Drug-induced drug resistance in cancer and evolution in structured population dynamics, with perspectives in control

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A few definitions: evolution or adaptation of cell populations

[Naive and utilitary definitions]

- **Evolution**: constitution of a new species (cell population of a new type) by genetic mutations (including single nucleotide substitutions, deletions, translocations...), i.e. irreversible modifications of the genome 'written in the marble of the genetic code', resulting in a new phenotype

- **Adaptation**: modification of a cell type also resulting in a new phenotype in a cell population, but reversible, i.e., amenable to complete restitution of the initial phenotype, with preservation of the intact genome (= of the initial sequence of base pairs)

Mutations, epimutations in cell populations

[Again, naive and utilitary definitions]

- **[Genetic] mutation**: irreversible modification of the genome (cf. Evolution)
- **Epigenetic modification** = 'epimutation': modification of the phenotype due to mechanisms that do not affect the genetic code, but are due to silencing of genes (that may be activators or inhibitors of the expression of other genes) by DNA methylation and histone methylation or acetylation

Cancer as an evolutionary disease

[See recent viewpoint by JC, P. Magal and V. Volpert in ESMTB bulletin (Eur. Comm. Math. Theor. Biol. 16:17-20, 2013): "Cancer as evolutionary process"]

What is, or is there, an "origin of cancer"? Could it be:

- ... just an abnormal cell clone emerging from a single "renegade" cell?
- ... resulting from a sequence of mutations/deletions/translocations? occurring in an inevitable way... or else should we ask rather:
- *"Do mutations beget cancers or do cancers beget mutations?"* (Prehn, Canc. Res. 1994)

An ideal scenario for 'cell environmental' therapies?

- An ecological-like answer could be: *Stochastic molecular events at the single cell level yield cells that are normally unfit in a healthy environment, and have no progeny; but in a disrupted environment (e.g., unbalanced w.r.t. metabolic resources, or under cytotoxic drug pressure), such newly generated cells thrive, eventually in organised populations, at the expense of their formerly more fit neighbours*

- Hence, conversely, in an ideally reestablished healthy environment, transformed cells - at least those that have not been able to gather into viable tumours - should disappear by lack of progeny

- To represent environmental resistance-inducing drug pressure and [environment correction or] drug delivery strategies that might be used to overcome such drug resistance, the cell population level is the best adapted to model and theoretically optimise these drug effects

Different viewpoints to represent antitumour therapies

- At the molecular level: representing specific molecular targets in cancer cells hit by targeted therapies; presently a popular viewpoint among cancer biologists
  Achievements: imatinib in chronic myelogenous leukaemia (CML), ATRA+anthracyclins in acute promyelocytic leukaemia (APL)
  Problems: (often very) relative specificity; toxicity to healthy tissues; not taking into account tumour heterogeneity (polyclonality) and emergence of resistance

- At the molecular level: taking into accounts *all* intracellular molecular pathways involved in proliferation, cell death and [de-]differentiation
  Advantages: exhaustive representation (a biocomputer scientist's point of view)
  Problems: scores of reaction networks, hundreds of parameters to estimate, not amenable to take into account evolution towards drug resistance

- At the cell population level: representing functional targets for drugs in controlled cell population dynamic models: PDEs or IDEs (integro-differential)
  "Functional?": i.e., by designing targets in controlled cell population dynamic models related to those fates that are considered as relevant for cell and tissue behaviour in cancer: proliferation, cell death, [de-]differentiation, motility
  Advantages: the right level to take into account population level effects (in particular emergence of resistance) and to design theoretical optimisation strategies for continuous drug delivery
  Problems: attributing to given drugs specific functional effects; macroscopic (cell cultures, ex-vivo and in-vivo) rather than molecular data (*but is it a drawback?*)

Drug resistance: a phenomenon common to various therapeutic situations

- In therapeutic situations where an external pathogenic agent is proliferating at the expense of the resources of an organism: antibiotherapy, virology, parasitology, target populations are able to develop drug resistance mechanisms (e.g., expression of β-lactamase in bacteria submitted to amoxicillin).

- In cancer, there is no external pathogenic agent (even though one may have favoured the disease) and the target cell populations share much of their genome with the host healthy cell population, making overexpression of natural defence phenomena easy (e.g., ABC transporters in cancer cells).

- Drug resistance may account for unexpected failures in targeted therapies.

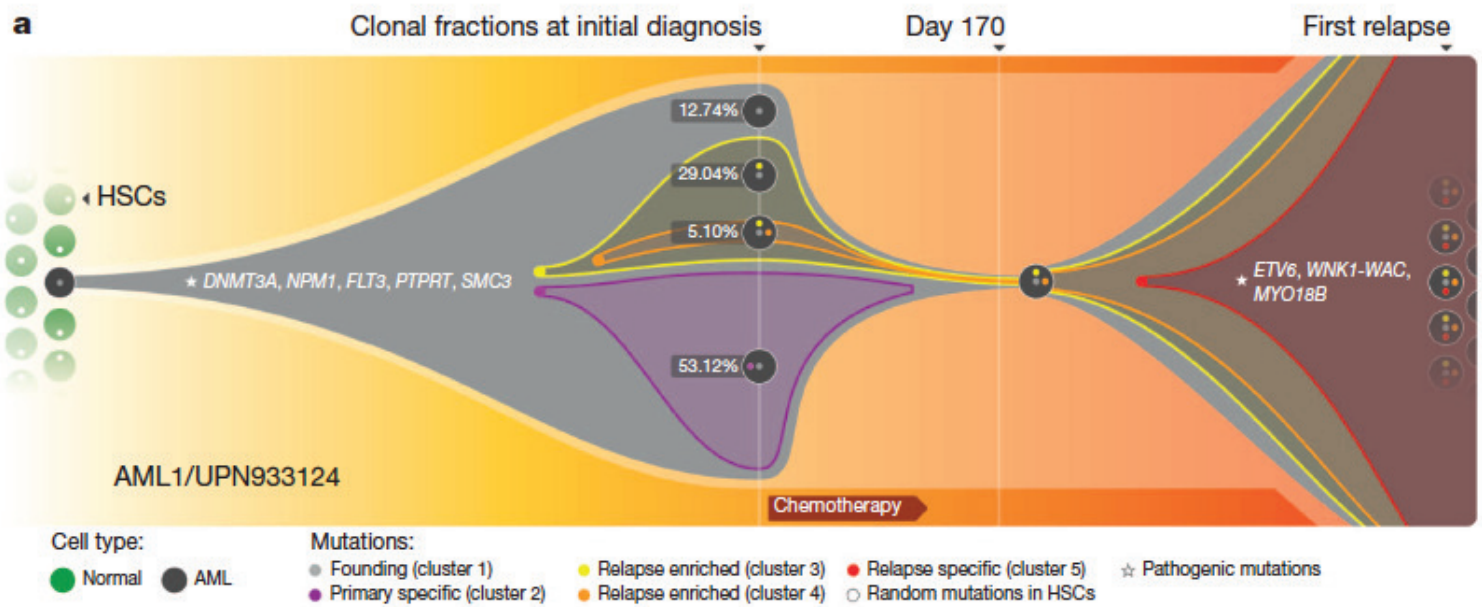
Drug resistance: how does it work?

- What was formerly assumed: 0-1 expression of genes (e.g., functional or inefficient p53 due to a mutation)
- Varying expressivity of genes in a cell population, or else degree of effectiveness of mutations (e.g., mutated EGFR)
- Varying activity of ABC transporters (e.g., P-gp), main effectors of drug efflux out of cells
- Darwinian effects of drug pressure selecting subpopulations in a heterogeneously constituted (by stochastic variations: bet hedging?) cell population
- *Transient adaptation to hostile environments by subclones in the cell population?* Note that we deal with *drug-induced*, not constitutive drug-resistance

Drug resistance: evolutionary bottlenecks in cancer

- Furthermore, animal genome (of the host to cancer) is rich and amenable to adaptation scenarios that may recapitulate developmental scenarios abandoned in the process of evolution from protozoa to metazoa (*Davies & Lineweaver 2011*).

- So that drug therapy may be followed, after initial success, by relapse due to selection of a resistant clone (*Ding et al. 2012*).



Molecular mechanisms at the single cell level vs. Phenotypes at the cell population level

- Overexpression of ABC transporters, of drug processing enzymes, decrease of drug cellular influx, etc. are relevant to describe resistance mechanisms at the single cell level.
- At the cell population level, representing drug resistance by a continuous variable x standing for a resistance phenotype (in evolutionary game theory: a strategy) is adapted to describe evolution from sensitivity ($x = 0$) towards resistance ($x = 1$).
- Is it due to sheer Darwinian selection of the fittest after cell division or, at least partially, due to adaptation of individual cells? Not clear.

1st IDE model, mutations, one cytotoxic drug: cancer cells

- $x =$ level of expression of a drug resistance phenotype (to a given drug)
- $n_H(x,t)$, $n_C(x,t)$ densities of cell populations (H=healthy, C=tumour)

$$\frac{\partial}{\partial t} n_C(x,t) = \Big[\overbrace{(1-\theta_C)\, r(x)}^{\text{growth}} - \overbrace{d(x)}^{\text{death}} - \overbrace{u(t)\mu_C(x)}^{\text{drug effect}} \Big] n_C(x,t)$$

$$+\theta_C \overbrace{\int r(y) M_{\sigma_C}(y,x) n_C(y,t)\, dy}^{\text{birth with mutation}}$$

- $r(x) =$ basic reproduction rate, $d(x) =$ basic death rate; we assume $r(0) > d(0) > 0, \qquad r'(\cdot) < 0, \qquad r(+\infty) = 0, \qquad d'(\cdot) > 0,$
- $0 \le \theta_{H,C} < 1$ ($\theta_C > \theta_H$) is the proportion of divisions with mutations,
- $\mu_{[H,C]}(x)$ (with $\mu'_C(\cdot) < 0$) represents the phenotype-dependent response to cytotoxic drug, with concentration $u(t)$, designed to target cancer cells.
- Note: assumptions $r(\cdot) > 0, \mu_C(\cdot) > 0$, $\mu'_C(\cdot) < 0$ and $r'(\cdot) < 0$ (cost of resistance: the higher is x, the lower is proliferation) represent an *evolutionary double bind on resistant cancer cell populations*, *i.e., an evolutionary trade-off between growing (thus getting exposed) and keeping still (thus surviving)*

1st IDE model, mutations, one cytotoxic drug: healthy cells

$$\frac{\partial}{\partial t} n_H(x,t) = \Big[\overbrace{\frac{1-\theta_H}{\left(1+\rho(t)\right)^{\beta}} r(x)}^{\text{growth with homeostasis}} - \overbrace{d(x)}^{\text{death}} - \overbrace{u(t)\mu_H(x)}^{\text{drug effect}} \Big] n_H(x,t)$$

$$+ \frac{\theta_H}{\left(1+\rho(t)\right)^{\beta}} \overbrace{\int r(y) M_{\sigma_H}(y,x) n_H(y,t) dy}^{\text{birth with mutation}},$$

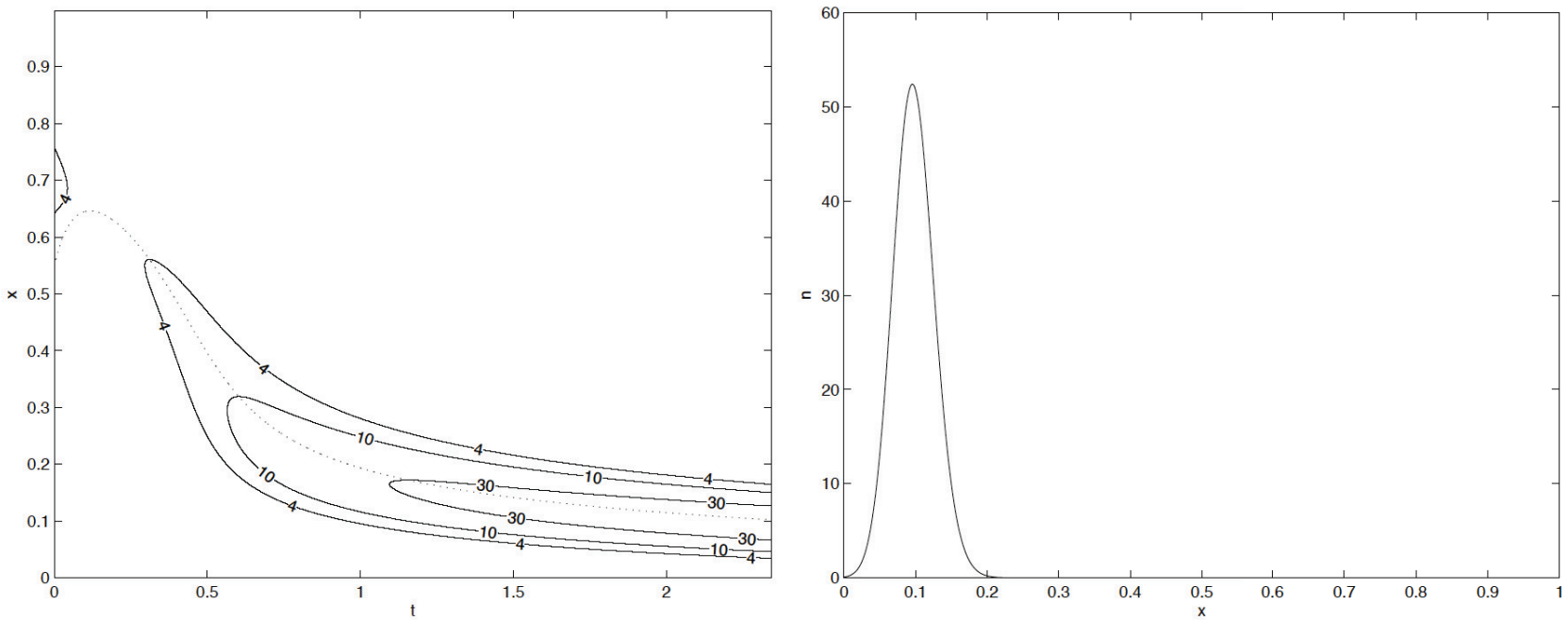
where the total population is defined as

$$\rho(t) = \rho_H(t) + \rho_C(t); \rho_H(t) = \int_{x=0}^{\infty} n_H(x,t)dx; \rho_C(t) = \int_{x=0}^{\infty} n_C(x,t)dx.$$

- $\beta > 0$ to impose healthy tissue homeostasis,
- $u(t)$ denotes the instantaneous dose (concentration) of chemotherapy. We assume in this model that its effect is cytotoxic, i.e., on the death term only.

IDE model, mutations, one cytotoxic drug: illustrations (1)

[Sensitive cell population case: illustration of Gause's exclusion principle]
Theorem: Monomorphic evolution towards drug sensitivity, illustrated here with $\theta_H = 0$, *(no mutations) and* $\mu_H = 0$ *(no drug-induced resistance)*

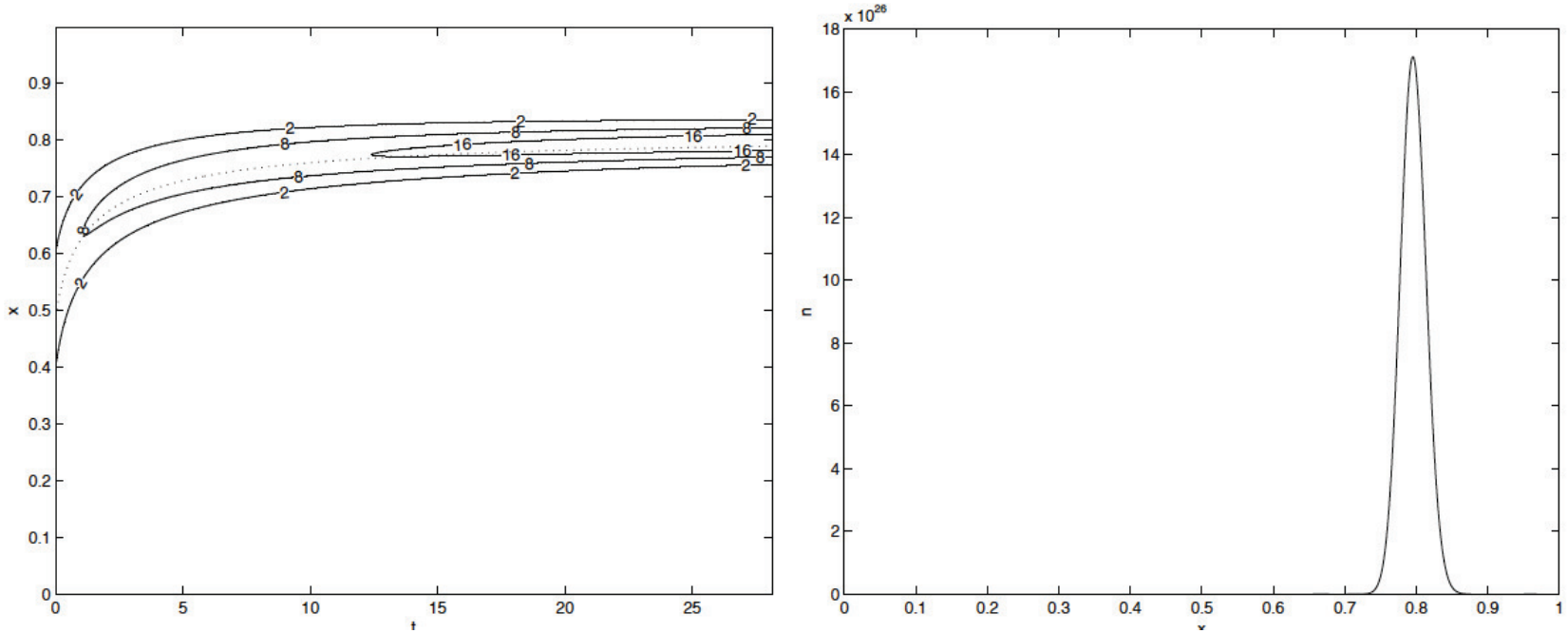


Left panel: starting from a medium phenotype $x = 0.5$, *level sets of a drug-sensitive population in the* (t, x) *plane. Right panel: asymptotic distribution of this drug-sensitive population according to the drug resistance phenotype* x.

(Lorz et al., M2AN 2013)

IDE model, mutations, cytotoxic drug: illustrations (2)

[Resistant cell population case: Gause's exclusion principle again]
Theorem: Monomorphic evolution towards drug-induced drug resistance, here with $\theta_C = 0$, $\mu_C(\cdot) > 0, r'(\cdot) < 0, \mu'_C(\cdot) < 0$ *(costly drug-induced resistance)*



Left panel: starting from a medium phenotype $x = 0.5$*, level sets of a drug- resistant population in the* (t, x) *plane. Right panel: asymptotic distribution of this drug-resistant population according to the drug resistance phenotype* x*.*

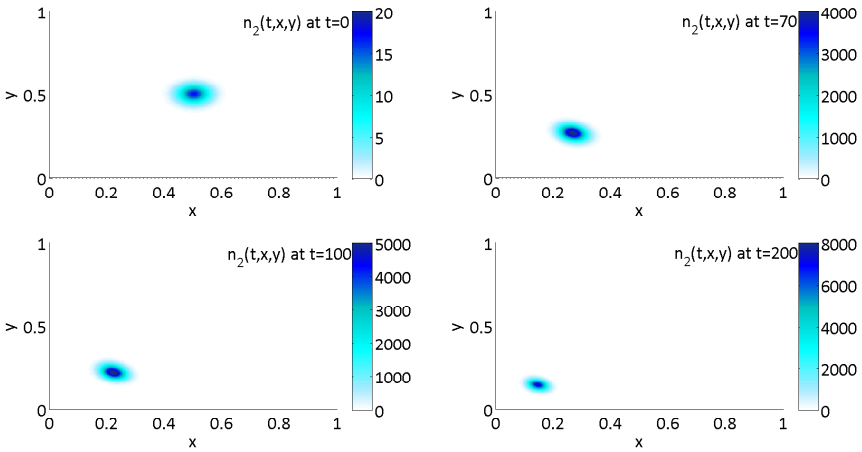
(Lorz et al., M2AN 2013)

2nd IDE model, 2 drugs, cytotoxic $u_1(t)$, cytostatic $u_2(t)$, bidimensional resistance phenotype (x, y), no mutations

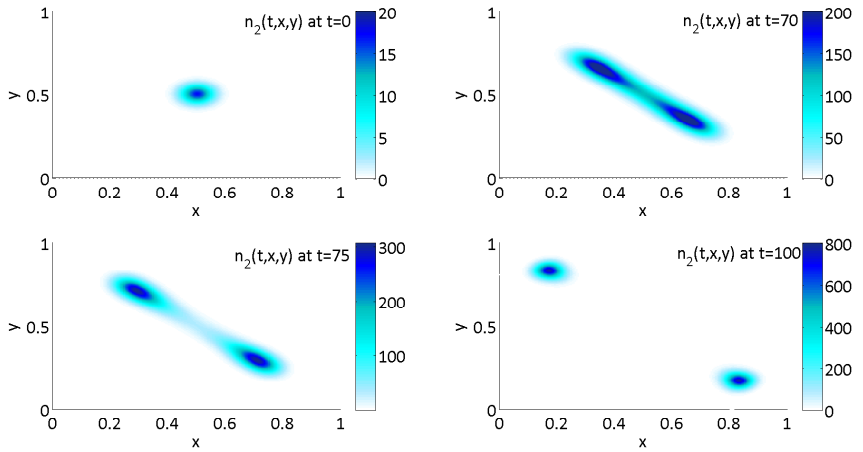
$$\frac{\partial}{\partial t} n_C(x,y,t) = \left[\frac{r_C(x,y)}{1 + k u_2(t)} - d_C(x,y) I_C(t) - u_1(t) \mu_C(x,y) \right] n_C(x,y,t)$$

Environment: $I_C(t) = \alpha \int_0^1 \int_0^1 n_C(x,y,t)\, dx\, dy + \beta \int_0^1 \int_0^1 n_H(x,y,t)\, dx\, dy$

Sensitive cell population case:



Resistant cell population case:



Convergence toward total sensitivity

Convergence toward 2 resistant phenotypes

(Tommaso Lorenzi, work underway)

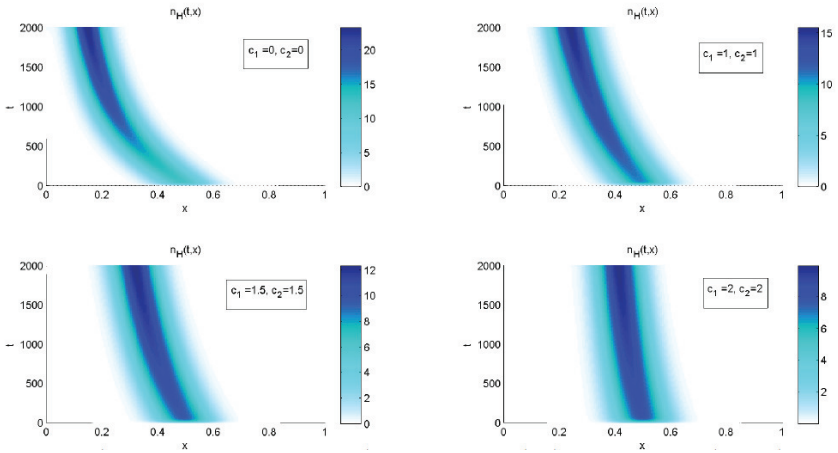
Now 2 drugs with one (scalar) resistance phenotype x

$$\frac{\partial}{\partial t} n_H(x,t) = \left[\frac{r_H(x)}{1 + k_H u_2(t)} - d_H(x) I_H(t) - u_1(t) \mu_H(x) \right] n_H(x,t)$$

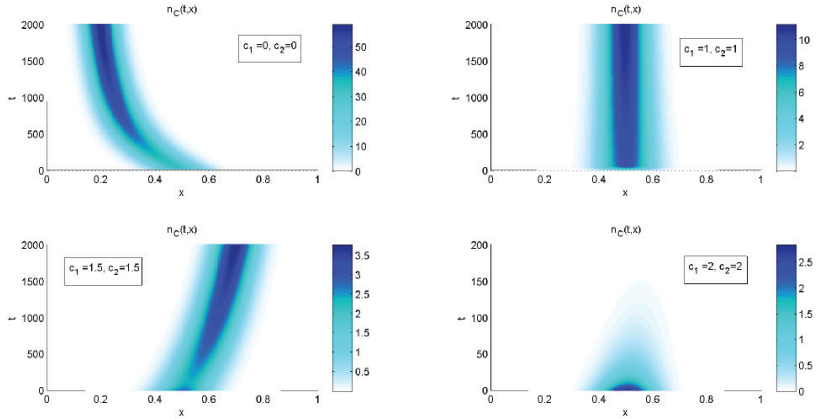
$$\frac{\partial}{\partial t} n_C(x,t) = \left[\frac{r_C(x)}{1 + k_C u_2(t)} - d_C(x) I_C(t) - u_1(t) \mu_C(x) \right] n_C(x,t)$$

Environment: $I_H(t) = a_{HH}.\rho_H(t) + a_{HC}.\rho_C(t)$, $I_C(t) = a_{CH}.\rho_H(t) + a_{CC}.\rho_C(t)$,
with $\rho_H(t) = \int_0^1 n_H(x,t)\,dx$, $\rho_C(t) = \int_0^1 n_C(x,t)\,dx$.

Simultaneous combinations of the 2 drugs, with increasing equal doses



Healthy cells: preserved



Cancer cells: eventually extinct

("Pedestrian's optimisation")

Optimisation algorithms to improve drug delivery in cancer cell populations (work by Emmanuel Trélat, LJLL, UPMC)

Same phenotype-structured model, but instead of a 'pedestrian's optimisation' (i.e., merely using grids), solving an optimal control problem: determining control functions u_1 and u_2 in $L^\infty(0,T)$, satisfying the constraints

$$0 \leq u_1(t) \leq u_1^{\max}, \qquad 0 \leq u_2(t) \leq u_2^{\max}, \tag{1}$$

and minimising the cost functional

$$C_T(u_1,u_2) = \int_0^1 n_C(x,T)\,dx + \gamma_1 \int_0^T u_1(t)\,dt + \gamma_2 \int_0^T u_2(t)\,dt, \tag{2}$$

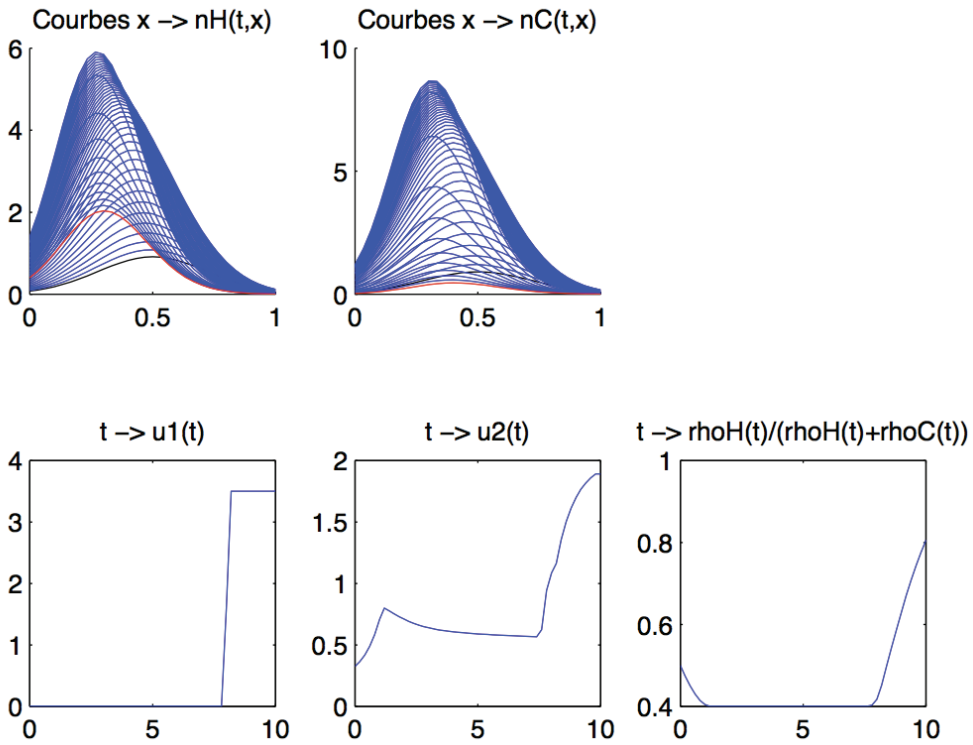
where $(n_C(\cdot,\cdot), n_H(\cdot,\cdot))$ is the unique solution of the system of PDEs corresponding to the controls u_1 and u_2, such that $n_H(0,\cdot) = n_H^0(\cdot)$ and $n_C(0,\cdot) = n_C^0(\cdot)$ and where the trajectory $t \mapsto (n_C(\cdot,t), n_H(\cdot,t))$ is subject to the dynamic state constraint

$$\frac{\rho_H(t)}{\rho_H(t)+\rho_C(t)} \geq \theta. \tag{3}$$

(here $\theta = 0.4$) We use a direct approach, discretising the whole problem and then solving the resulting constrained optimisation problem with AMPL (automatic differentiation) combined with IPOPT (expert optimisation routine)

Numerical solution to this first optimisation problem

Distribution of populations according to phenotype (black: initial; red: final; blue: intermediate steps of the optimisation algorithm)

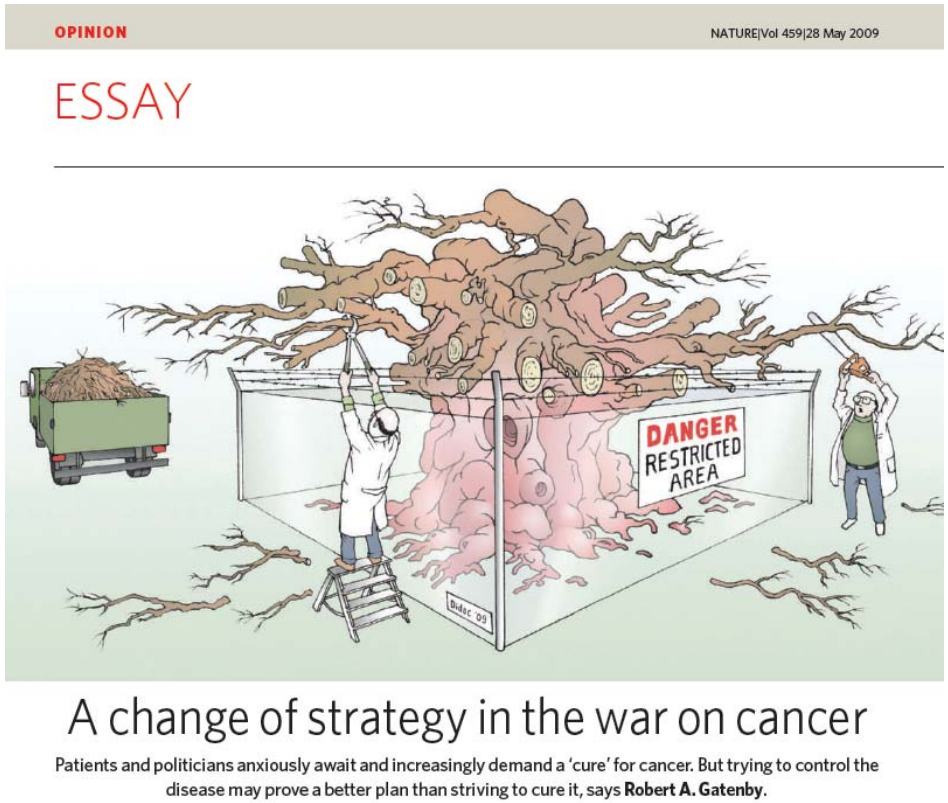


Left and centre panels: optimal drug flows for $u_1(t)$ (cytotoxic) and $u_2(t)$ (cytostatic)

Right panel: satisfaction of dynamic constraint

Introducing 'adaptive therapy', following Robert Gatenby

- Principle: keep alive an objective ally in the enemy place
- Relies on competition for resources between resistant (weakly proliferative) and sensitive cancer cells in the tumour
- Aim: avoid extinction of sensitive tumour cells, that are able to outcompete resistant tumour cells provided that not too high doses of a drug are delivered
- Method: deliver relatively low doses of the drug to prevent thriving of too many sensitive cells and limit emergence of too many (unbeatable) resistant cells
- Objective: controlling total (sensitive + resistant) tumour cell population
- Caveat: not necessarily applicable in the case of fast growing tumours (e.g., acute myeloblastic leukaemia)



Second optimisation problem, same IDE model (1)

Environment: $I_H(t) = a_{HH}.\rho_H(t) + a_{HC}.\rho_C(t), I_C(t) = a_{CH}.\rho_H(t) + a_{CC}.\rho_C(t)$,
with $\rho_H(t) = \int_0^1 n_H(x,t)\,dx, \rho_C(t) = \int_0^1 n_C(x,t)\,dx$.

Same IDE model with evolution in phenotype x due to effects of cytotoxic drug $u_1(t)$

$$\frac{\partial}{\partial t} n_H(x,t) = \left(\frac{r_H(x)}{1+\alpha_H u_2(t)} - d_H(x) I_H(t) - u_1(t)\mu_H(x) \right) n_H(x,t)$$
$$\frac{\partial}{\partial t} n_C(x,t) = \left(\frac{r_C(x)}{1+\alpha_C u_2(t)} - d_C(x) I_C(t) - u_1(t)\mu_C(x) \right) n_C(x,t)$$

$$0 \le u_1(t) \le u_1^{\max}, \qquad 0 \le u_2(t) \le u_2^{\max}$$

$$\min C_T(u_1, u_2) = \rho_C(T) = \int_0^1 n_C(x,T)\,dx$$

under the additional constraints

$$\frac{\rho_H(t)}{\rho_H(t)+\rho_C(t)} \ge \theta_H, \qquad \rho_H(t) \ge \rho_H(0)$$

Second optimisation problem, same model (2)

Furthermore, we add the "adaptive" constraint

$$\frac{\rho_{CS}(t)}{\rho_C(t)} \geq \theta_{CS}, \quad \textit{where}$$

$$\rho_{CS}(t) = \int_0^1 (1-x) n_C(t,x)\, dx$$

may be seen as the total number at time t of tumour cells that are sensitive, and

$$\rho_{CR}(t) = \int_0^1 x n_C(t,x)\, dx$$

as the total number at time t of tumour cells that are resistant.

Of course, sensitivity/resistance being by construction a non-binary variable, the weights x and $1-x$ are here to stress in a simple way a partition between a sensitive class and a resistant class in the cancer cell population; other choices with $\rho_C(t) = \rho_{CS}(t) + \rho_{CR}(t)$ might be made for these weights, e.g., x^2 and $1-x^2$, .

Second optimal control problem: theoretical results

Theorem

Under these conditions, the optimal trajectory in large time $T > 0$ consists of 3 arcs:

1. A first transient **short-time** arc, consisting of reaching the boundary $\frac{\rho_H(t)}{\rho_H(t)+\rho_C(t)} = \theta_H$, with $u_1 = 0$ and with an appropriate control u_2.
2. A middle **long-time** arc: $u_1 = 0$, $u_2 \simeq \mathrm{Cst}$, this constant being tuned so that

$$\frac{\rho_H(t)}{\rho_H(t) + \rho_C(t)} = \theta_H.$$

   At the end of this long-time arc, we have

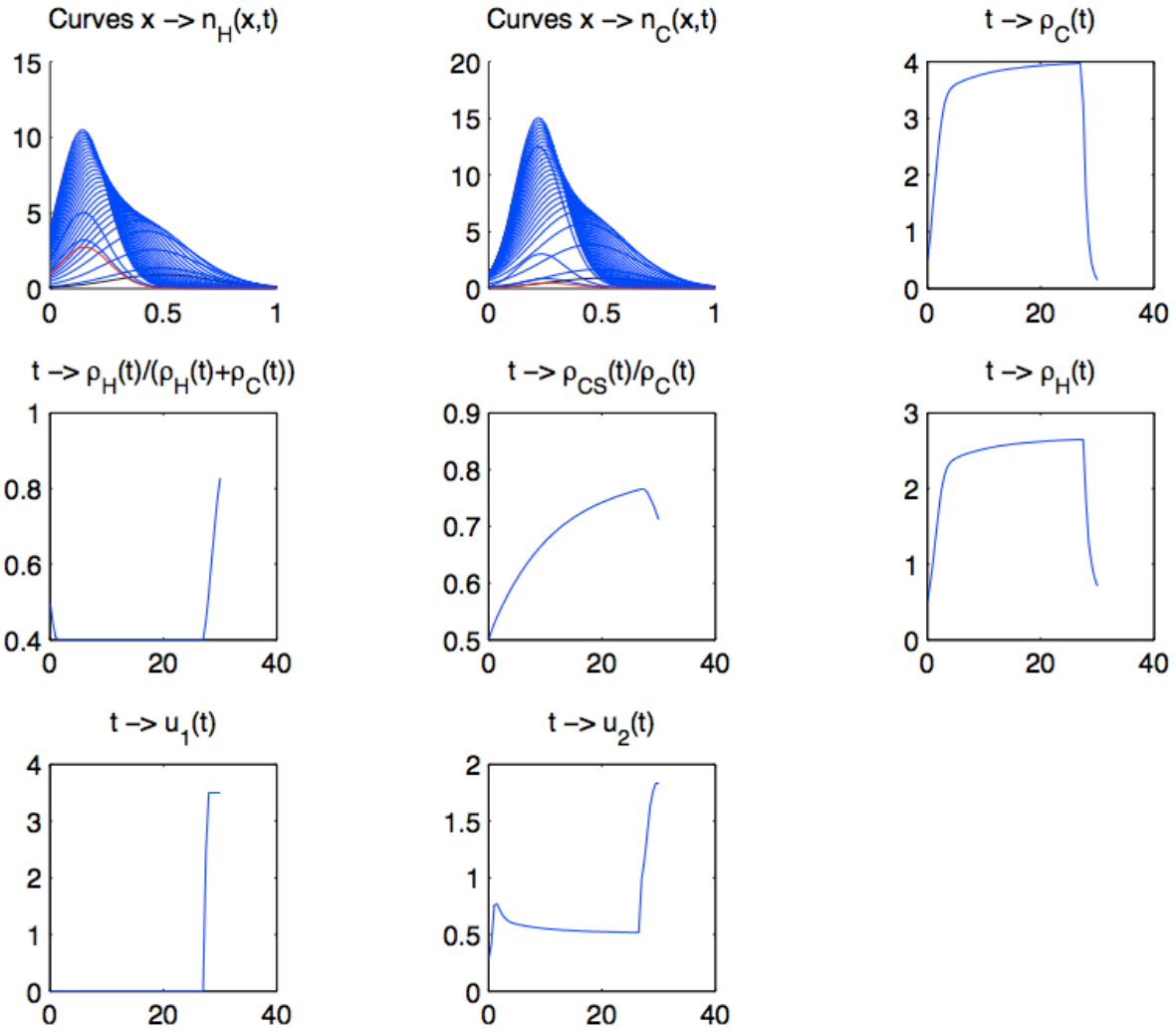
$$\boxed{n_H(\cdot, t) \simeq \delta_{x_H^\infty}, \quad n_C(\cdot, t) \simeq \delta_{x_C^\infty}} \quad (\delta_{x_{[H,C]}^\infty} \textit{ Dirac masses})$$

   i.e., healthy and tumour cells have concentrated at some given respective phenotypes x_H^∞ and x_C^∞.
3. A last transient **short-time arc**: $u_1 = u_1^{\max}$, $u_2 = u_2^{\max}$, along which the population of healthy and of tumour cells is very quickly decreasing.

Simulations illustrating this theorem

Simulation with $T = 30$

(optimisation using AMPL-IPOPT)



Interpretation

Neglecting the first transient arc, in a first approximation the optimal trajectory is made of two parts, the first one with $u_1 = 0$ and the second one with $u_1 = u_1^{\max}$.

Main idea:

1. Let the system naturally evolve to a phenotype concentration (long-time phase).
2. Then, apply the maximal quantity of drugs, during a short-time phase, in order to eradicate as many tumour cells as possible.

The second short-time phase is all the more efficient as the phenotypes are concentrated (hence, as the time T is large).

The proof of the theorem relies on two main facts.

First fact: asymptotic behaviour

Lemma

Assume that $u_1 = \mathrm{Cst} = \bar{u}_1$ and that $u_2(t) = \mathrm{Cst} = \bar{u}_2$. Then, $\forall (n_H(\cdot,0), n_C(\cdot,0))$

$$n_H(\cdot,t) \underset{t\to+\infty}{\longrightarrow} \delta_{x_H^\infty}, \quad n_C(\cdot,t) \underset{t\to+\infty}{\longrightarrow} \delta_{x_C^\infty},$$

i.e., healthy and tumour cells concentrate at some given respective phenotypes x_H^∞ and x_C^∞ (which can be characterised and computed).

Proof.

We show that $(\rho_H(t), \rho_C(t))$ satisfies integral *inequalities* with at each bound the solutions of a coupled system of (non-explosive) Riccati equations

$$\dot{z}_1(t) = z_1(t)(a_1 - b_{11}z_1(t) - b_{12}z_2(t))$$
$$\dot{z}_2(t) = z_2(t)(a_2 - b_{22}z_2(t) - b_{21}z_1(t)).$$

with nonnegative constant coefficients. This implies their convergence. The concentration follows from the exponential behaviour of $n_H(\cdot,t)$ and $n_C(\cdot,t)$. □

Second fact: an alternative optimal control problem

Lemma

Consider the following optimal control problem on the short-time interval $[t_1, T]$:

> *Find the best possible distribution* $n_C(\cdot, t_1)$ *such that, applying along* $[t_1, T]$ *the maximal quantity of drugs, we minimise the quantity* $\rho_C(T)$.

The answer is a Dirac mass:

$$n_C(\cdot, t_1) = \delta_{x_C^\infty}.$$

This lemma implies that, in order to kill as many tumour cells as possible, the drugs are most efficient when the tumour cells are concentrated on a given phenotype.

These two facts, combined with other remarks (showing for instance that T must be large, that the controls must be almost constant, etc.), allow to prove the theorem.

Comparison with "almost periodic" curative strategies

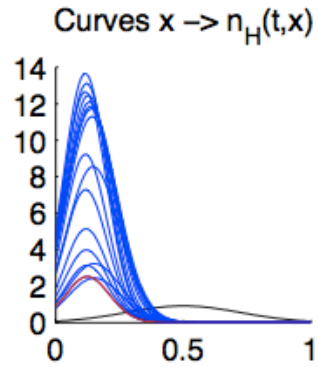
On the right: drugs given almost periodically, within $T = 60$.
$\rightarrow$ Far less efficient!!

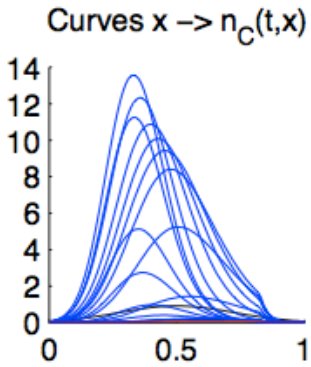
$$\rho_C(T) \simeq 0.03$$

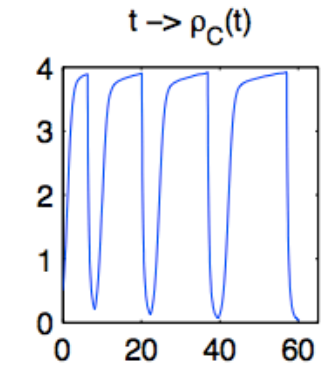
whereas using the previous strategy we had

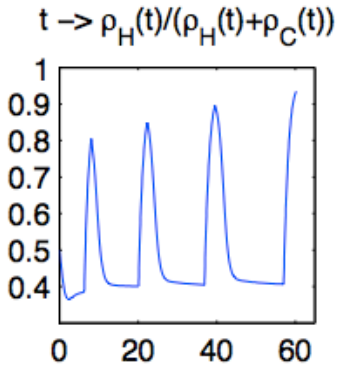
$$\rho_C(T) \simeq 10^{-6}$$

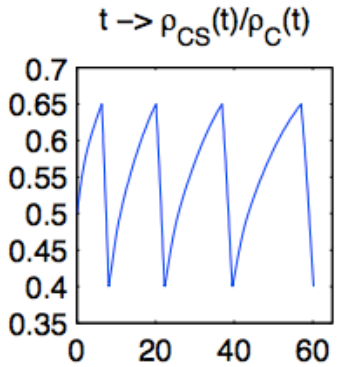
(optimisation using AMPL-IPOPT)

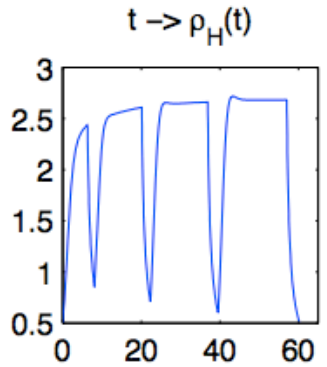


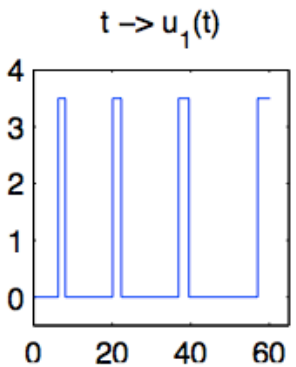


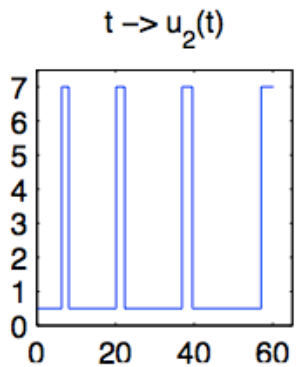






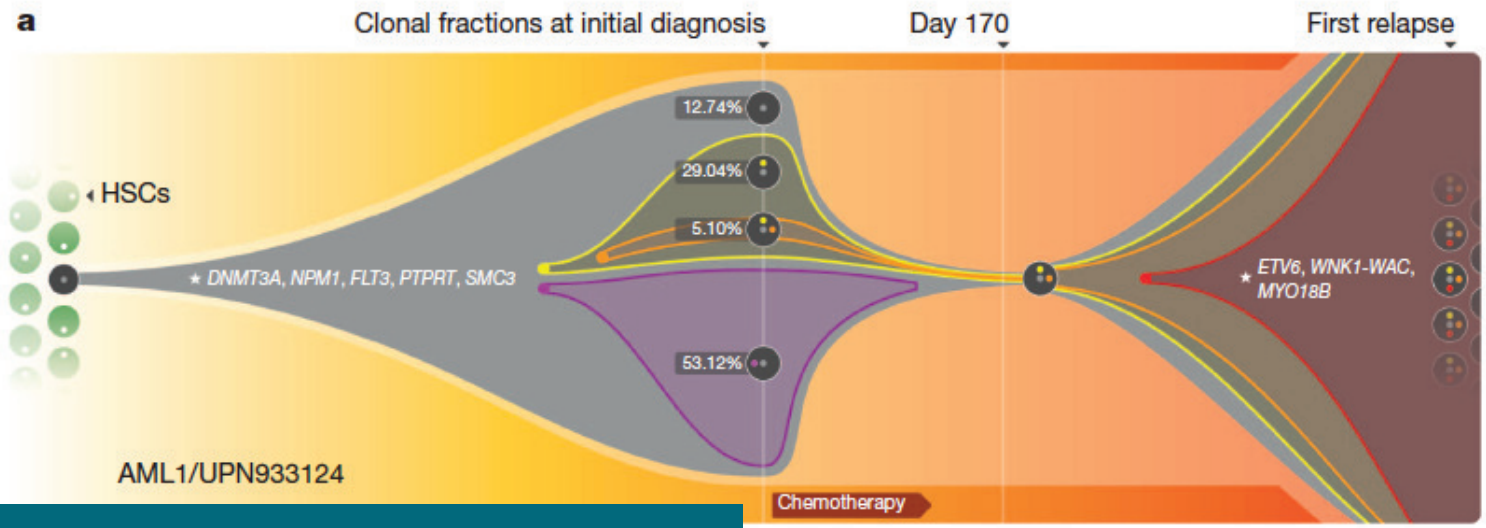




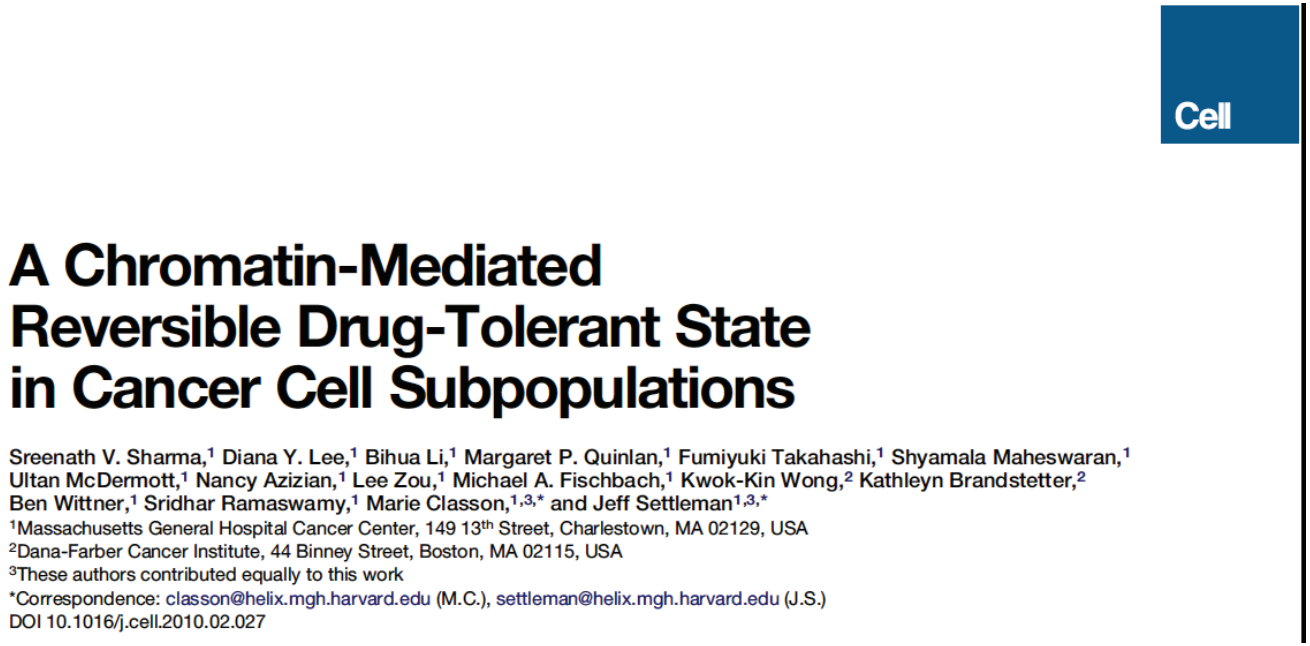


Limitations of this optimisation procedure, owing to the fact that the trait represents resistance to only one drug

- The model assumes *one* trait of resistance corresponding to *one* cytotoxic drug.
- However, overcoming resistance using this strategy may not be successful if too many types of resistance coexist, due to high phenotype heterogeneity.
- Phenotype heterogeneity within the tumour may thus reduce such strategy to nothing, unless a multidimensional phenotype may be considered.
- ... Unless also one could act very early to avoid the development of transient drug-resistant cell clones by epigenetic drugs or metabolism modifying strategies.



Extension of the IDE model to include a 2D phenotype



Cell

A Chromatin-Mediated Reversible Drug-Tolerant State in Cancer Cell Subpopulations

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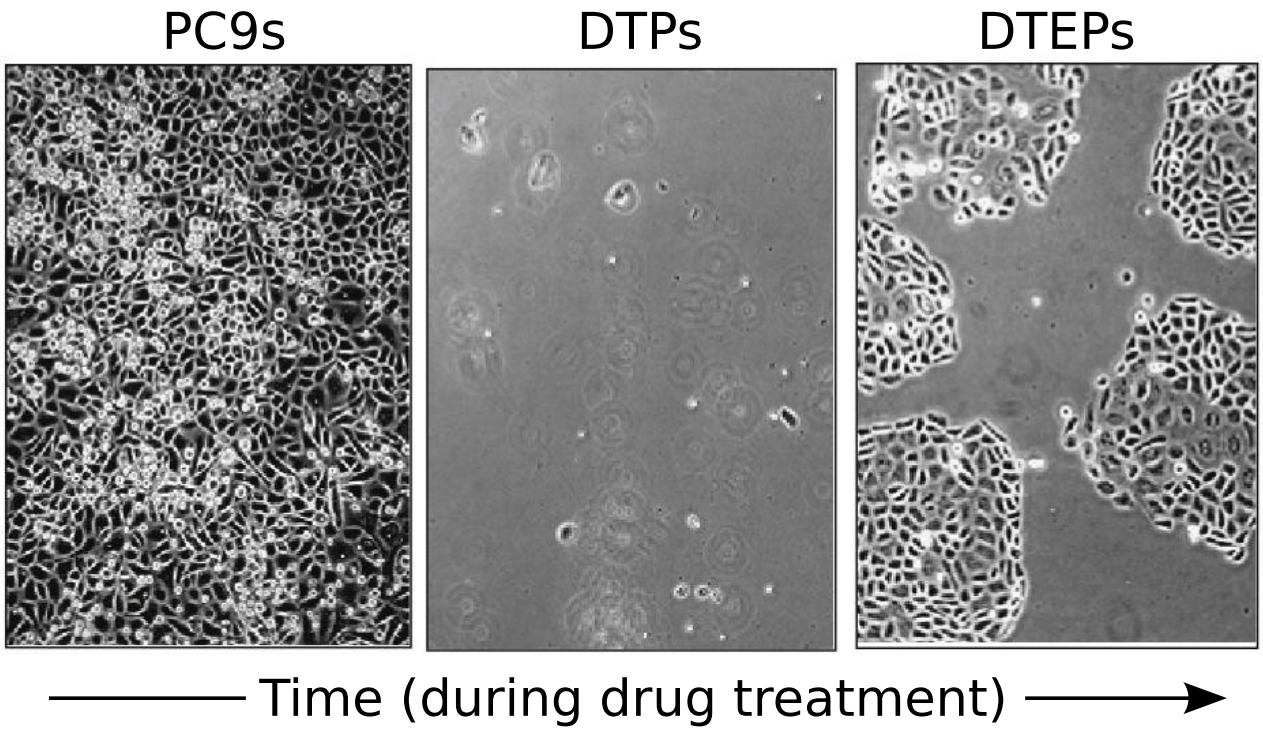
- Motivation: to account for biological observations on a reversible drug-resistant phenotype in cancer cell populations: *Sharma et al. 141:69–80, Cell 2010*
- Underlying hypothesis: epigenetic modifications affect differently survival and proliferation potentials in cancer cell populations submitted to high drug doses
- 2 proposed traits: x, stress survival potential ($\sim$ apoptosis inhibition?) and y, proliferation potential ($\sim$ cell division cycle enhancement?), both reversible
- An agent-based (AB) model shows the same behaviour for the cancer cell population

Sum-up of the *Sharma et al.* paper

- Population of PC9 (NSCLC) cells under high doses of drugs (e.g., gefitinib)
- 99.7% cells die, .3% survive in this maintained hostile drug environment: DTPs
- In the same hostile environment, 20% of DTPs resume proliferation: DTEPs
- Total reversibility to drug sensitivity is obtained by drug withdrawal, occurring after 9 doubling times for DTPs, and 90 doubling times for DTEPs
- Inhibition of epigenetic enzyme KDM5A blocks emergence of DTPs



2D phenotype-structured IDE model

- Initial (PC9) cancer cell population structured by 2 continuous variables:
  $x \in [0,1]$: normalised expression level of survival-potential, and
  $y \in [0,1]$: normalised expression level of proliferation-potential
  (both biologically relying on, e.g., levels of methylation in DNA and histones)

- Population density of cells with phenotypic expression (x, y) at time t, $n(x, y, t)$ satisfies

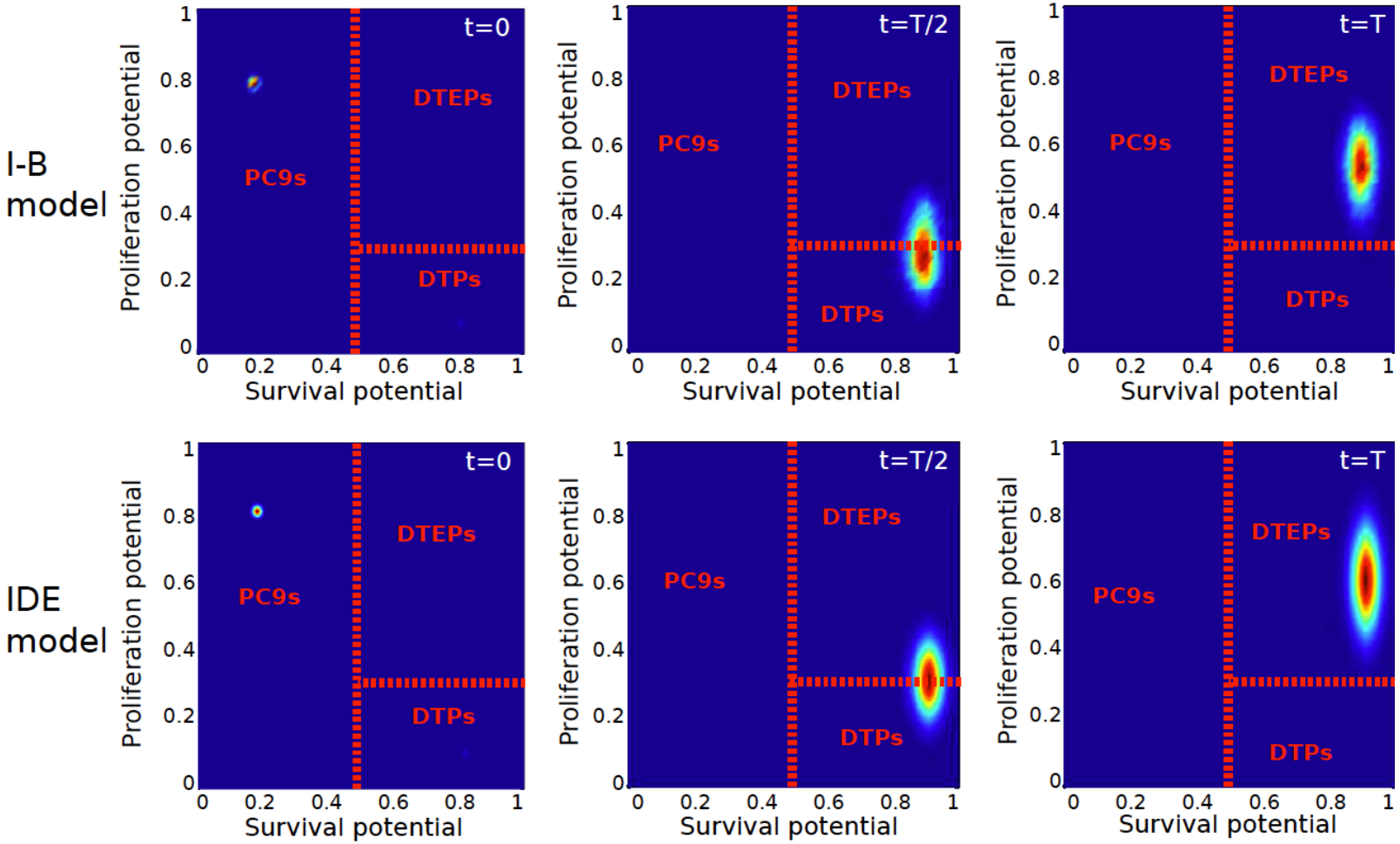
$$\frac{\partial n}{\partial t}(x,y,t) + \underbrace{\frac{\partial}{\partial y}\Big(v(x,c(t);\bar{v})n(x,y,t)\Big)}_{\substack{\text{stress-induced adaptation}\\ \text{of the proliferation level}}} = \underbrace{\Big[p(x,y,\varrho(t)) - d(x,c(t))\Big]n(x,y,t)}_{\text{selection}} + \underbrace{\beta\Delta n(x,y,t).}_{\substack{\text{non-genetic}\\ \text{phenotype instability}}}$$

- $\varrho(t) = \int_0^1 \int_0^1 n(x,y,t)\,\mathrm{d}x\mathrm{d}y$ is the global population density
- No-flux BCs

(*Chisholm et al., in revision, 2014*)

AB model and IDE model recover phenotype dynamics

e.g., during drug treatment (PC9s and DTPs present initially)

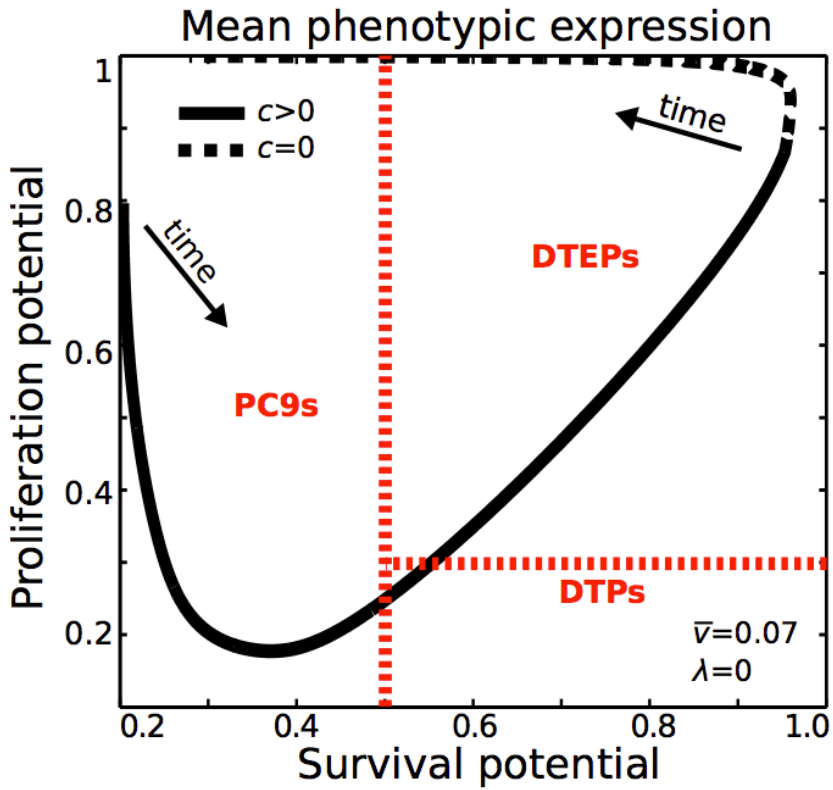


T is the simulation end-time: $0 \leq t \leq T$

(*Chisholm et al., in revision, 2014*)

AB model and IDE model recover phenotype dynamics

e.g., during drug exposure and after drug withdrawal: total recovery of drug sensitivity (here only PC9s present initially)



Adaptation is present $v < 0$

(*Chisholm et al., in revision, 2014*)

Use IDE model to address two questions

Q1. What can we expect if the drug dose is low?

Q2. Could genetic-mutations generate similar dynamics?

Consider $c(\cdot) = c$ and two scenarios:

(i) Only PC9s initially, adaptation is present ($v < 0$)

(ii) PC9s and a few DTPs initially, adaptation is absent ($v = 0$)

Q1. What can we expect if the drug dose is low?

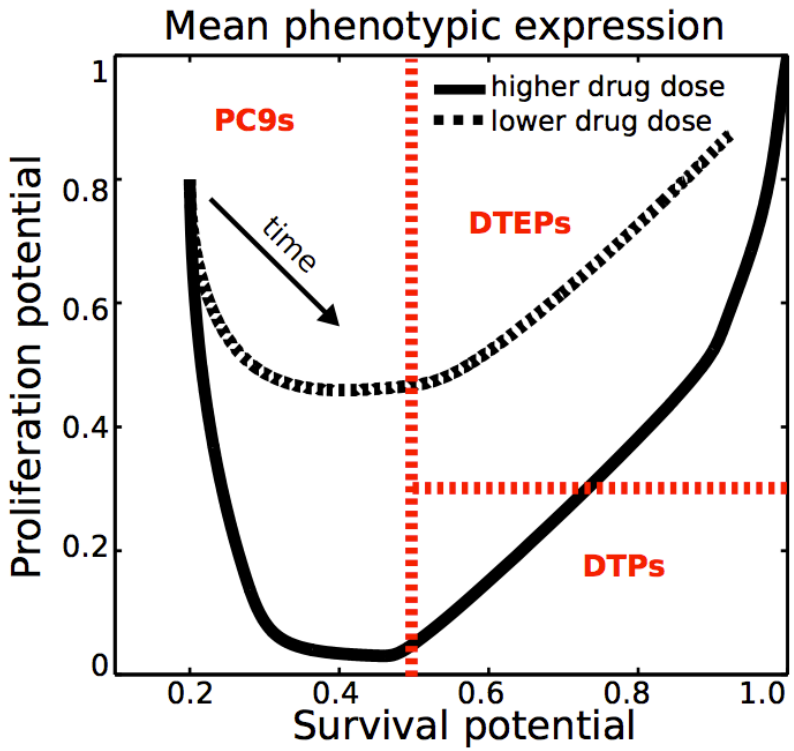
Definition (LC_γ dose)

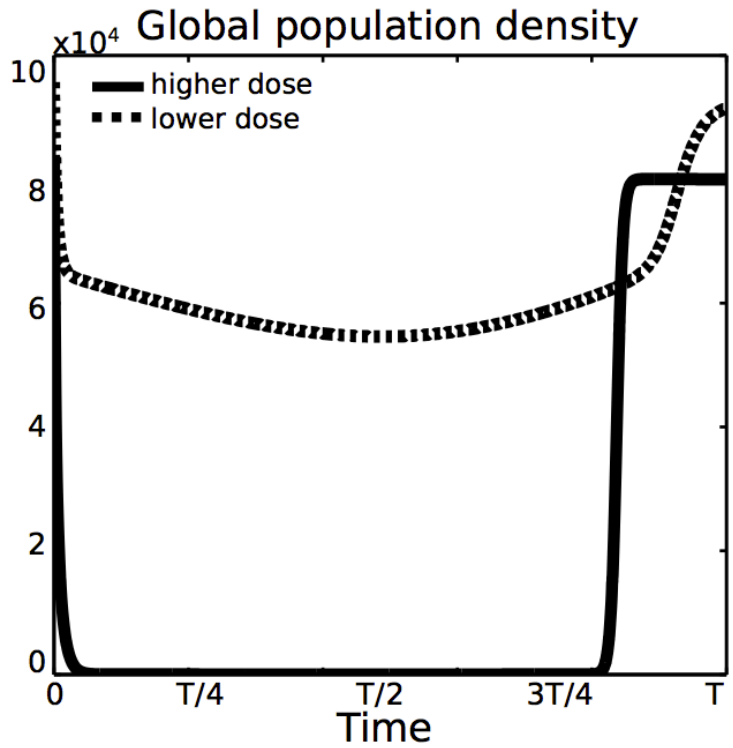
The drug dose required to kill γ% of the total cell population, in the initial stage of drug therapy, before the population starts to recover

- High c: $c \geq LC_{90}$ dose
- Low c: $c \leq LC_{50}$ dose

A1. High dose of cytotoxic drugs is necessary for the transient dominance of DTPs

Only PC9s initially



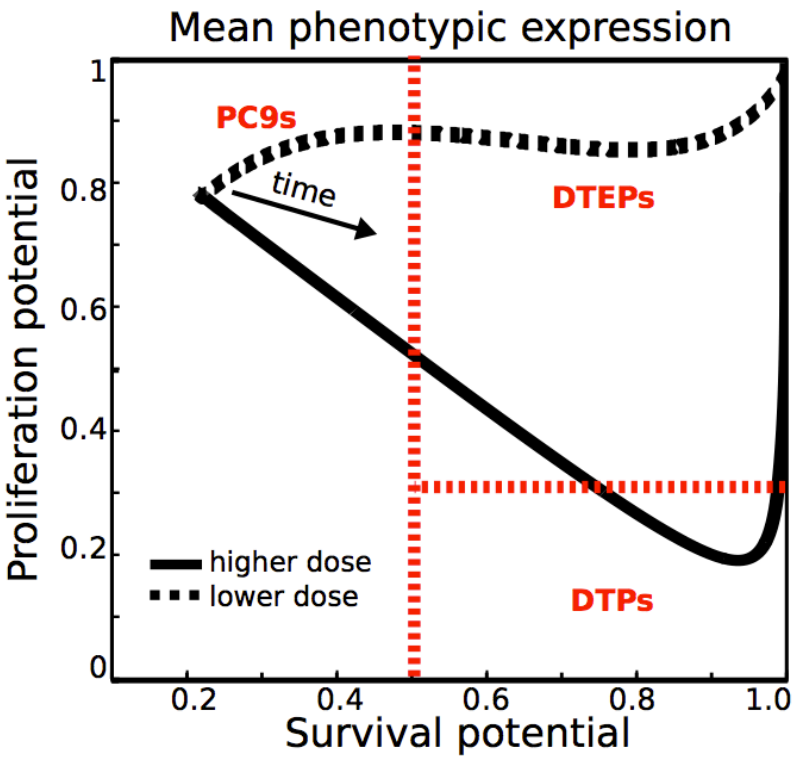


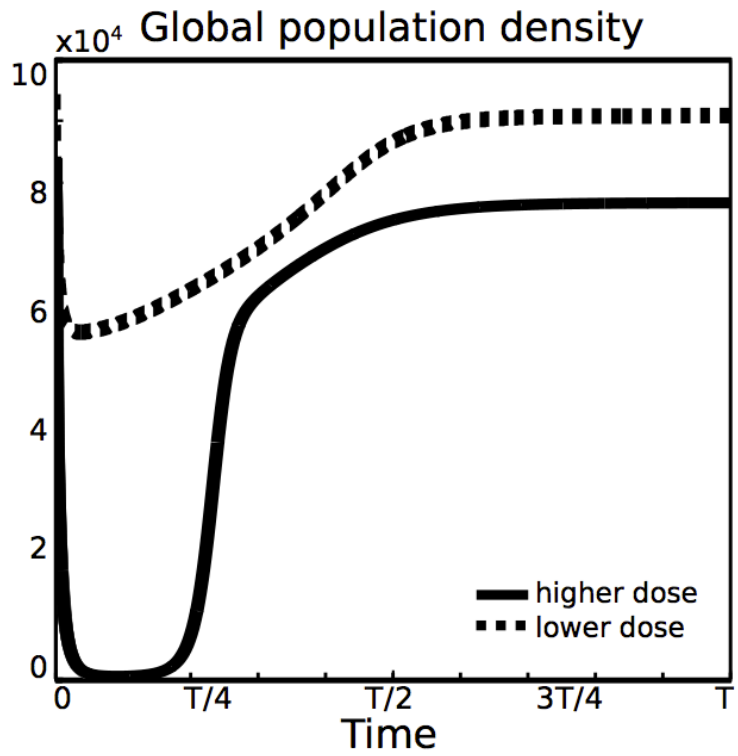
Adaptation is present $v < 0$

(*Chisholm et al., in revision, 2014*)

A1. High dose of cytotoxic drugs is necessary for the transient dominance of DTPs

DTPs and PC9s initially





Adaptation is absent $v = 0$

(*Chisholm et al., in revision, 2014*)

Q2. Could genetic mutations generate similar dynamics?

Consider the pure mutation model (*no diffusion, no stress-induced adaptation drift*)

$$\frac{\partial n}{\partial t}(x,y,t) = \underbrace{\Big[(1-\alpha)p(x,y,\varrho(t)) - d(x,c(t))\Big] n(x,y,t)}_{\text{birth and death term due to sheer selection}} + \underbrace{\alpha \int_0^1 \int_0^1 p(\xi,\eta,\varrho(t)) M(x,y|\xi,\eta;\sigma) n(\xi,\eta,t) d\xi\, d\eta}_{\text{birth term due to genetic mutations}},$$

where the mutation kernel is defined as,

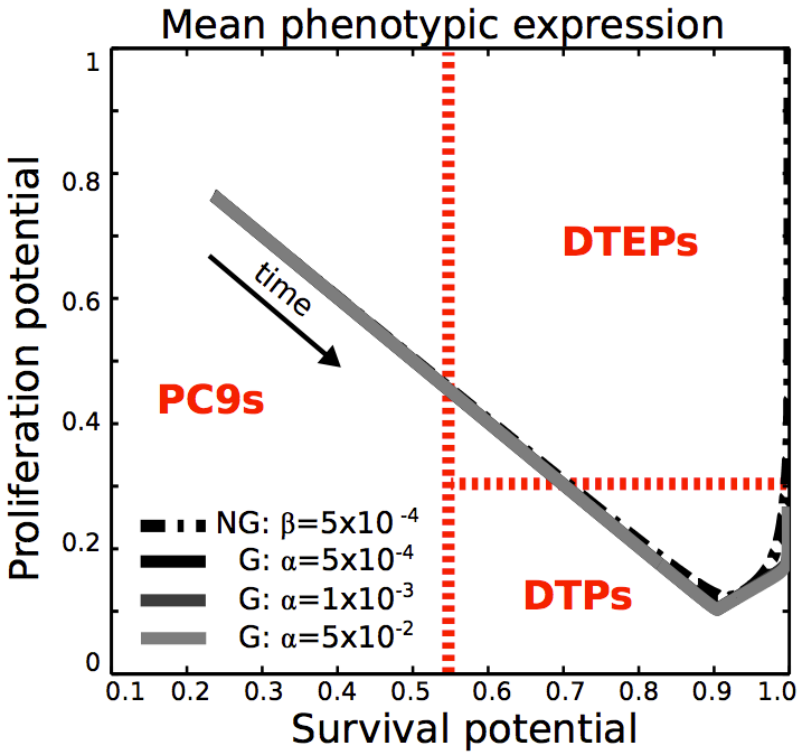
$$M(x,y|\xi,\eta;\sigma) := C_M e^{-\frac{(x-\xi)^2}{\sigma}} e^{-\frac{(y-\eta)^2}{\sigma}},$$

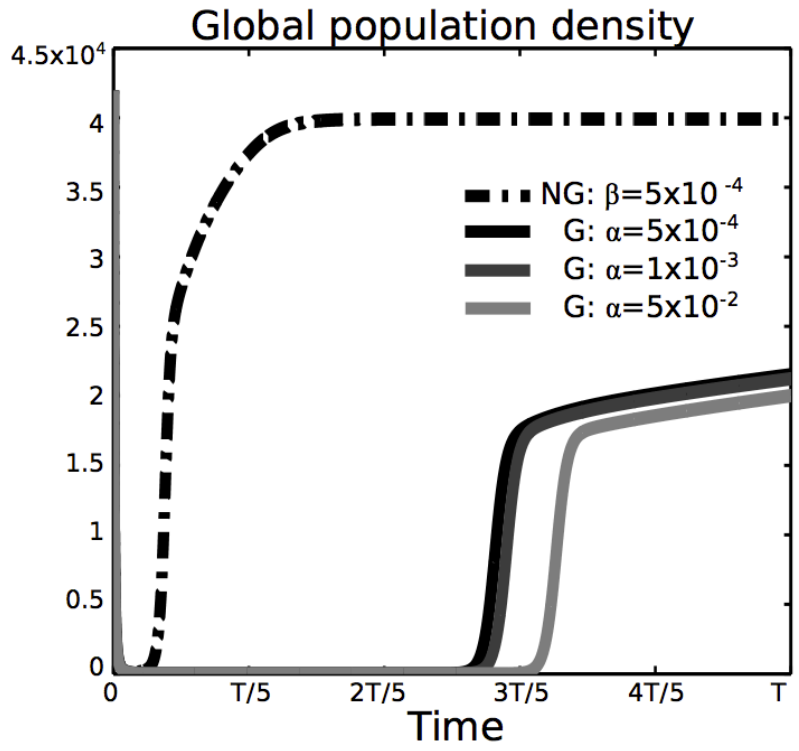
and C_M is a normalisation constant such that

$$\int_0^1 \int_0^1 M(x,y|\cdot,\cdot;\cdot)\mathrm{d}x\mathrm{d}y = 1.$$

A2. Genetic mutations cannot generate similar dynamics

- Initially there are DTPs and PC9s.
  - G: mutations and selection vs.
  - NG: non-genetic instability and selection

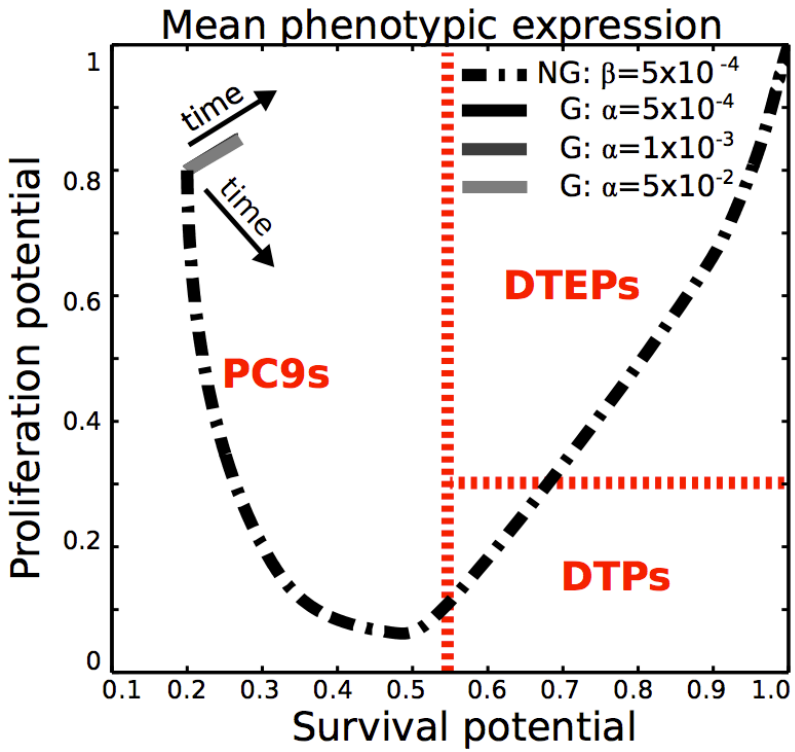


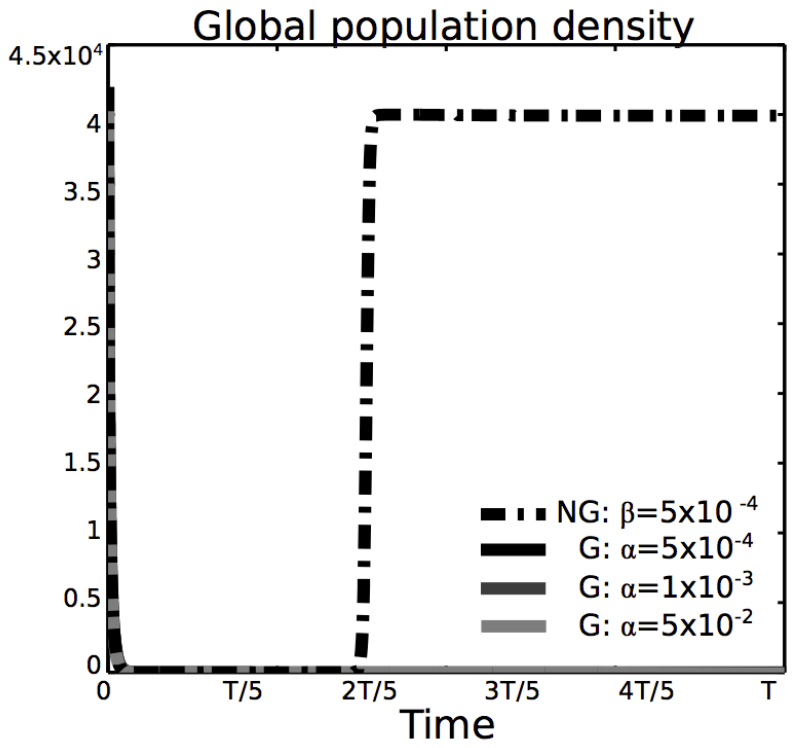


(*Chisholm et al., in revision, 2014*)

A2. Genetic mutations cannot generate similar dynamics

- Initially there are only PC9s.
  - G: mutations and selection vs.
  - NG: non-genetic instability, adaptation and selection





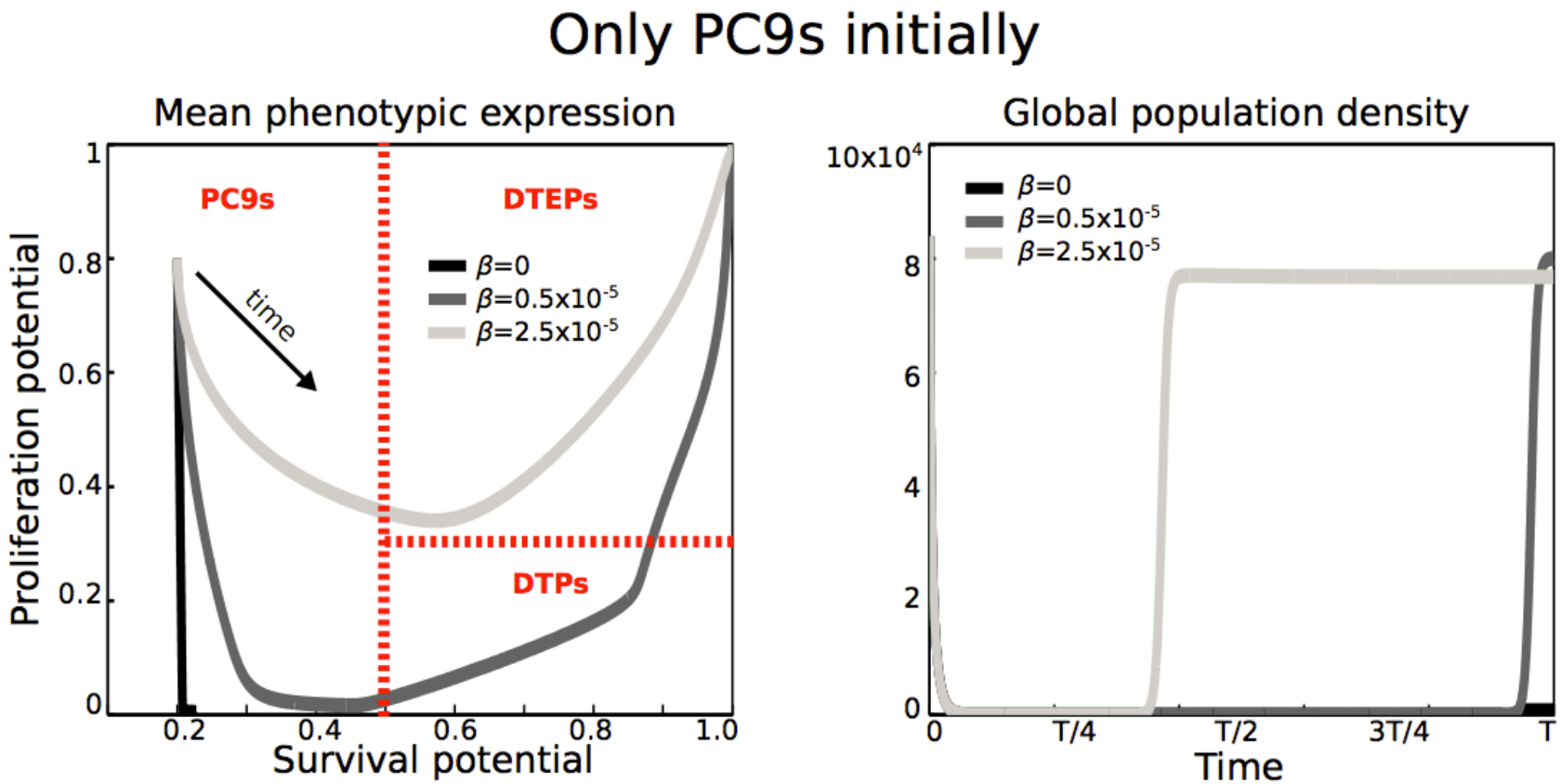
(*Chisholm et al., in revision, 2014*)

References

- Chisholm, RH, Lorenzi, T, Almeida, L, JC, Escargueil, A, Lorz, A. Emergence of drug tolerance in cancer cell populations: an evolutionary outcome of selection, non-genetic instability and stress-induced adaptation. *In revision*, 2014.
- Lorz, A, Lorenzi, T, JC, Escargueil, A, Perthame, B. Effects of space structure and combination therapies on phenotypic heterogeneity and drug resistance in solid tumors. *In revision*, 2014.
- Lorz, A, Lorenzi, T, Hochberg, ME, JC, Perthame, B. Populational adaptive evolution, chemotherapeutic resistance and multiple anticancer therapies. *ESAIM: Mathematical Modelling and Numerical Analysis*, 47(02):377-399, 2013.
- Lorz, A, Mirrahimi, S, Perthame, B. Dirac mass dynamics in multidimensional nonlocal parabolic equations. *Communications in Partial Differential Equations*, 36(6):1071-1098, Jan 2011.
- Perthame, B, Barles, G. Dirac concentrations in Lotka-Volterra parabolic PDEs. *Indiana University Mathematics Journal*, 57(7):3275-3302, 2008.
- Trélat, E, Lorz, A, JC. Optimal therapy in cancer treatment. *Work in progress*, 2014.

A0. Non-genetic instability is crucial for the emergence of DTEPs

[Q0. Is non-genetic instability necessary in the development of drug-tolerance?]

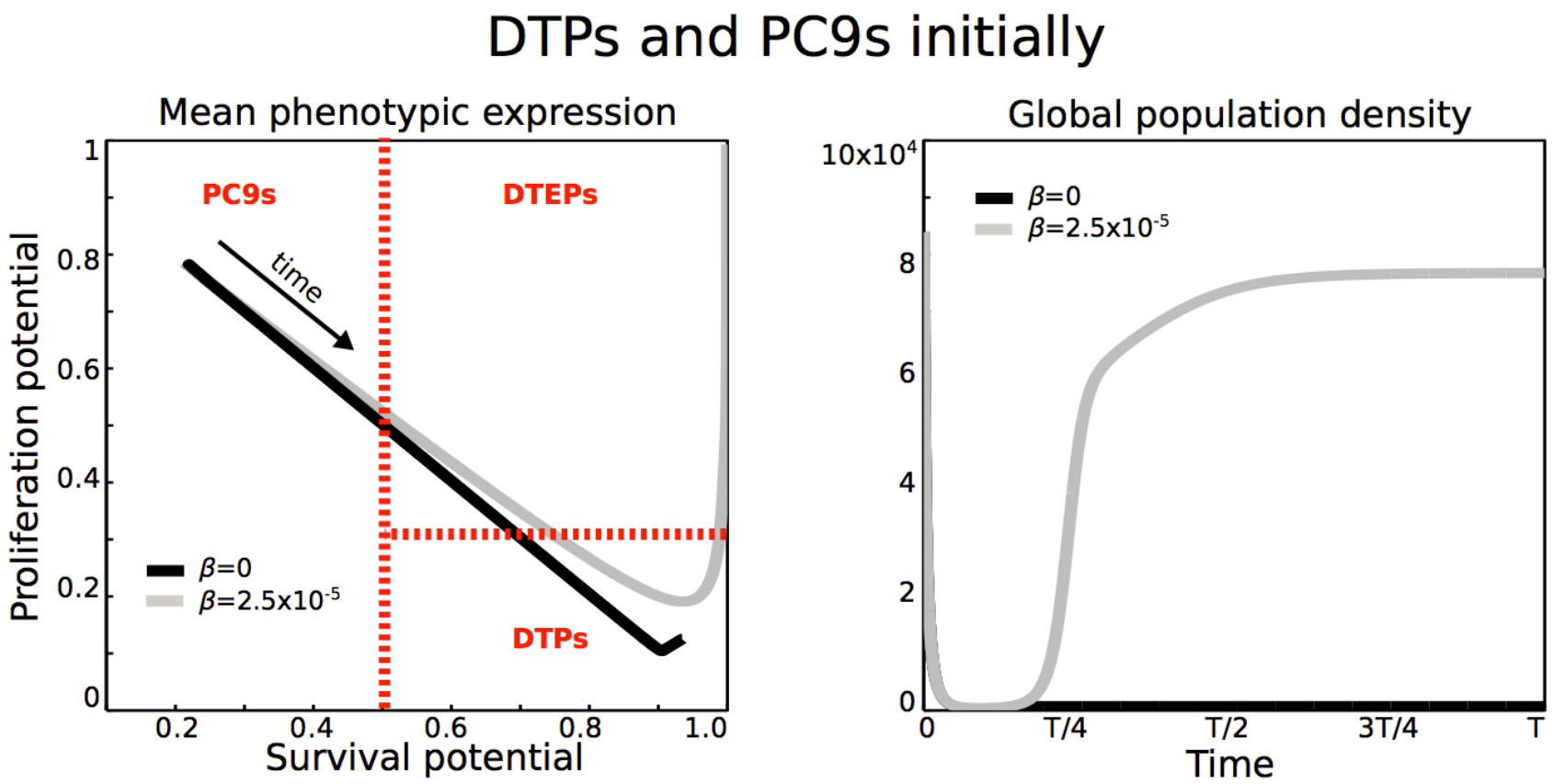


Adaptation is present $v < 0$

(*Chisholm et al., in revision, 2014*)

A0. Non-genetic instability is crucial for the emergence of DTEPs

[Q0. Is non-genetic instability necessary in the development of drug-tolerance?]



Adaptation is absent $v = 0$

(*Chisholm et al., in revision, 2014*)

Notes about the 'cooking recipes' used in the simulations (1)

In this version of the simulations (used throughout in the sequel)

$$r_H(x) = \frac{1.5}{1+x^2}, \quad r_C(x) = \frac{3}{1+x^2},$$

$$d_H(x) = \frac{1}{2}(1-0.1x), \quad d_C(x) = \frac{1}{2}(1-0.3x),$$

$$u_1^{\max} = 3.5, \quad u_2^{\max} = 7,$$

and the initial data are

$$n_H(0,x) = C_0 \exp(-(x-0.5)^2/\varepsilon), \quad n_C(0,x) = C^0 \exp(-(x-0.5)^2/\varepsilon),$$

with $\varepsilon > 0$ small (typically, we will take either $\varepsilon = 0.1$ or $\varepsilon = 0.01$), and where $C_0 > 0$ is such that

$$\rho_H(0) + \rho_C(0) = 1.$$

Notes about the 'cooking recipes' used in the simulations (2)

The closer to 1 is the variable x, the more resistant are the tumour cells. The choice done in *Lorz et al. 2013* is

$$\mu_H(x) = \frac{0.2}{0.7^2 + x^2}, \quad \mu_C(x) = \frac{0.4}{0.7^2 + x^2}.$$

Note that, with this choice of functions, if we take constant controls u_1 and u_2, with

$$u_1(t) = \mathrm{Cst} = u_1^{\max} = 3.5, \qquad u_2(t) = \mathrm{Cst} = 2,$$

then we can kill all tumour cells (at least, they decrease exponentially to 0), and no optimisation is necessary.

Notes about the 'cooking recipes' used in the simulations (3)

The environment variables $I_{[H,C]}(t)$ defined by

$$\begin{aligned} I_H(t) &= a_{HH}\rho_H(t) + a_{HC}\rho_C(t), \\ I_C(t) &= a_{CH}\rho_H(t) + a_{CC}\rho_C(t), \end{aligned} \tag{4}$$

and

$$\rho_H(t) = \int_0^1 n_H(x,t)\,dx, \qquad \rho_C(t) = \int_0^1 n_C(x,t)\,dx.$$

have been chosen such that

$$a_{HH} = 1, \quad a_{CC} = 1, \quad a_{HC} = 0.07, \quad a_{CH} = 0.01, \quad \alpha_H = 0.01, \quad \alpha_C = 1,$$

which means in particular that in the limiting logistic terms in the model, intraspecific competition is overwhelmingly higher than interspecific competition, i.e., cell growth is mainly limited by access to resources, and very little by frontal competition between cancer and healthy cells, a choice done on biological grounds (*cancer cells and healthy cells are not thriving on the same metabolic niche, e.g., aerobic vs. glycolytic metabolisms*). As a consequence, as in classical Lotka-Volterra models with competition, the choice of these parameters will lead in the simulations to asymptotic coexistence of the two species, healthy and cancer, in a non trivial equilibrium state.

1st IDE model, asymptotic behaviour, details on the proof: convergence

Assume that $u_1 = \mathrm{Cst} = \bar{u}_1$, and that $u_2(t) = \mathrm{Cst} = \bar{u}_2$. Then, for any initial population of healthy and of tumour cells, $(\rho_H(t), \rho_C(t))$ converges to the equilibrium point $(\rho_H^\infty, \rho_C^\infty)$, which can be exactly computed as follows.
Let $a_1 \geq 0$ and $a_2 \geq 0$ be the smallest nonnegative real numbers such that

$$\frac{r_H(x)}{1+\alpha_H \bar{u}_2} - \bar{u}_1 \mu_H(x) \leq d_H(x) a_1 \quad \text{and} \quad \frac{r_C(x)}{1+\alpha_C \bar{u}_2} - \bar{u}_1 \mu_C(x) \leq d_C(x) a_2. \qquad (1)$$

Then $(\rho_H^\infty, \rho_C^\infty)$ is the unique solution of the system (invertible as a consequence of the fact that intraspecific competition is assumed higher than interspecific competition)

$$a_{HH}\rho_H^\infty + a_{HC}\rho_C^\infty = a_1,$$
$$a_{CH}\rho_H^\infty + a_{CC}\rho_C^\infty = a_2.$$

Furthermore, if $A_H \subset [0,1]$ (resp., $A_C \subset [0,1]$) is the set of all points such that equalities hold in (1), then the supports of the probability measures $\nu_H(t) = \frac{n_H(x,t)}{\rho_H(t)}\,dx$ and $\nu_C(t) = \frac{n_C(x,t)}{\rho_C(t)}\,dx$ converge respectively to A_H and A_C. In particular, if A_H is reduced to a singleton x_H^∞, and if A_C is reduced to a singleton x_C^∞ (cases of our simulations), then $\nu_H(t)$ and $\nu_C(t)$ converge for the vague topology respectively to the Dirac masses $\delta_{x_H^\infty}$ and $\delta_{x_C^\infty}$ for some $x_H^\infty \in [0,1]$ and $x_C^\infty \in [0,1]$ as t tends to $+\infty$.

Asymptotic behaviour, other details : concentration on $x^\infty_{[H,C]}$

Indeed, by integration, we have

$$n_H(x,t) = n_H^0(x)\exp\left(\left(\frac{r_H(x)}{1+\alpha_H\bar{u}_2} - \bar{u}_1\mu_H(x)\right)t - d_H(x)\left(a_{HH}\int_0^t \rho_H(s)\,ds + a_{HC}\int_0^t \rho_C(s)\,ds\right)\right),$$

$$n_C(x,t) = n_C^0(x)\exp\left(\left(\frac{r_C(x)}{1+\alpha_C\bar{u}_2} - \bar{u}_1\mu_C(x)\right)t - d_C(x)\left(a_{CH}\int_0^t \rho_H(s)\,ds + a_{CC}\int_0^t \rho_C(s)\,ds\right)\right).$$

Then, since for large t, we have $\int_0^t \rho_H(s)\,ds \sim \rho_H^\infty t$ and $\int_0^t \rho_C(s)\,ds \sim \rho_C^\infty t$, the asymptotic behaviour of $n_H(x,t)$ and of $n_C(x,t)$ depends on the functions

$$b_H(x) = \frac{r_H(x)}{1+\alpha_H\bar{u}_2} - \bar{u}_1\mu_H(x) - d_H(x)(a_{HH}\rho_H^\infty + a_{HC}\rho_C^\infty),$$

$$b_C(x) = \frac{r_C(x)}{1+\alpha_C\bar{u}_2} - \bar{u}_1\mu_C(x) - d_C(x)(a_{CH}\rho_H^\infty + a_{CC}\rho_C^\infty),$$

whose maxima on $[0,1]$ may be shown to be both zero, with the choices made for the coefficients $a_{[H,C][H,C]}$. The points at which these maxima are attained (A_H and A_C, generically singletons x_H^∞ and x_C^∞) are the supports of the announced Dirac masses.

Second fact: an alternative optimal control problem

Lemma

Consider the following optimal control problem on the short-time interval $[t_1, T]$:

> *Find the best possible distribution* $n_C(\cdot, t_1)$ *such that, applying along* $[t_1, T]$ *the maximal quantity of drugs, we minimise the quantity* $\rho_C(T)$.

The answer is a Dirac mass:

$$n_C(\cdot, t_1) = \delta_{x_C^\infty}.$$

This lemma implies that, in order to kill as many tumour cells as possible, the drugs are most efficient when the tumour cells are concentrated on a given phenotype.

These two facts, combined with other remarks (showing for instance that T must be large, that the controls must be almost constant, etc.), allow to prove the theorem.

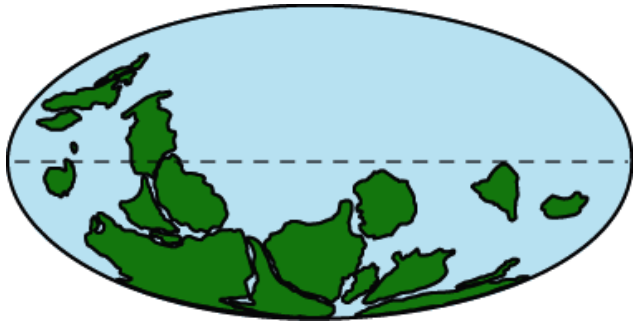
What could be models of cancer cell population dynamics identifiable from genome samples in single cells?

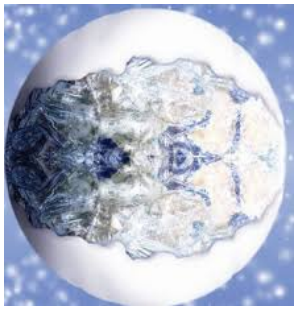
We need several modules (not all of them presently at hand) to design such a model:

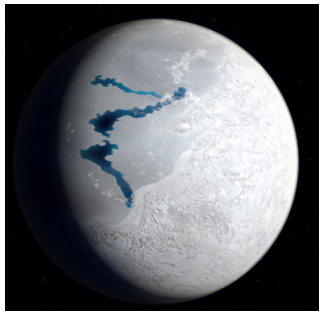
- A (time) dynamic deterministic structured model of cell population behaviour with phenotype variability and evolutionary (relevant trait) dynamics; we have experience of such PDE models: transport, reaction-diffusion, integro-differential
- Intracellular molecular deterministic models for the concentration of relevant mRNAs and proteins to determine cell fates, e.g., of nodal antagonist pairs X,Y such as transcription factors PU.1/GATA1 for the choice of myeloid vs. erythroid lineages in HSCs: relatively easy to design and classic by sets of ODEs
- For each antagonism, a stochastic process Z at the gene expression level, where would lie the (epigenetic?? TET2, etc.) source of phenotypic heterogeneity, randomly determining ODE parameters and whose parameters would themselves depend on tissue environment variables; prototypes by Mackey and Yvinec
- Upscaling principles to integrate models from cell ODEs to tissue physiologically structured PDEs, making phenotype signatures from single cell genome samples
- Environment variables would result from integration, at the tissue level, of such "readouts" from single cell characteristics; their concentrations would determine phenotypes in cell populations; see e.g., Friedman et al. J Diff Eq 2009, 2012

From local to global, and back: a metaphor from geophysics

- At the planet level, albedo ratio: reflection (cooling) vs. refraction (warming) of sunbeams on ice crust vs. ocean water, plus greenhouse effect (warming)
- At the most elementary level (here simplified): $H_2O + CO_2 \leftrightharpoons H^+ + HCO_3^-$, i.e., CO_2 emission (greenhouse warming) vs. CO_2 sequestration (cooling)
- Environment variable, from global to local: temperature (of the reaction)
- Global cooling: state of the Earth 650 million years ago ("Snowball Earth")

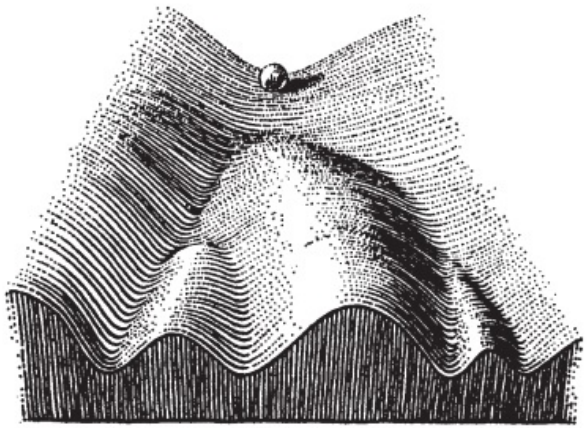




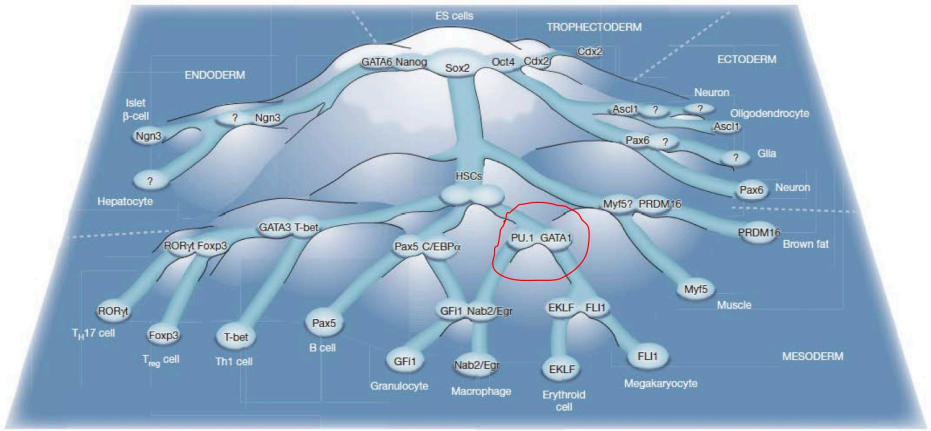


- *NB: Stable equilibrium? (M. Budyko)... yes, but only if one does not take into account volcanic activity, that can pierce the ice crust and release enormous quantities of gases (CO_2, CH_4), contributing to re-establishing the greenhouse effect, which actually happened (ice melt -635 My?) and led to the Cambrian multicellular explosion about 540 million years ago, from which we were begotten*

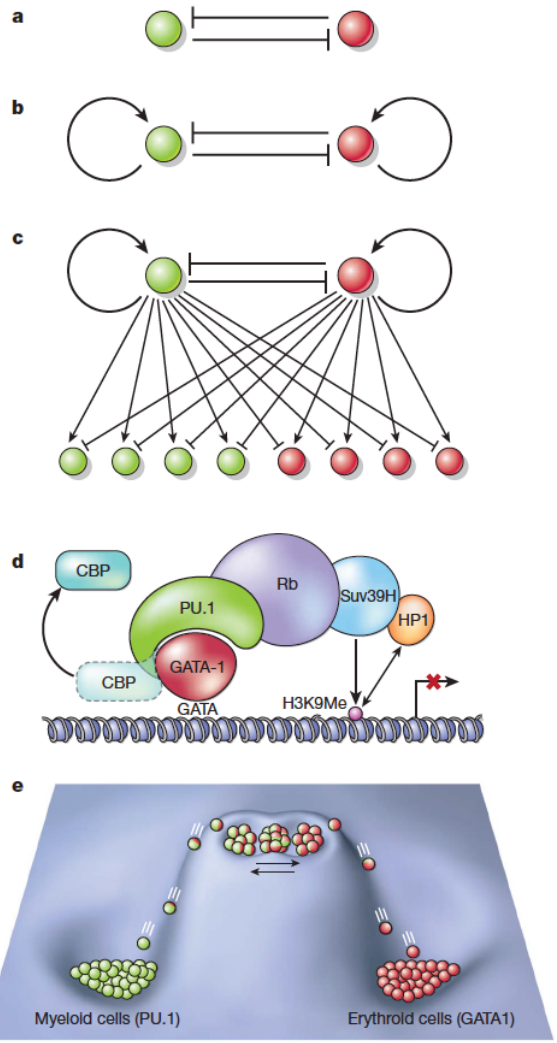
Illustrations from Graf & Enver, Nature 2009



[Classic Waddington landscape]



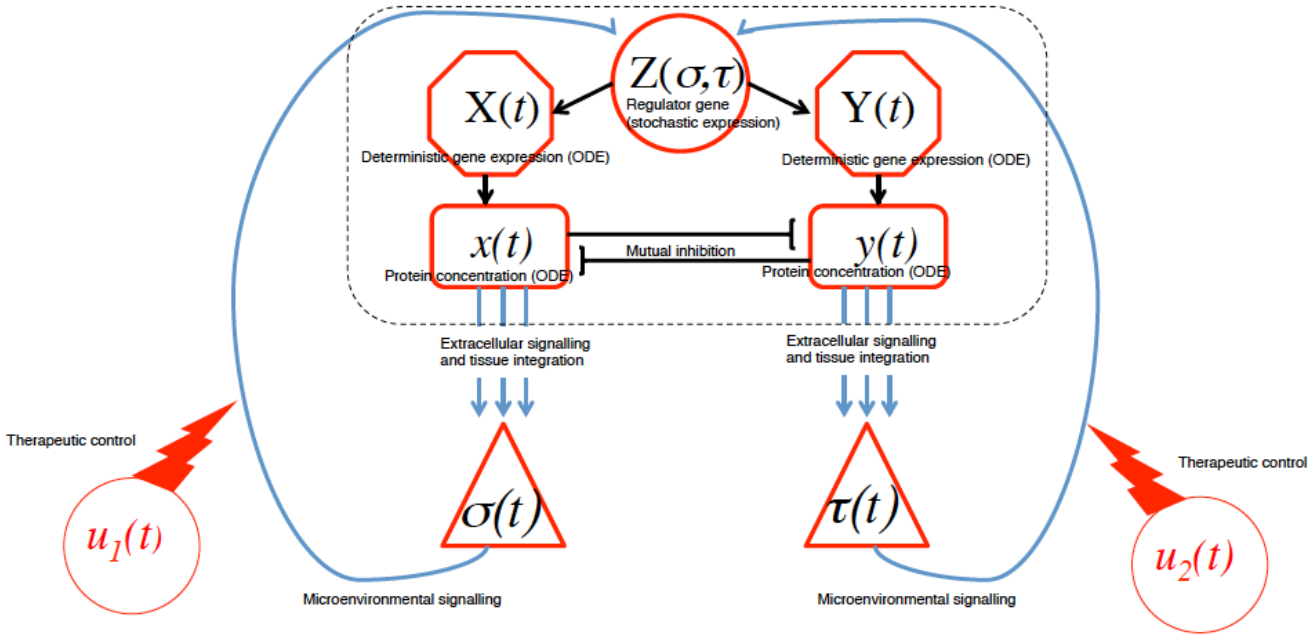
Stem cell fate: modern version by Tariq Enver



Zoom on the PU.1/GATA1 node

Sketched candidate model for 2 antagonistic genes X, Y

- Stochastic process Z to represent regulator gene expression (epigenetic? TET2?)
- ODEs (or possibly intracellular reaction-diffusion PDEs) for mRNA expression of antagonistic genes X, Y and for resulting synthesised proteins x, y
- Environment (=tissue) signal production by integration of intracellular protein concentrations x and y (or of their extracellular outputs),
- Extracellular signals σ and τ (possibly controlled by therapeutic molecules $u_1(t)$, $u_2(t)$) go to the nucleus to control stochastic expression of regulator gene Z



- Dynamics of cell population density $\varphi(X, Y, Z, t)$, structured in traits X, Y, Z

$$\frac{\partial \varphi}{\partial t} + \frac{\partial}{\partial X}\left(\varphi \frac{dx}{dt}\right) + \frac{\partial}{\partial Y}\left(\varphi \frac{dy}{dt}\right) + \mathcal{L}_Z \varphi = R.\varphi$$

Possible candidate equations for the dynamics of the model

- Z stochastic process controlled by $\sigma(t), \tau(t), u_1(t), u_2(t)$, with outputs on transcription $v(Z), w(Z)$ for bursting frequency ($V_a = V_{a_0}.v(Z)$, $W_a = W_{a_0}.w(Z)$: effects on launching transcription), and $f(Z), g(Z)$ amplification terms representing bursting magnitude (mRNA concentrations $Xf(Z)$ and $Yf(Z)$ in RHS representing bursting amplitude as seen on transcriptional effects on protein concentrations x, y) and

$$\mathcal{L}_Z\varphi = -\lambda(\sigma,\tau,Z)\varphi(t,X,Y,Z) + \int_0^Z \lambda(\sigma,\tau,\zeta)\varphi(t,X,Y,\zeta)\kappa(\zeta,Z)\,d\zeta + \frac{\partial}{\partial Z}(-\theta Z\varphi)$$

- X, Y: zero-order ultrasensitivity switches representing bursting of transcription in genes X and Y ($0 \leq X, Y \leq 1$), with $\frac{V_a}{V_i}$ and $\frac{W_a}{W_i}$ around threshold 1 ($\frac{V_a}{V_i}$ or $\frac{W_a}{W_i} > 1$: gene on; $\frac{V_a}{V_i}$ or $\frac{W_a}{W_i} < 1$: gene off, with steep switch):

$$\frac{dX}{dt} = V_a.\frac{1-X}{J_a+1-X} - V_i.\frac{X}{J_i+X}, \qquad \frac{dY}{dt} = W_a.\frac{1-Y}{K_a+1-Y} - W_i.\frac{Y}{K_i+Y}$$

- x, y: intracellular protein concentrations with mutual inhibition of synthesis:

$$\frac{dx}{dt} = -\mu x + \frac{\alpha_1 x^n}{k_1+x^n}.\frac{1}{1+\frac{y}{\gamma_1}} + Xf(Z), \qquad \frac{dy}{dt} = -\nu x + \frac{\alpha_2 y^n}{k_2+y^n}.\frac{1}{1+\frac{x}{\gamma_2}} + Yg(Z)$$

- σ, τ: tissue signalling (including therapeutic control) obtained by extracellular efflux of proteins x and y and their integration at the cell population level:

$$\sigma(t) = \frac{u_1(t) + \int\int x\varphi(t,X,Y)\,dX\,dY}{\int\int \varphi(t,X,Y)\,dX\,dY}, \qquad \tau(t) = \frac{u_2(t) + \int\int y\varphi(t,X,Y)\,dX\,dY}{\int\int \varphi(t,X,Y)\,dX\,dY}$$

Therapeutic means of action $u_1(t)$, $u_2(t)$ to be optimised

- Classical drugs acting on proliferation: with mechanisms more or less known at the individual cell level (cytotoxic, cytostatic, redifferentiating agents) or at the tissue level (antiangiogenic, supporting tissue modifiers)
- "Epigenetic" drugs acting on DNA methylation by tissue metabolism modifications or on histone acetylation (HDAC inhibitors): mechanisms not well known, nor sufficiently clinically assessed thus far, but clinical essays underway
- IPS therapies? Dedifferentiating cancer cells (using Yamanaka's 4 genes Oct3/Oct4, SOX2, KLF4 and c-myc, plus NANOG or other), then need to guide (= control) redifferentiation from induced pluripotent stem cells to normal cells
- Possible pitfalls of IPS therapies (i.e., designing guidelines to establish constraints for optimal control): non viability, non mastered proliferation, remnants of initial cell phenotypes in IPS cells, errors at nodes in going down phylogenetic trees...
- Objectives in optimal control strategies: targeting phenotypic signatures characteristic ("phylognomonic") of the desired cell population phenotypes