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

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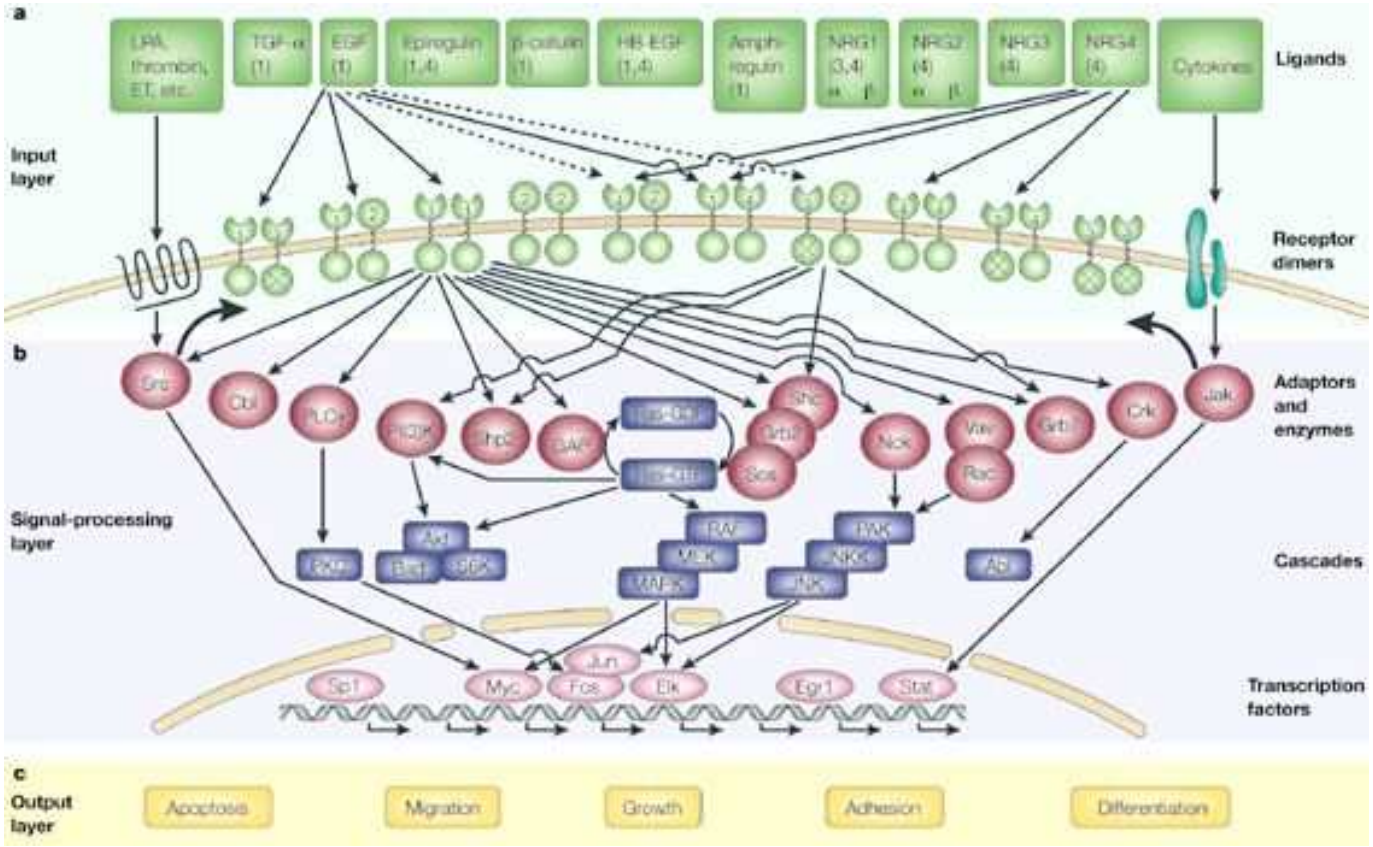
Outline of talk

- 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



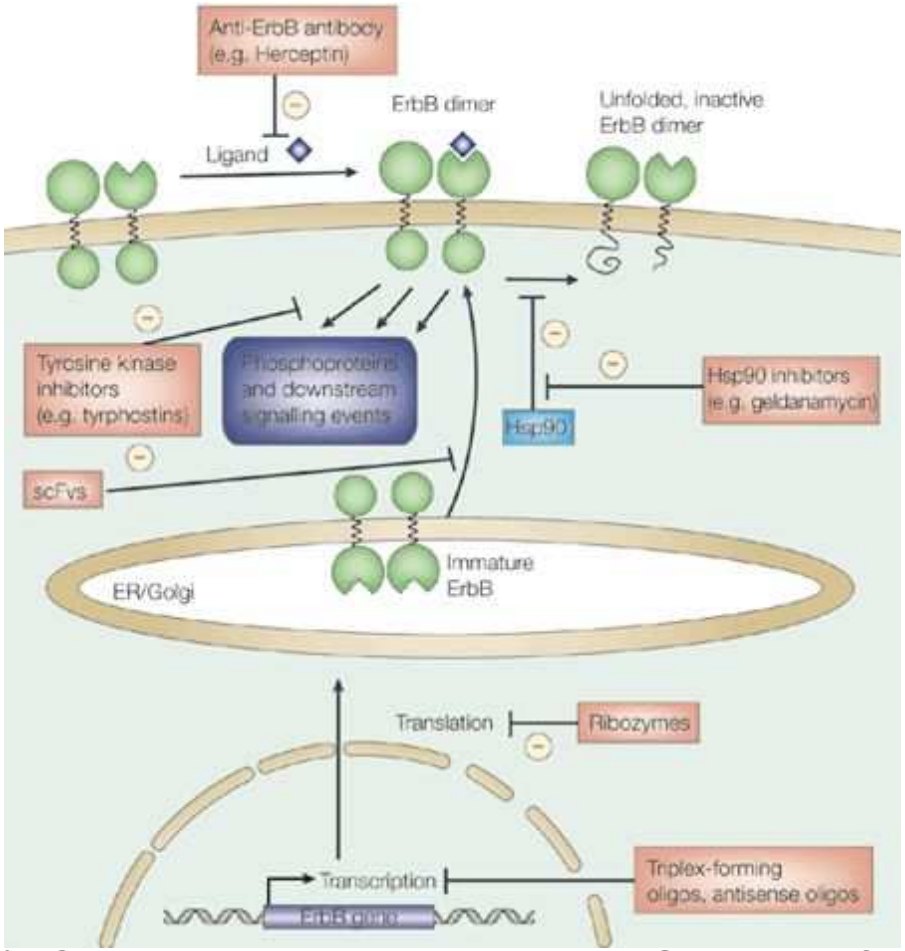
Yarden & Sliwkowski, *Nat. Rev. Mol. Cell Biol.* **2**:127–137

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).

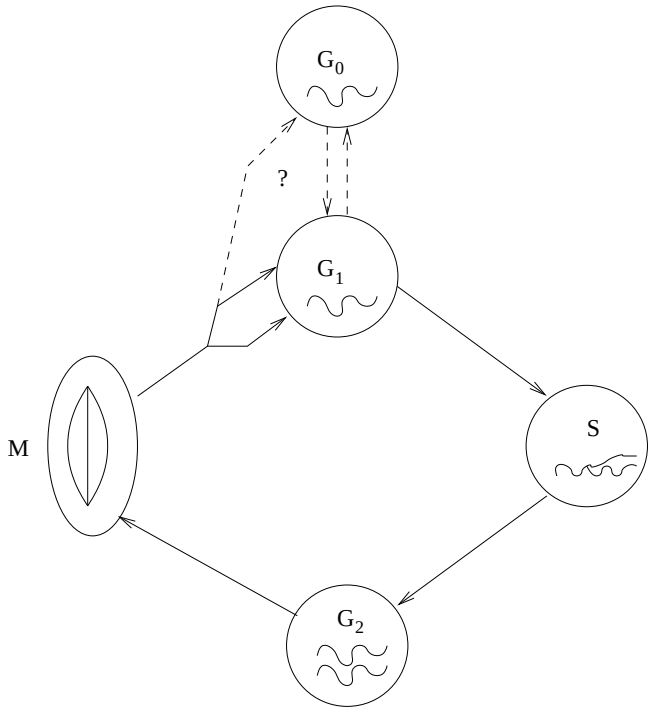
The role of lapatinib



Yarden & Sliwkowski, *Nat. Rev. Mol. Cell Biol.* **2**:127–137

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

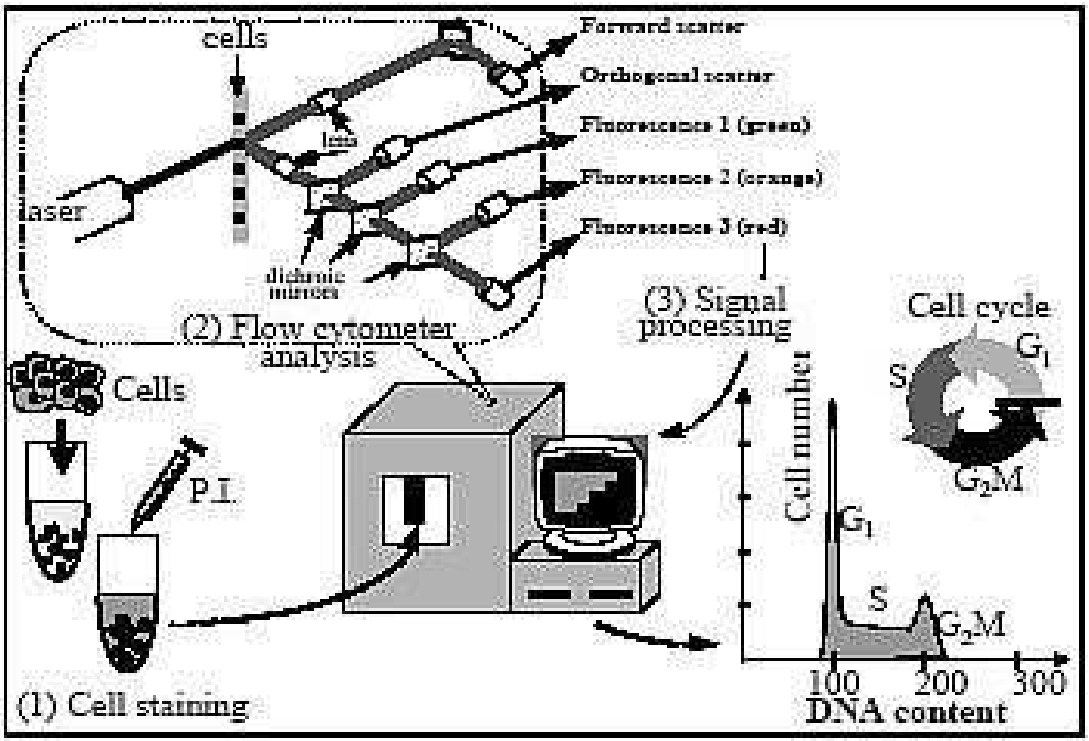
We wanted to

- 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.

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**:323–335

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 begin of experiment) and $a \in [0, a_m]$ denote 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 do have a "maturity", they just do not progress anymore and do not give rise to offspring.

Model equations for an exponentially growing population

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

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

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

Mitosis occurs at a rate β that depends on maturity.

Model equations for untreated cells

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

The function $\tilde{\mu}$ realizes the transition from the proliferating to the nonproliferating class.

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 $1 - \delta(a,t)$
- additional mortality $\epsilon(t)$.

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.

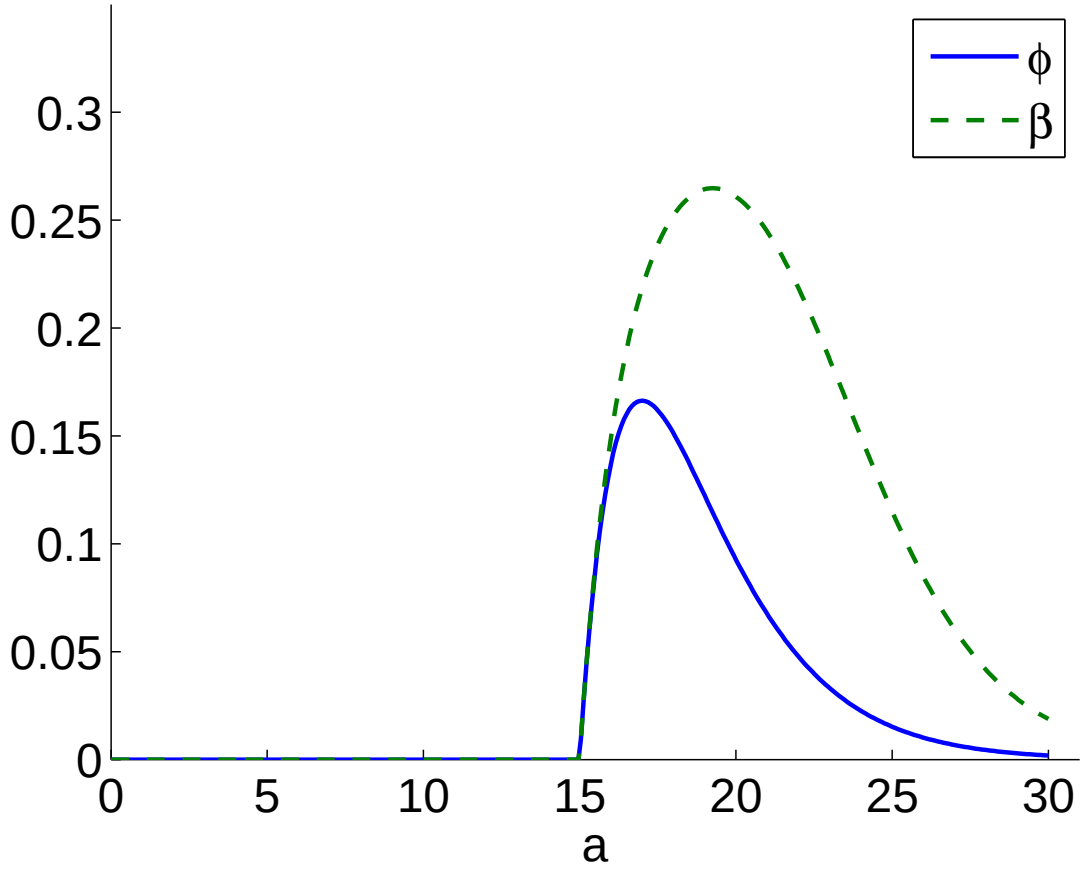
Choice of the age space

Let

$$a_{G_1} = 7,$$
$$a_S = 11,$$
$$a_m = 30.$$

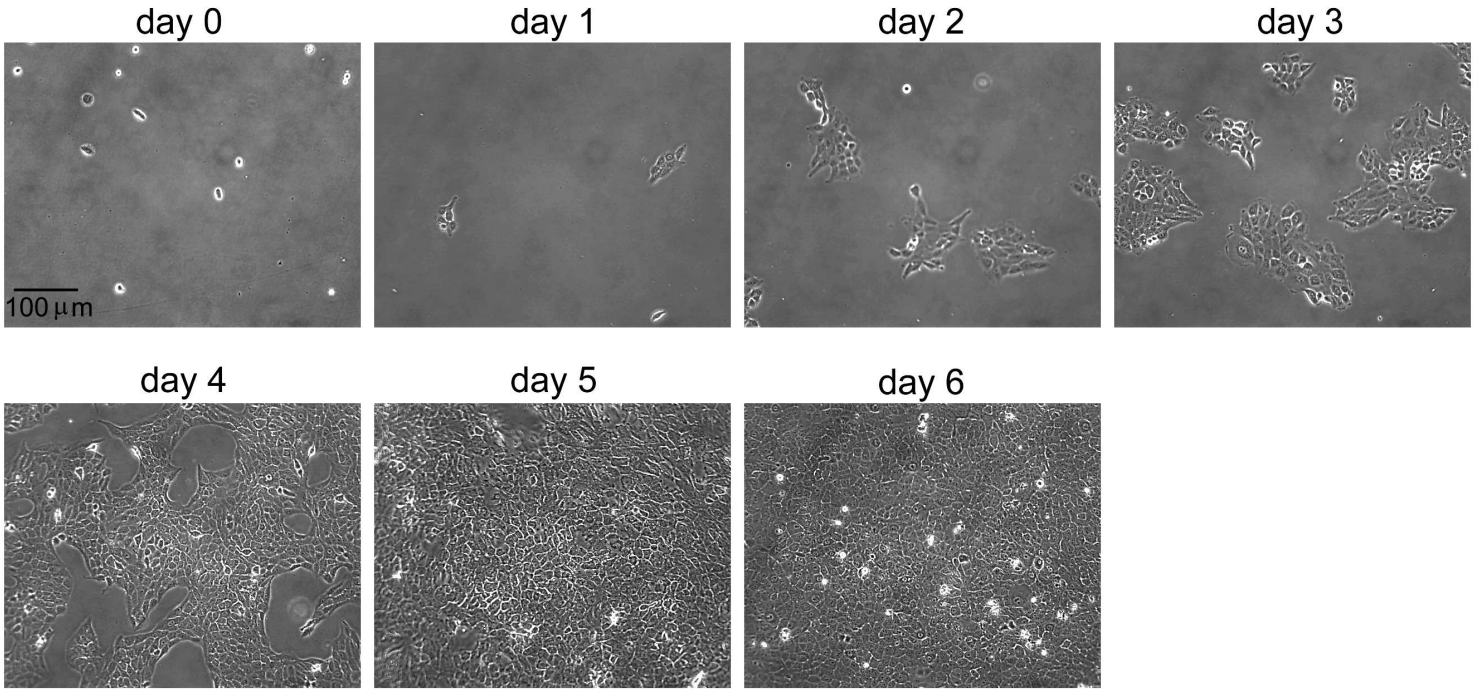
If no cytostatic effects are present, cells age as time progresses. Then these values are *hours after mitosis*. The control scenario supports our choices.

Choice of the proliferation rate



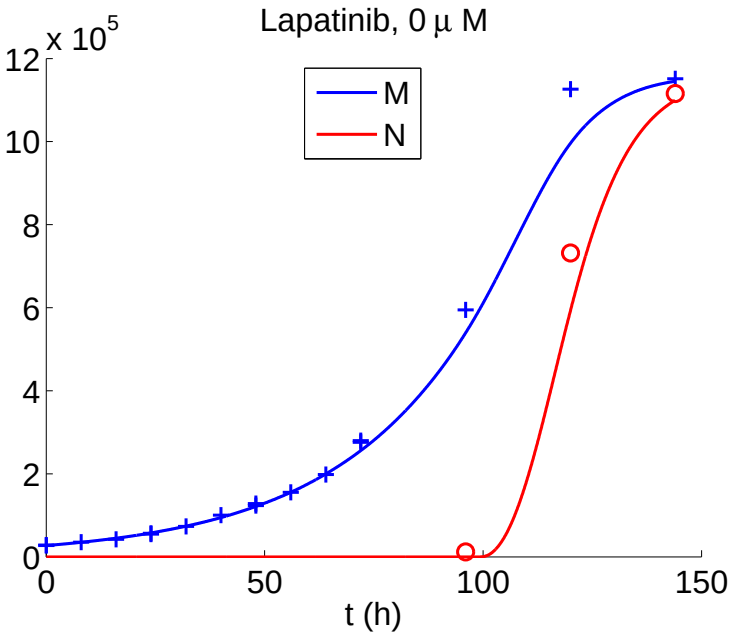
The distribution of intermitotic times ϕ is a shifted Γ–distribution $\Gamma(\cdot - 15; 2, 2)$ with mean 19 h (Dibrov et al. *Math. Biosci.* **66**:167–185).

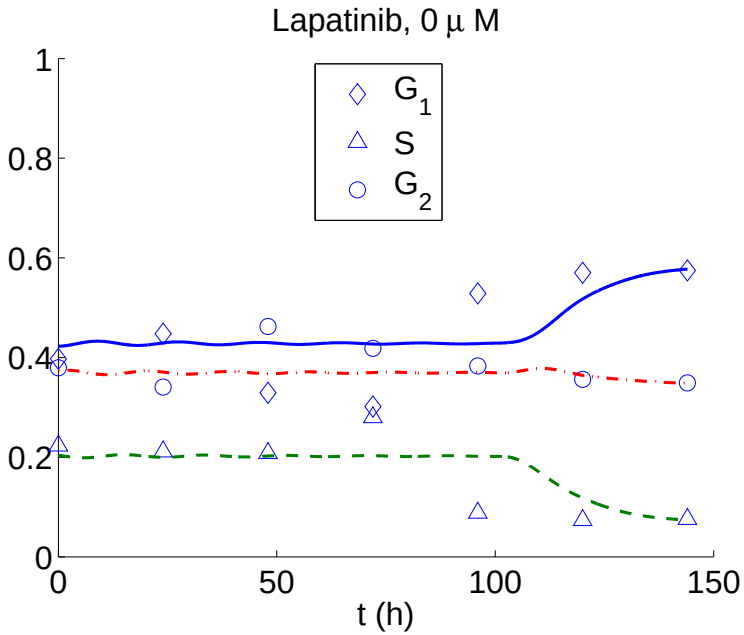
Control scenario



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

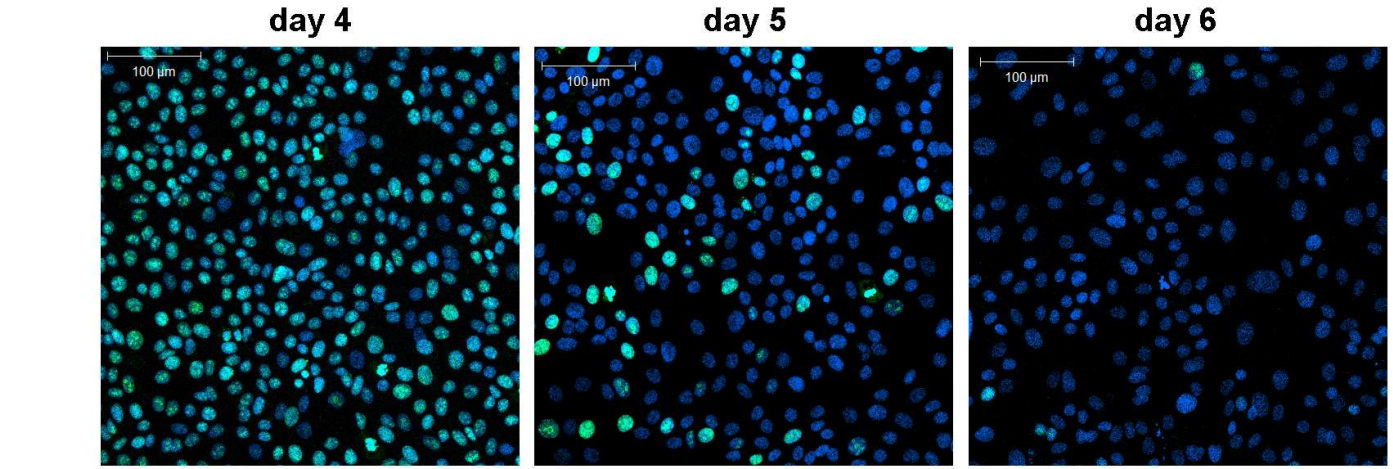
Control scenario





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.

Control scenario



Staining of untreated cells for marker of proliferation Ki–67 (green) on days 4 to 6. The simulations predict 100 %, 40% and 4% proliferating fraction on days 4, 5 and 6, respectively.

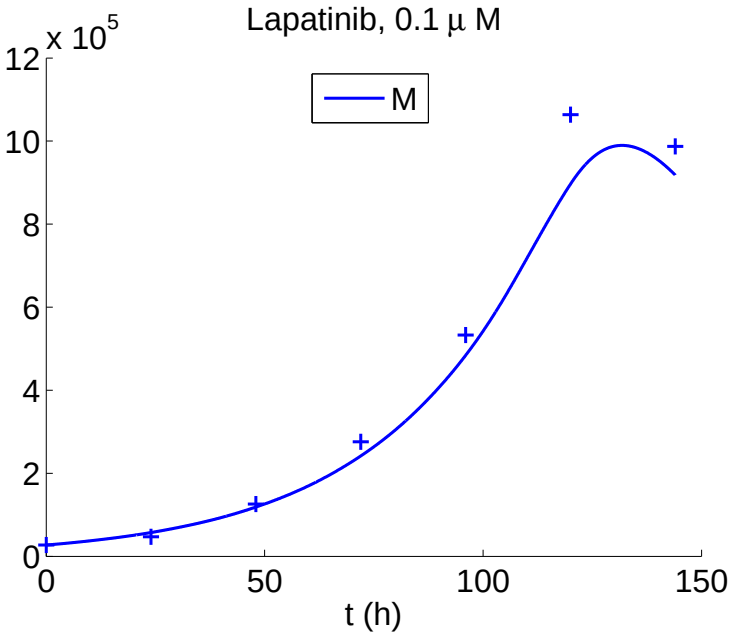
Cell–cycle specificity of delay effect

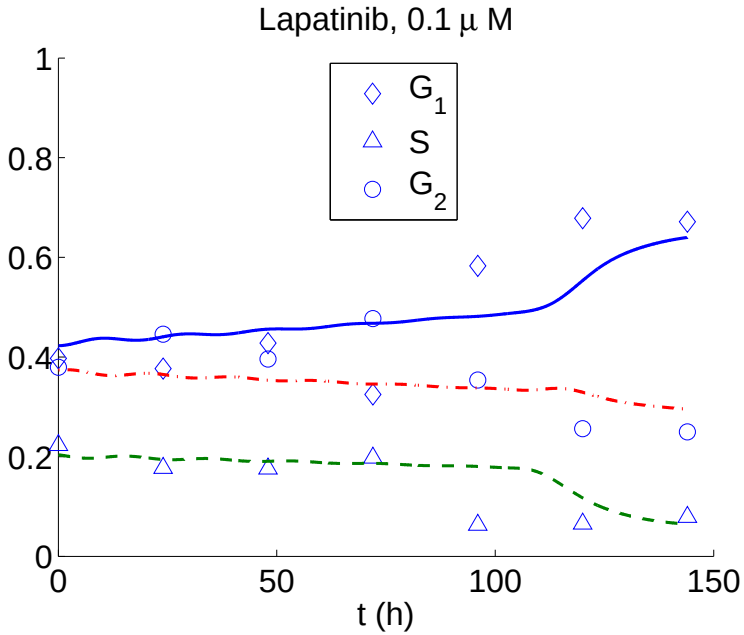
We want to test the hypothesis that lapatinib affects chiefly cells in G_1 phase. Moreover, the cytostatic effects increase with time.

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

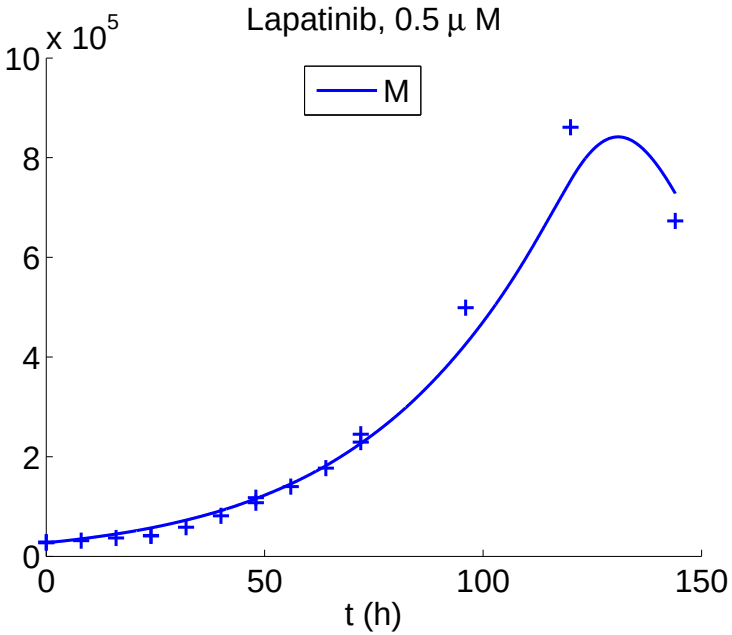
A sudden onset of cytostatic effects would cause oscillations in the percentages that are not seen in the experimental data.

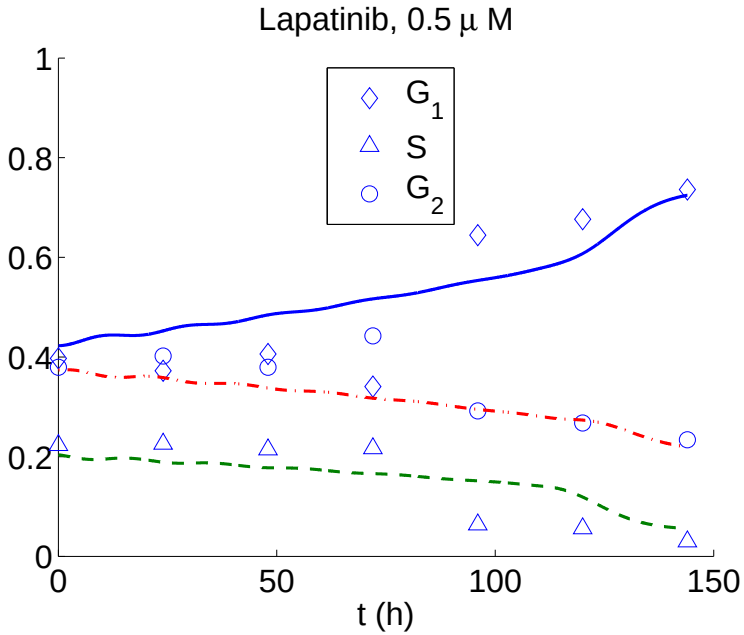
0.1 μM lapatinib



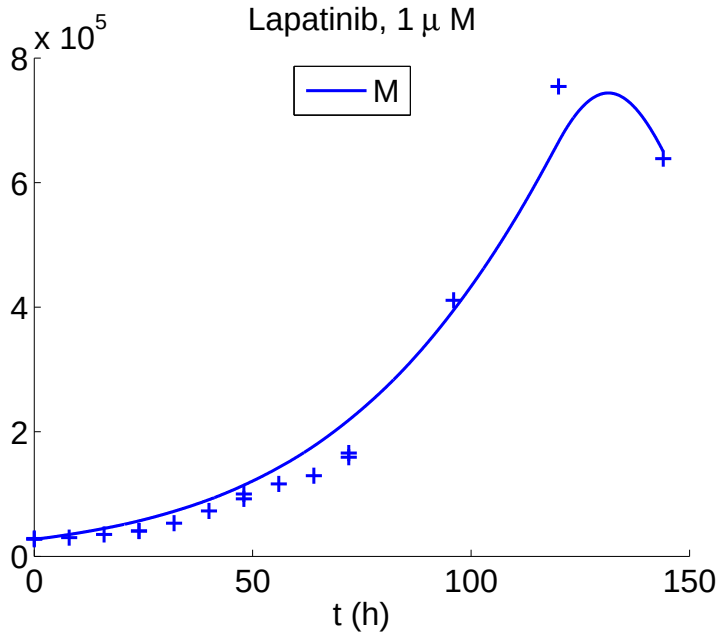


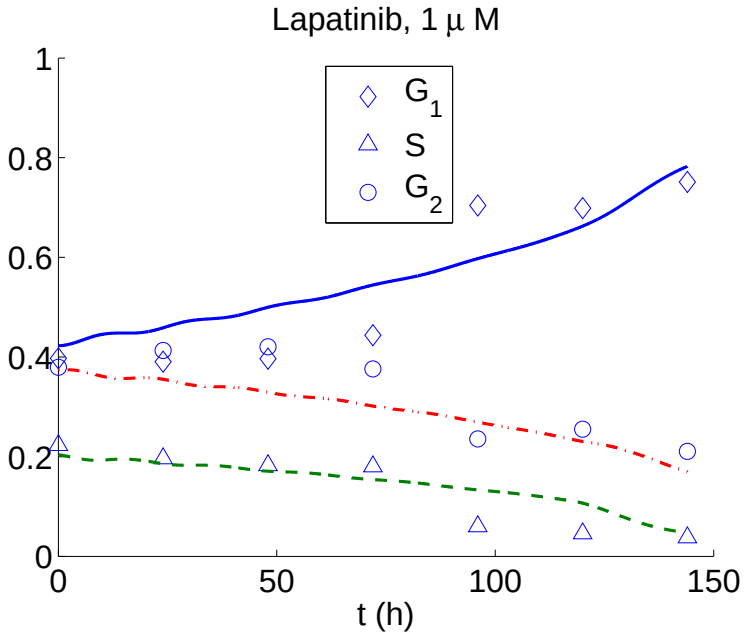
0.5 μM lapatinib



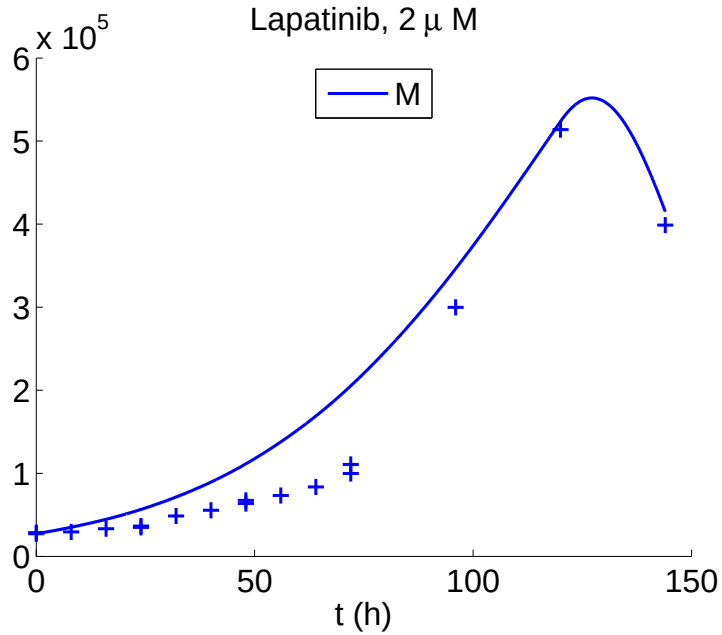


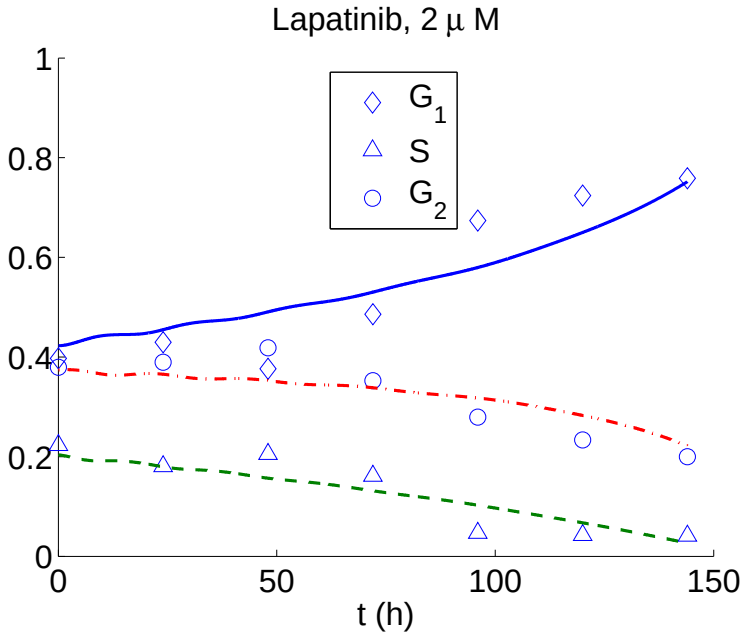
1 μM lapatinib



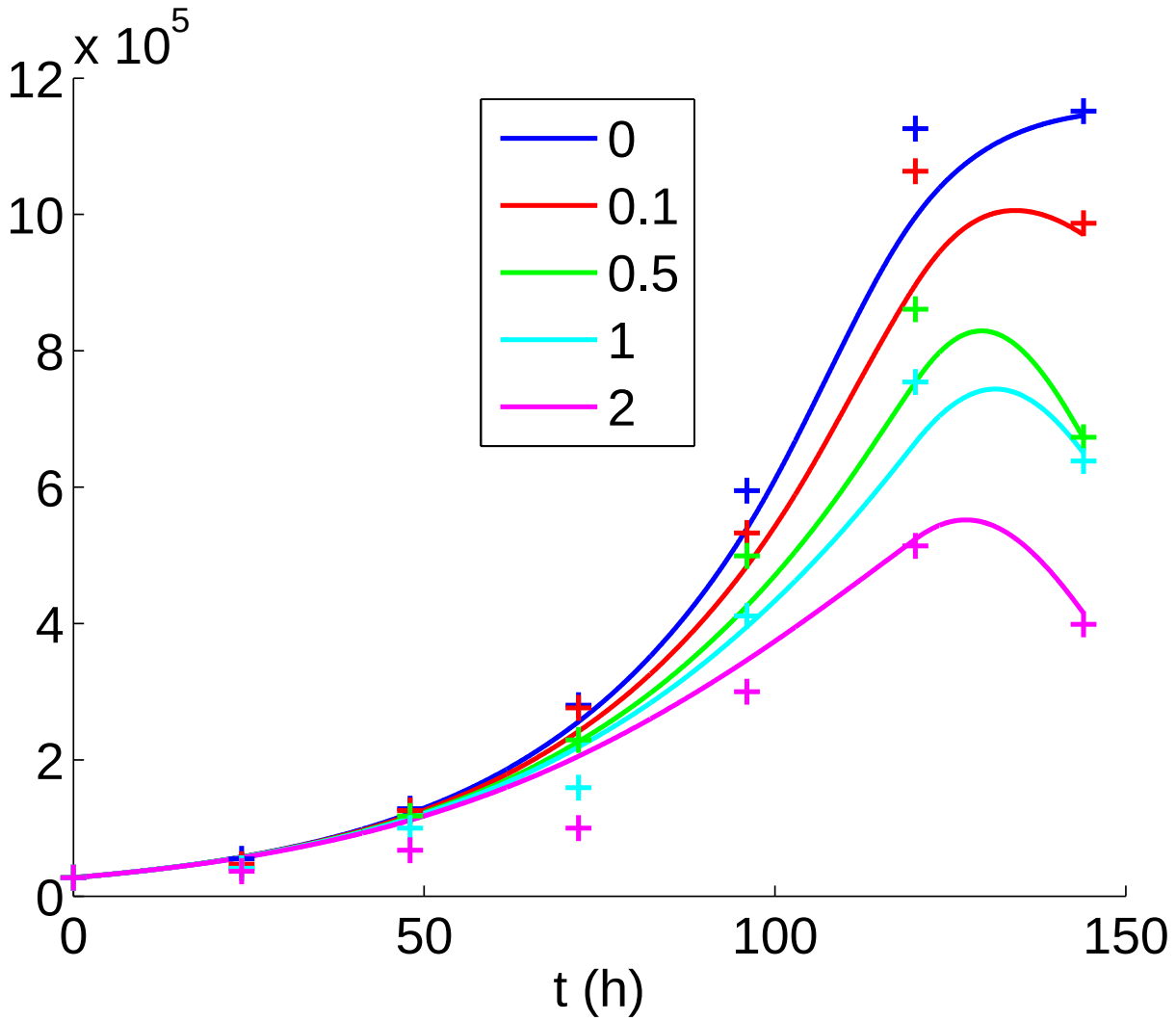


2 μM lapatinib





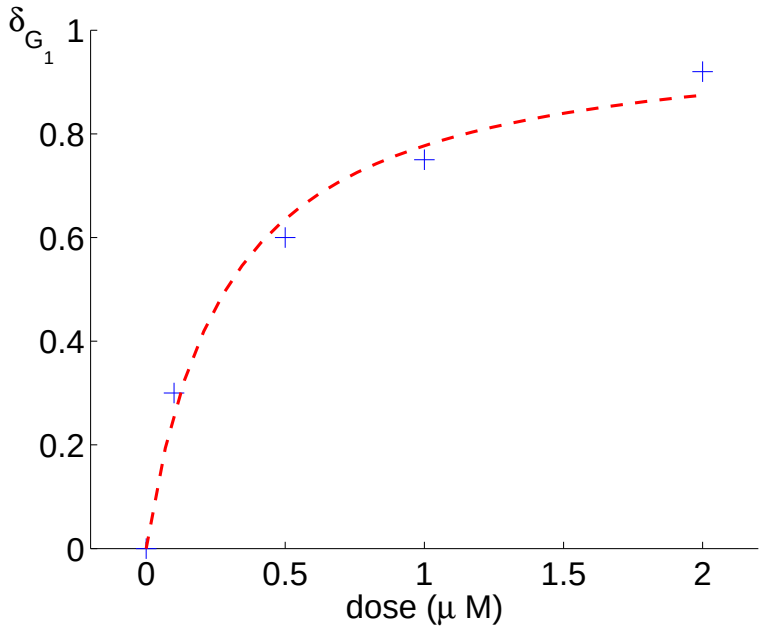
Combined growth curves



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 kinetics.
- 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`

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Thank you for your attention.