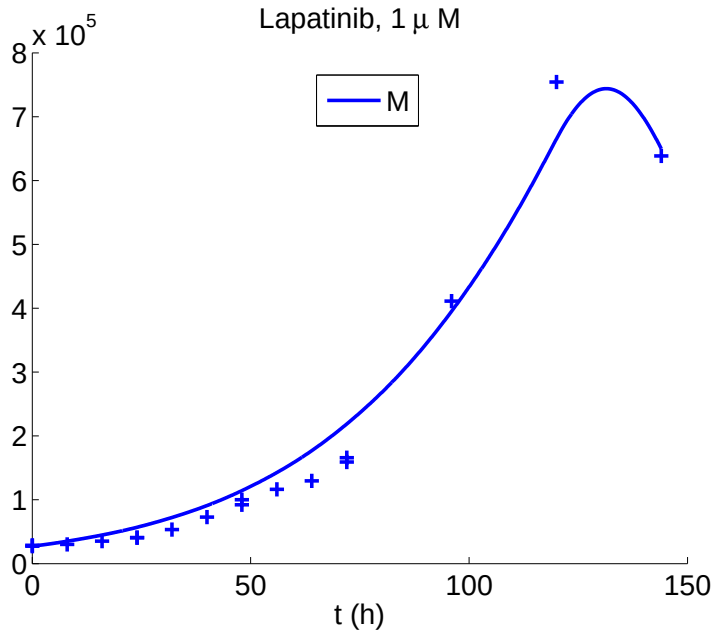
Notice decline in the total population after day 5.

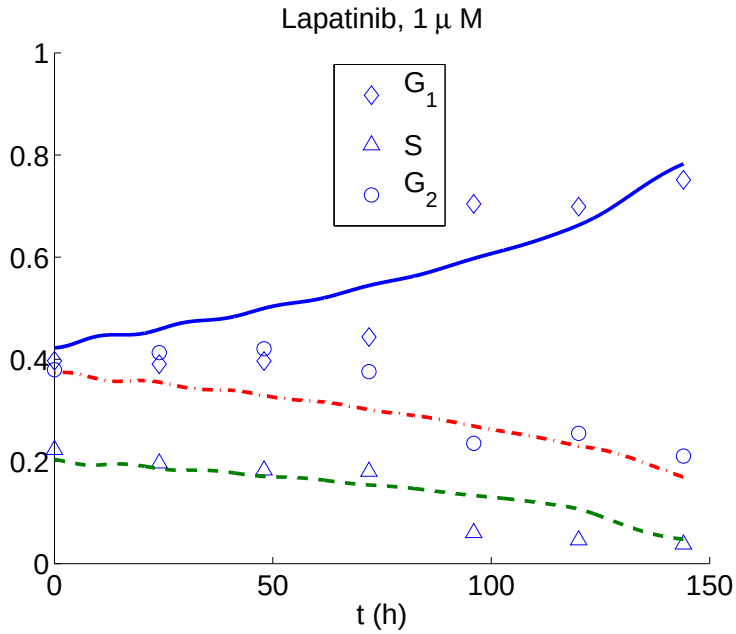
0.5 μM lapatinib



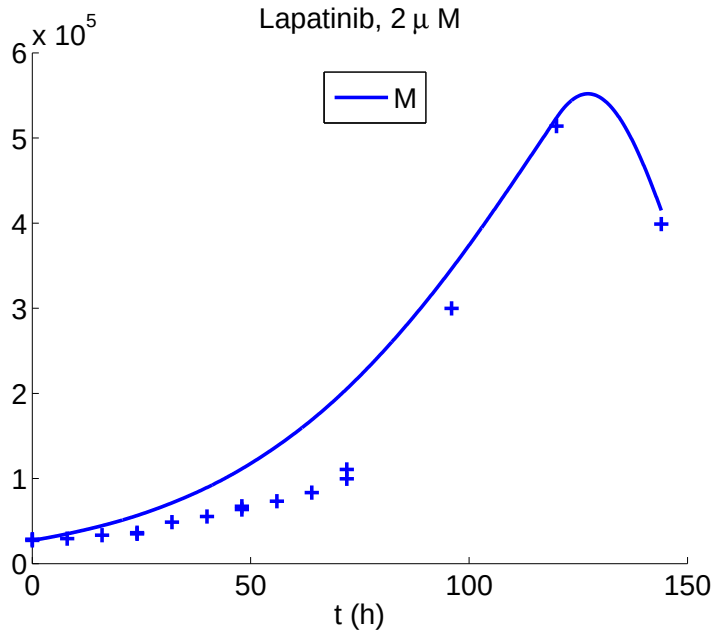


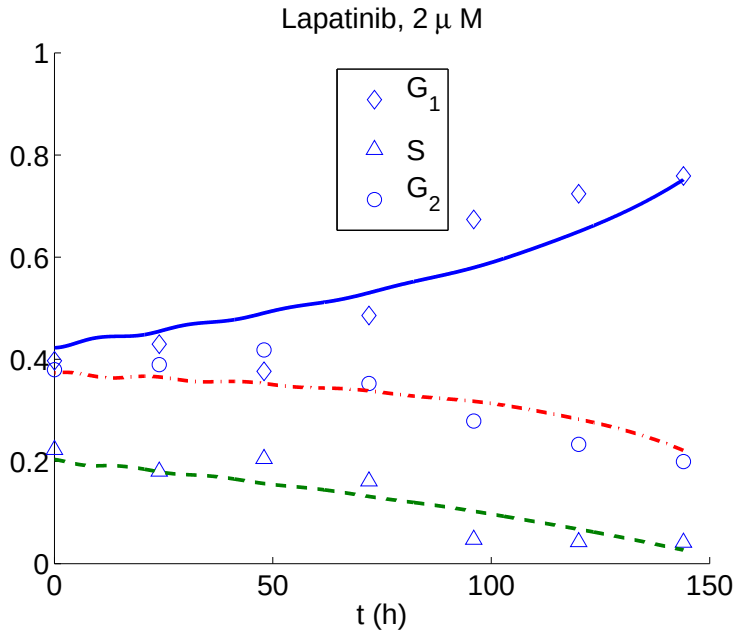
1 μM lapatinib



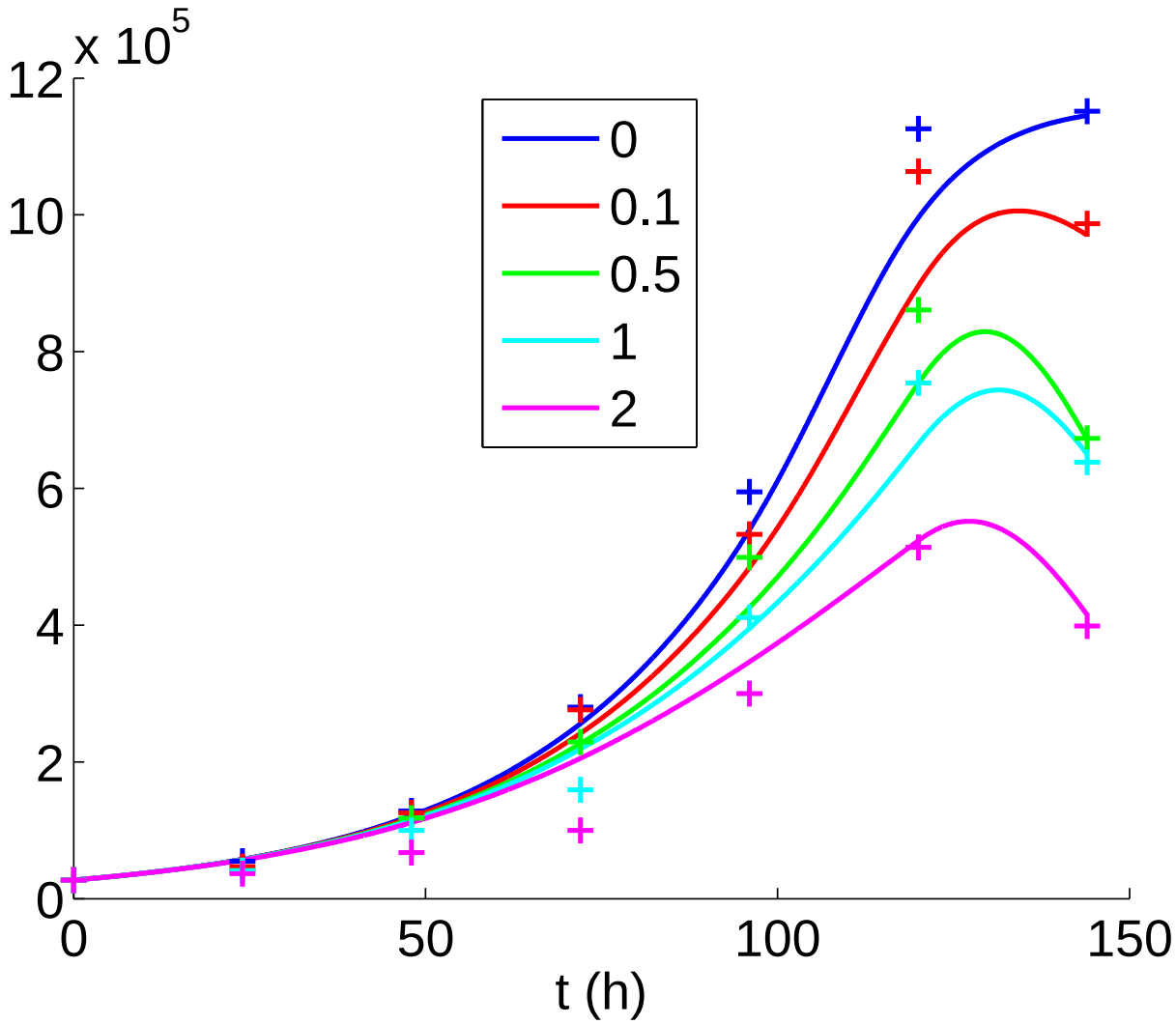


2 μM lapatinib





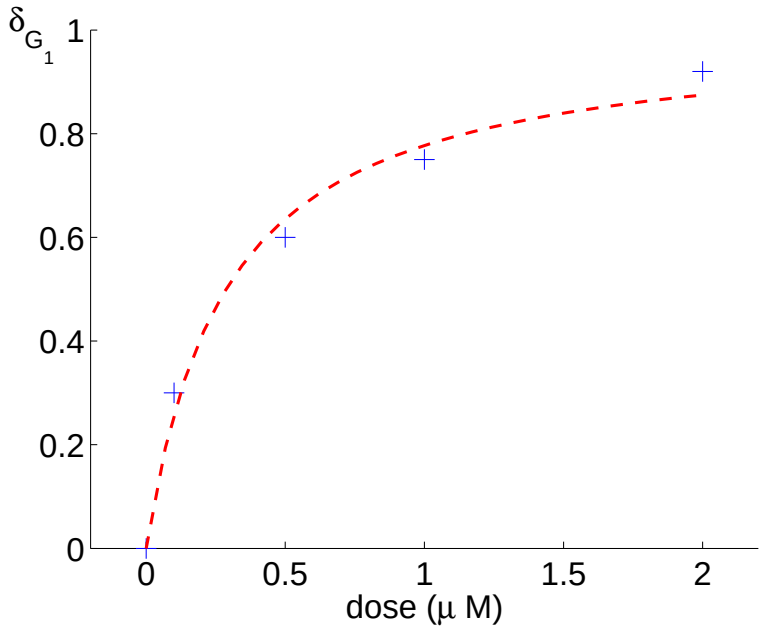
Combined growth curves



Conclusions

- In monolayer growth culture, lapatinib affects preferentially cells in G_1 phase.
- The strength of the cytostatic effects depends on the drug dosage and shows saturation at high drug concentrations.
- The cytostatic effect does not set in immediately but increases over the course of the experiment.
- The cytotoxic effects occur in all treatment cases, however only after day 5.

Conclusions



The strength of the delay in G_1-phase δ_{G_1} as function of dose is well described by the equation

$$\delta_{G_1}(d) = \frac{c_1 d}{1 + c_1 d}$$

with $c_1 = 3.5$.

Conclusions

- Our model can be applied to interpret cytostatic and cytotoxic effects of cell cycle specific drugs.
- The fully continuous model uses few parameters and these parameters have a straightforward biological interpretation.
- A refined model may be used to study an *in vivo* situation.
- It is advisable to combine lapatinib with cytotoxic therapeutic agents that kill not only proliferating cells but also quiescent cells (e.g. alkylating agents).

Reference, Acknowledgments


P. Hinow, S. E. Wang, C. Arteaga, and G. F. Webb. A mathematical model separates quantitatively the cytostatic and cytotoxic effects of a HER2 tyrosine kinase inhibitor. Theor. Biol. Med. Model. **4**:14; `http://www.tbiomed.com`

This work was supported by the NCI Integrative Cancer Biology Program (U54–CA113007) and the Vanderbilt Integrative Cancer Biology Center (VICBC).

Thank you for your attention.