Drug resistance in cancer cell populations: Mathematical and biological assessment

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Drug resistance in cancer cell populations as evolutionary phenomenon

- *"Nothing in biology makes sense except in the light of evolution"* (Thedosius Dobzhansky, The American Biology Teacher 35 (3): 125-129, 1973)
- Cancer cell populations, under the pressure of anticancer drug stress, evolve as any population of living individuals to ensure their survival as a whole
- But just what is *evolution*, and what is mere *adaptation*?

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, with changes in the sequence of base pairs in the DNA (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

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.

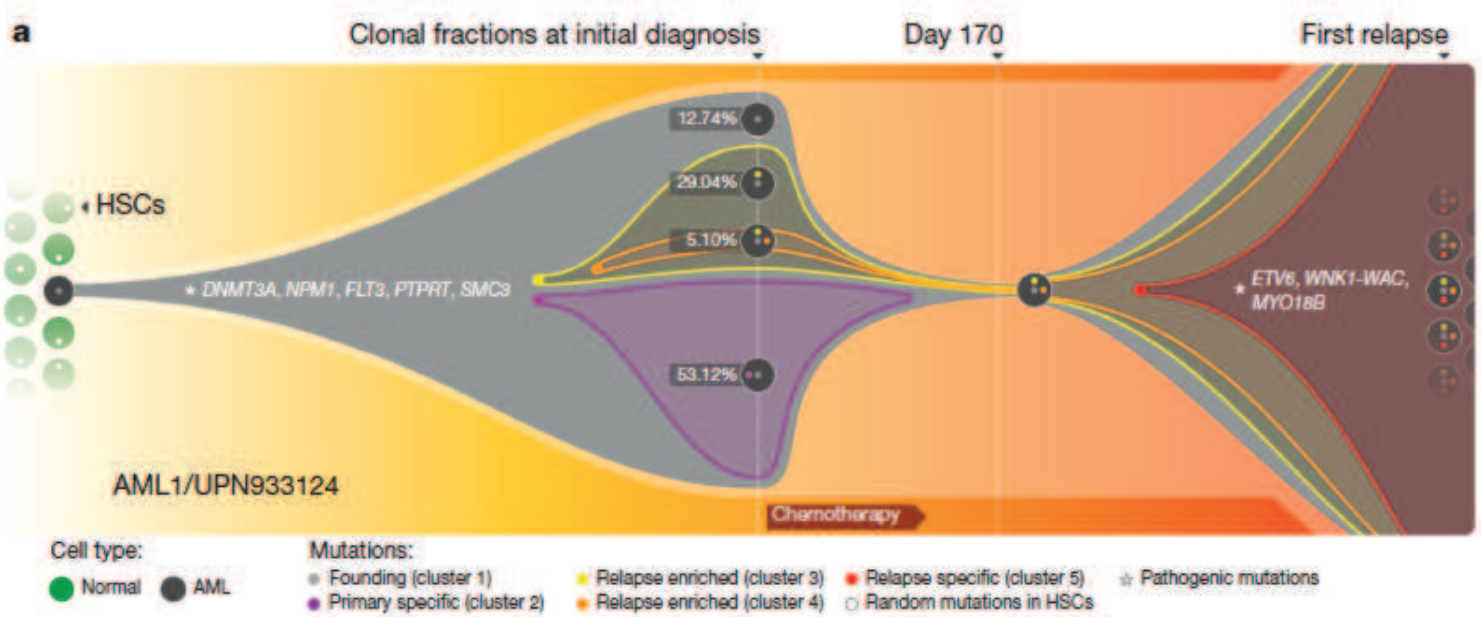
Drug resistance: how does it work?

- 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 ATP transporters, 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?*

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 ($x = 1$).

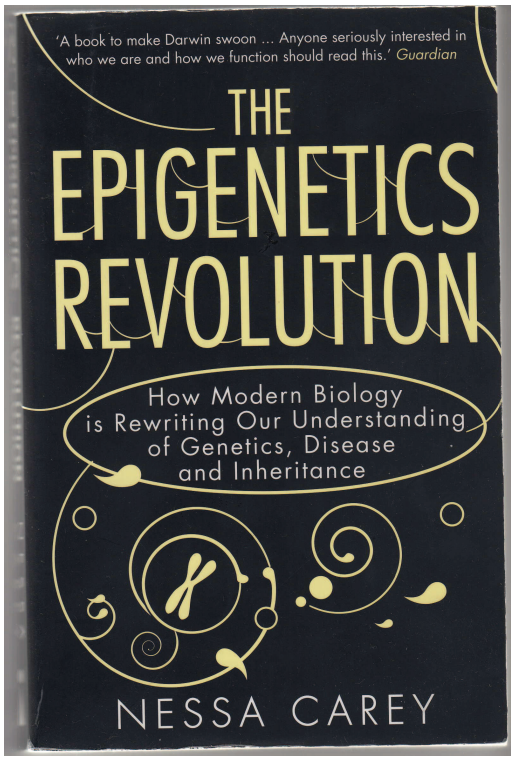
- Hypothesised biological basis for this structure variable representing relevant variability inside a population of cells: degree of methylation silencing normally present tumour suppressor genes, that arrest the cell cycle and send a cell towards programmed death

- 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.

Drug resistance: a genetic or epigenetic phenomenon?

In the same way as one can ask to what extent evolution towards malignancy in premalignant cell populations is genetic (irreversible, due to mutations) or epigenetic (reversible, due to *epimutations*), we can ask whether, in cancer cell populations, drug-induced evolution towards drug resistance is genetic or epigenetic.

- hence, is it irreversible or reversible?
- and if it is reversible:
- can we design combined drug strategies to overcome it?



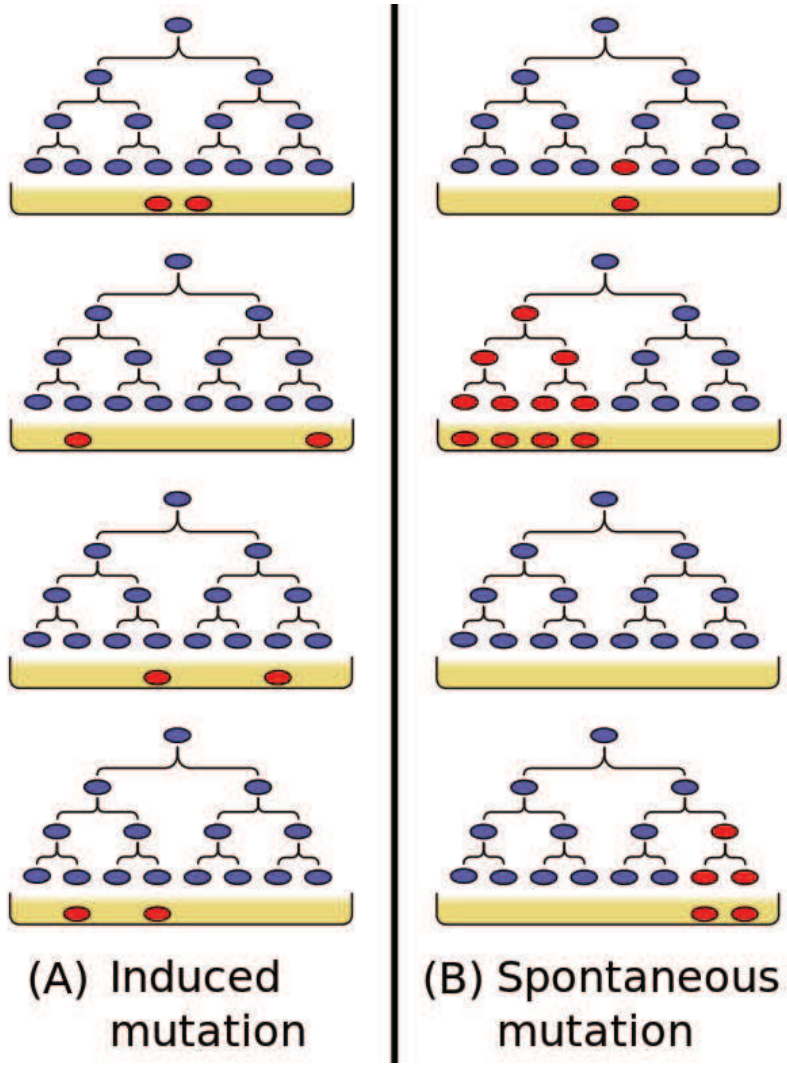
Can it be assessed by biological experiments? (1)

First hint: cell heterogeneity in Luria and Delbrück's experiment (1943)

Bacterial populations proliferating freely, then submitted to a phage environment: some will show resistance to the phages

Question: Is resistance induced by the phage environment (A)? Or was it preexistent in some subclones, due to random mutations at each generation, and selection by the phages (B)?

The answer is always (B):
preexistent mutations before selection.



Can it be assessed by biological experiments? (2)

Yes, it can! Example from *Sharma et al., Cell April 2010*: Fast *and reversible*, chromatin-mediated, acquisition of tolerance to anticancer drugs in a genetically homogeneous cell population:

- PC9 cells (NSCLC cells) submitted to various drugs (gefitinib, erlotinib...) at very high doses yielded 0.3% alive cells constituting a resistant clone (Drug Tolerant Persisters = DTPs), among which 20% were able to proliferate in maintained high doses of the drug (Drug Tolerant Expanded Persisters = DTEPs).
- The resistant phenotype was reversed, after drug withdrawal, in 9 doubling times for DTPs, in 30 doubling times for DTEPs.
- Evidence of necessity to obtain these persisters of the involvement of KDM5A (histone 3 lysine 4 demethylase, an epigenetic enzyme)
- Possible explanation: likely pre-existing random epimutations, i.e., Epigenetic heterogeneity in a population of genetically homogeneous cells
- *... But can it be completely excluded that stress-induced adaptation might have yielded emergent, non pre-existing DTPs from PC9 cells?*

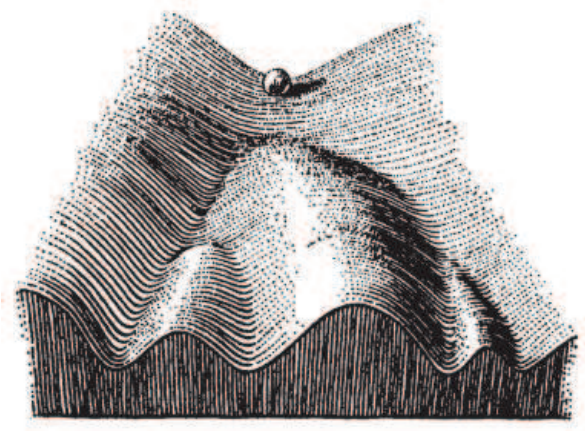
Can it be assessed by biological experiments? (3)

... Not meaning that all drug resistance should be only epigenetic: this 'dynamic phenotypic heterogeneity of non genetic nature' might be only a first step towards drug tolerance of a genetic nature if drug pressure is maintained.

- Multidrug tolerance in Sharma's experiment is without cell efflux (no ABC transporters)
- Transient clone (DTPs) of cells with *stem-cell like characteristics* (CD133, CD24, CD44): called for first-aid rescue?
- During transition from DTPs to DTEPs, stem-cell like markers are lost.
- Note that this is not the usual way (that is more gradual, but is it for this reason less reversible?) in biology labs to select resistant cell lineages.
- Work underway: evolutionary models, based on integro-differential equations structured by a 2D phenotype to account for the observations of Sharma et al.

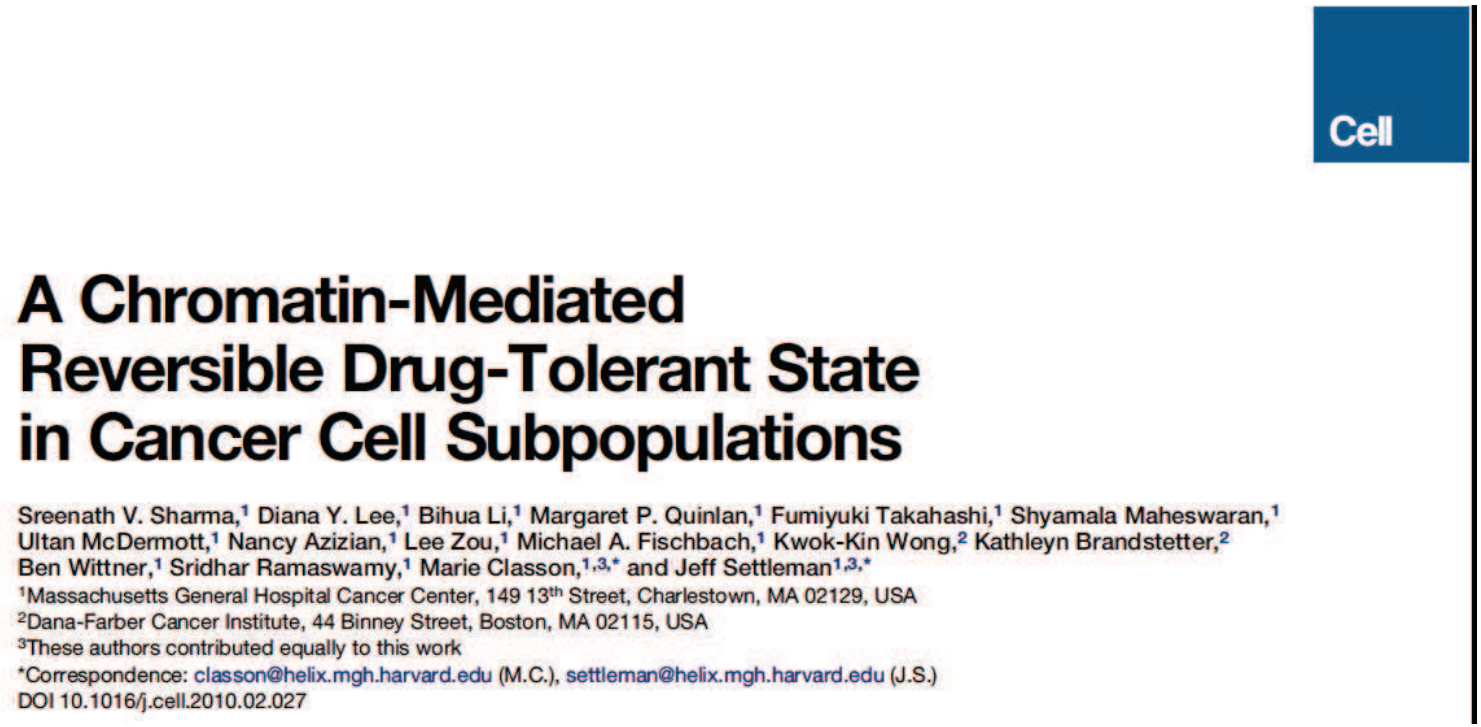
A metaphoric tale, in the manner of Waddington

- A cell population is differentiating (taking branch lines), and thriving following an evolutionary valley; the slope of the valley is the velocity of evolution.
- Suddenly, a flood occurs, limited to the hosting valley, drowning the population; the flood does not withdraw and remains slack for a long time.
- Some cells are not deadly drowned: those, stem-like, that were running on the sides of the valley and had enough adaptability to jump and reach a parallel, unflooded, valley.
- In this neighbouring valley, conditions are harsh, but it is still possible to survive, and even for some of these cells, to proliferate; from the crest between the two valleys, the forsaken valley can be watched over: it is still flooded.
- However, at some point, the flood recedes: these cells that were not committed in proliferation in this hostile valley, very quickly, freely following a hillside path, go back to the old valley where life is easier.
- Then the other cells, proliferating at high cost in the hostile valley, change their costly adapted programs, which takes some time, but eventually also go back to the old valley.



What could therapeutic implications be?

- Suggested by *Sharma et al.*: Conventional chemotherapies with epigenetic drugs to prevent chromatin intervention
- Also suggested: 'drug holiday' and re-treatment (now a popular concept among oncologists)
- More classically, association of drugs with different modes of action, to limit emergence of multi-drug resistance



Cell

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

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Different viewpoints to represent tumour therapies

- At the molecular level: hitting specific molecular targets in cancer cells by "targeted therapies". Presently the most popular point of view among cancer biologists. Achievements: imatinib (gleevec) in chronic myelogenous leukaemia (CML), ATRA+anthracylins in acute promyelocytic leukaemia (APL)
  Problems: (often very) relative specificity; toxicity to healthy tissues; not taking into account emergence of resistance.

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

- At the cell population level: choosing functional targets for drugs in a *qualitative* population dynamics model with added external control: PDEs or IDEs (integro-differential equations). *"Functional"*: i.e., by designing built-in model targets related to those fates that are considered as relevant for cell and tissue behaviour in cancer: proliferation, cell death, [de-]differentiation
  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?)

PDE model, one cytotoxic drug: equations for 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)*

Continued, one cytotoxic drug: equations for healthy cells

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

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

where the total population is defined as

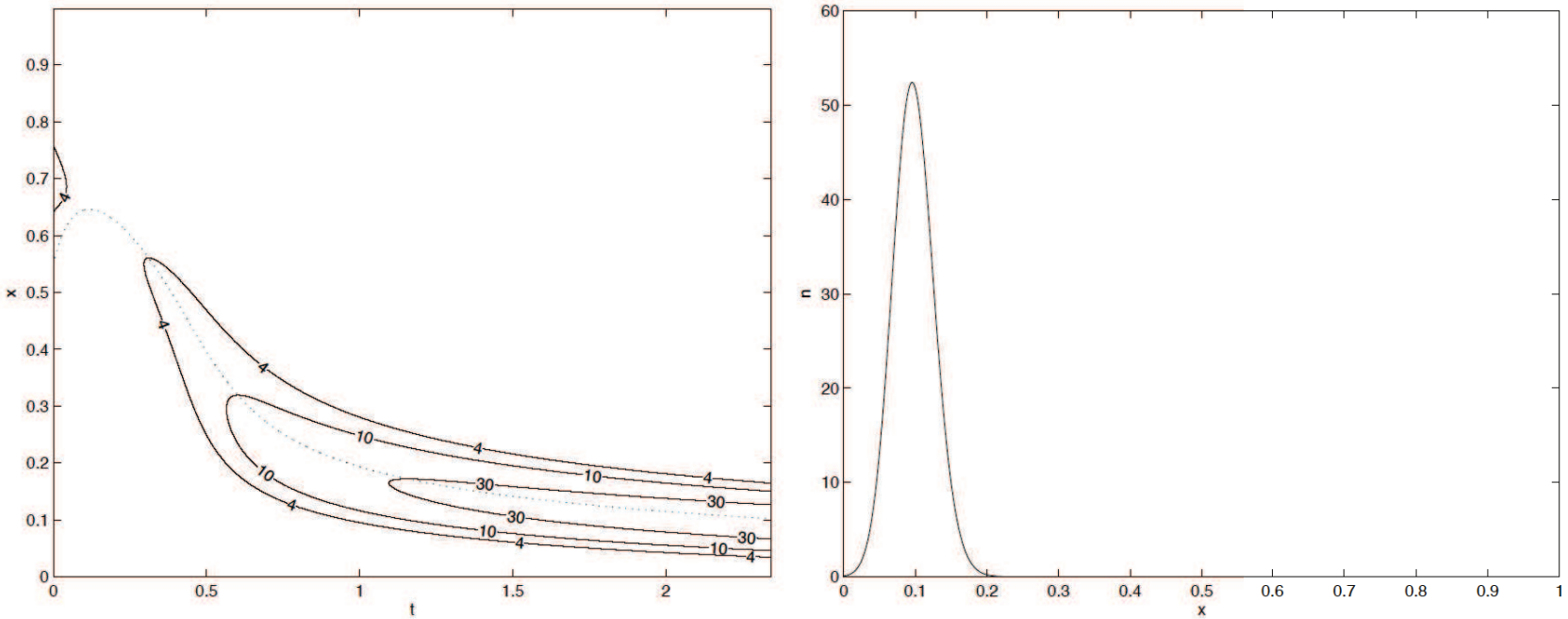
$$\rho(t) = \rho_H(t) + \rho_C(t); \rho_H(t) = \int_{x=0}^{\infty} n_H(x,t)dx; \rho_C(t) = \int_{x=0}^{\infty} n_C(x,t)dx.$$

- $\beta > 0$ 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.

PDE model with 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)*



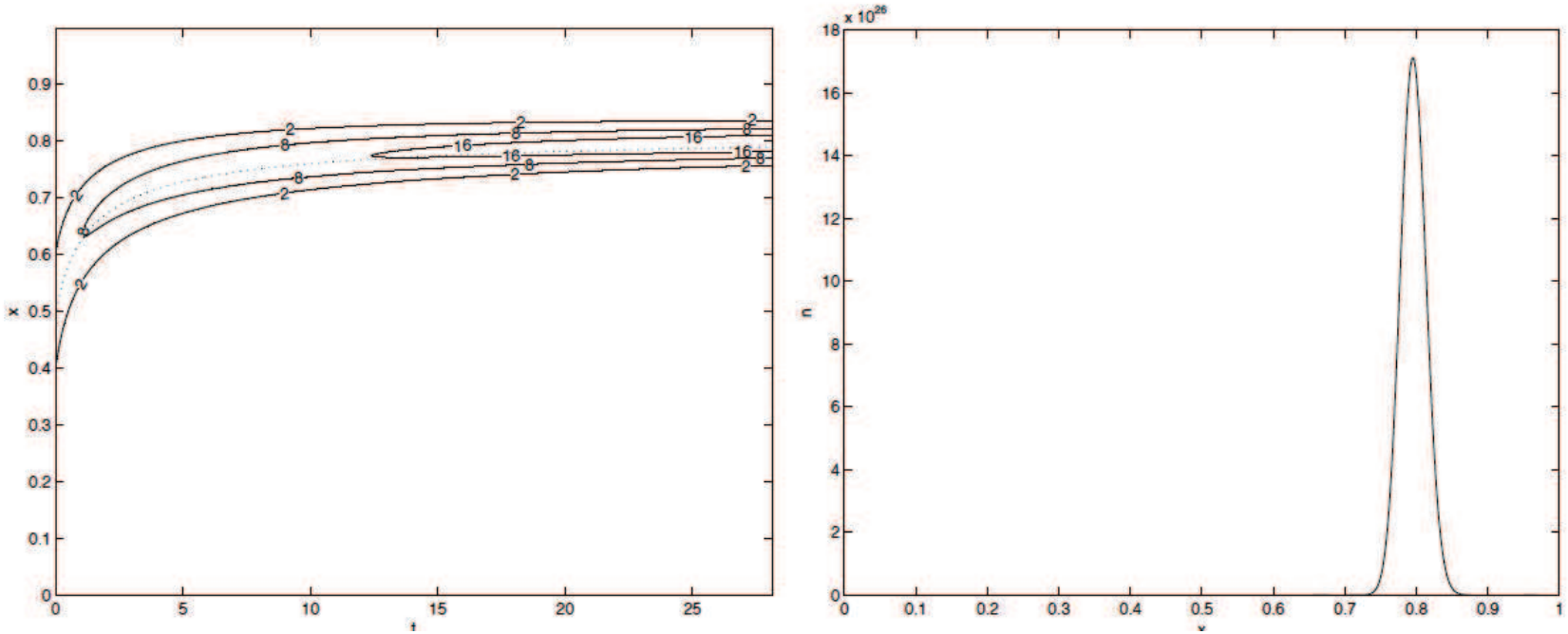
Left panel: starting from a medium phenotype $x = 0.5$, *level sets of a drug-sensitive population in the* (t, x) *plane. Right panel: asymptotic distribution of this drug-sensitive population according to the drug resistance phenotype* x.

(Lorz et al., M2AN 2013)

PDE model with one 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*.*

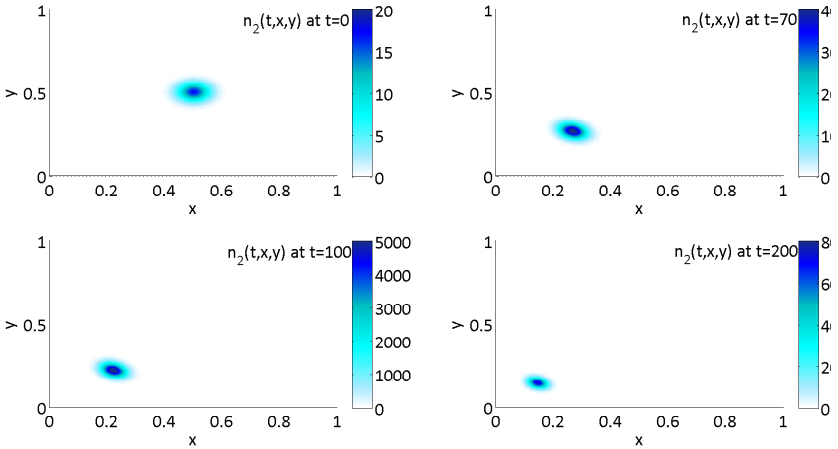
(Lorz et al., M2AN 2013)

Another model: 2 drugs, cytotoxic $u_1(t)$, cytostatic $u_2(t)$ with bidimensional resistance phenotype (x, y)

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

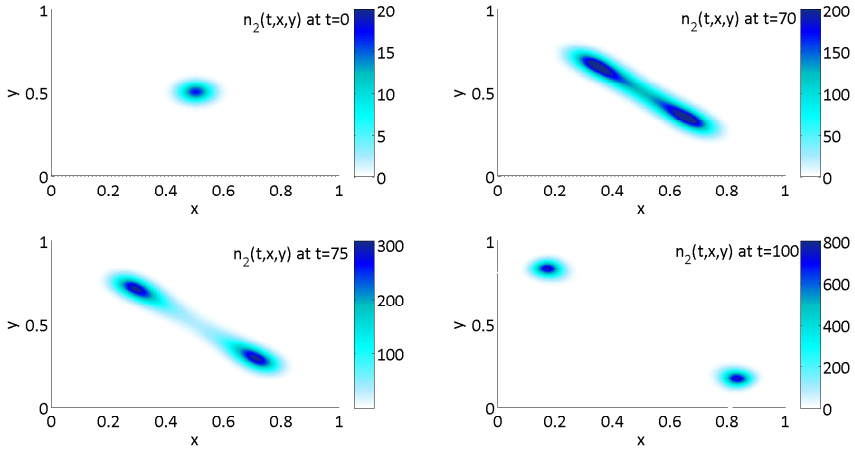
Environment: $I_C(t) = \alpha \int_0^1 \int_0^1 n_C(x,y,t)\, dx\, dy + \beta \int_0^1 \int_0^1 n_H(x,y,t)\, dx\, dy$

Sensitive cell population case:



Convergence toward total sensitivity

Resistant cell population case:



Convergence toward 2 resistant phenotypes

(Tommaso Lorenzi, work underway)

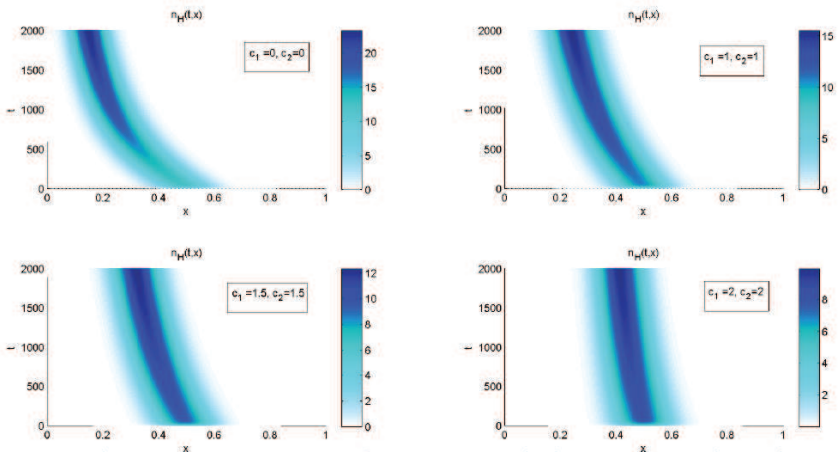
Now 2 drugs with one (scalar) resistance phenotype x

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

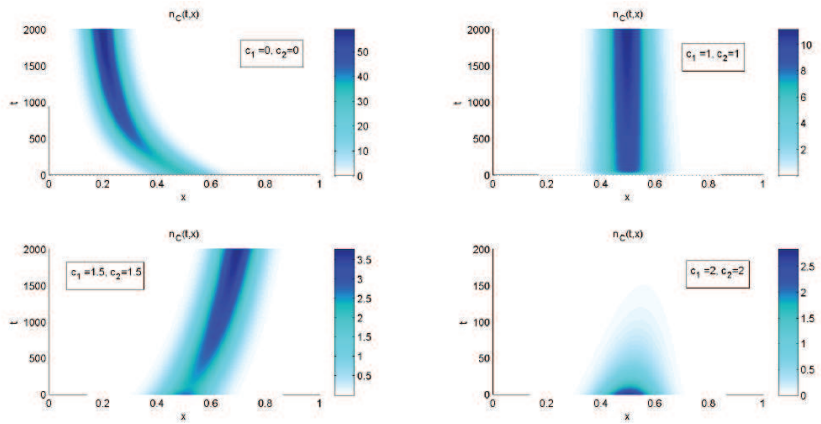
$$\frac{\partial}{\partial t} n_C(x,t) = \left[\frac{r_C(x)}{1 + k_C u_2(t)} - d_C(x) I_C(t) - u_1(t) \mu_C(x) \right] n_C(x,t)$$

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")

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

$$\begin{aligned} I_H(t) &= a_{HH}\rho_H(t) + a_{HC}\rho_C(t), \\ I_C(t) &= a_{CH}\rho_H(t) + a_{CC}\rho_C(t), \end{aligned} \tag{1}$$

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.

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

$$0 \leq u_1(t) \leq u_1^{\max}, \qquad 0 \leq u_2(t) \leq u_2^{\max}, \tag{2}$$

and minimising the cost functional

$$C_T(u_1,u_2) = \int_0^1 n_C(x,T)\,dx + \gamma_1 \int_0^T u_1(t)\,dt + \gamma_2 \int_0^T u_2(t)\,dt, \tag{3}$$

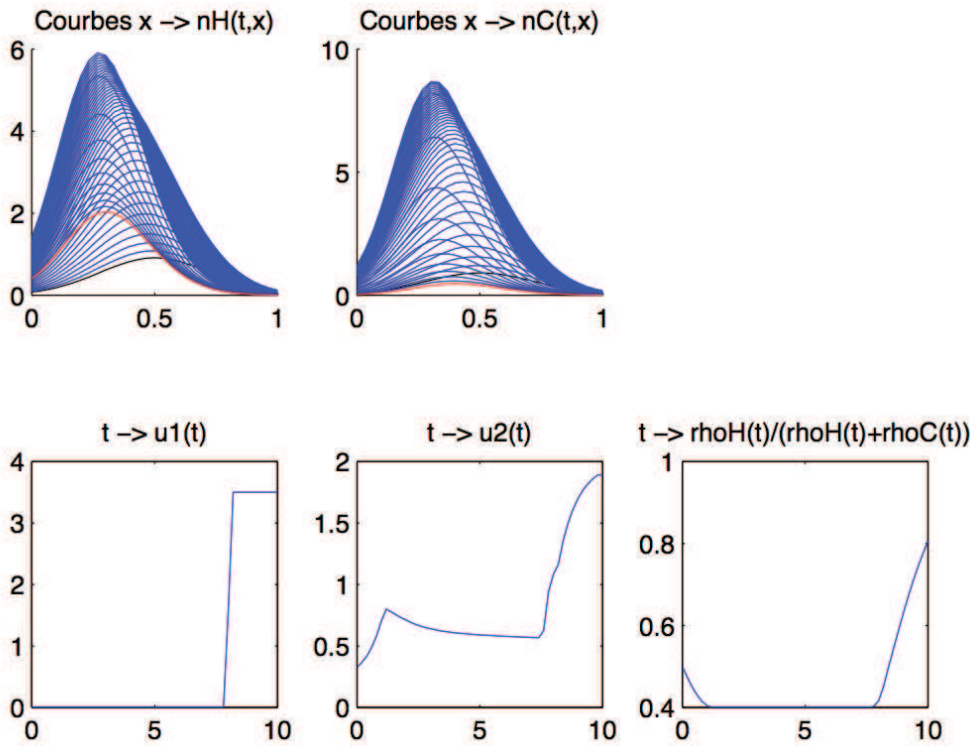
where $(n_C(\cdot,\cdot), n_H(\cdot,\cdot))$ is the unique solution of the system of PDEs corresponding to the controls u_1 and u_2, such that $n_H(0,\cdot) = n_H^0(\cdot)$ and $n_C(0,\cdot) = n_C^0(\cdot)$ and where the trajectory $t \mapsto (n_C(\cdot,t), n_H(\cdot,t))$ is subject to the dynamic state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

$$\frac{\rho_H(t)}{\rho_H(t)+\rho_C(t)} \geq \theta. \tag{4}$$

(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)

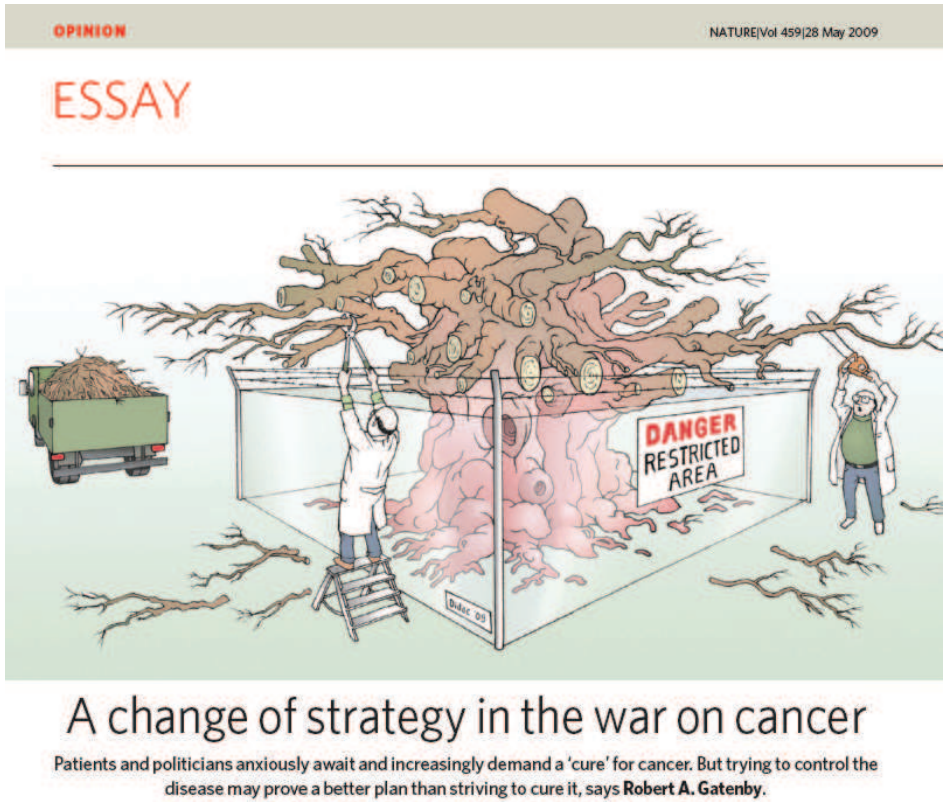


Left panels: optimised drug flows for $u_1(t)$ (cytotoxic) and $u_2(t)$ (cytostatic)

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

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

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

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

under the additional constraints

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

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 might be made for these weights, e.g., x^2 and $1-x^2$. Note that $\rho_C(t) = \rho_{CS}(t) + \rho_{CR}(t)$.

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

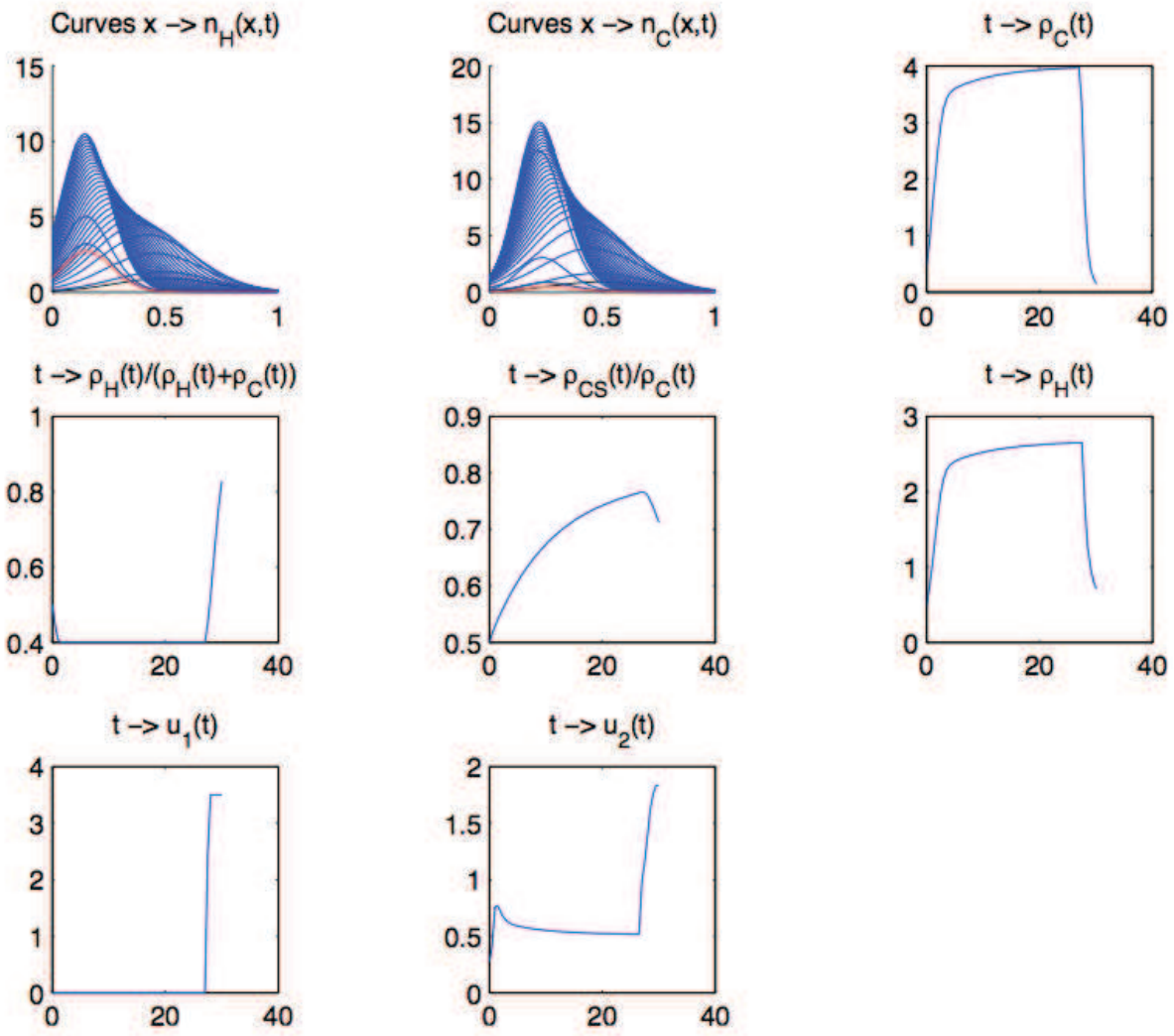
$$\boxed{n_H(\cdot, t) \simeq \delta_{x_H^\infty}, \quad n_C(\cdot, t) \simeq \delta_{x_C^\infty}} \quad (\delta_{x_{[H,C]}^\infty} \textit{ Dirac masses})$$

   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 $u_1 = u_1^{\max}$.

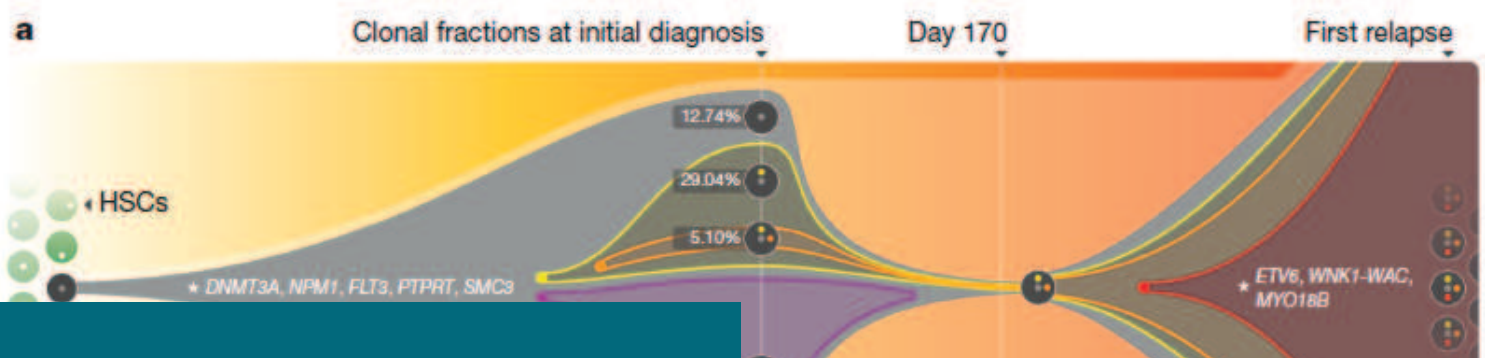
Main idea:

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).

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 resistant tumour cell population may thus reduce such strategy to nothing, unless a multidimensional phenotype may be found, corresponding to drugs specific of each scalar phenotype.
- ... Unless also it may be possible, more generally, in the perspective of Sharma et al., to avoid the development of transient drug-resistant cell clones, whatever the drug used, assuming that these transient clones are mandatory to obtain genetically established drug resistance, such avoidance being achieved by epigenetic drugs (e.g., HDAC inhibitors) or metabolism modifying strategies.



Back to evolution towards resistance: pending questions, possible tracks to enrich the model

- Is there a succession of events from a population dynamics point of view between an epigenetic, reversible, state of drug resistance, followed by a possibly acquired, genetic, unbeatable state of resistance to a given drug?
- Is there a way to measure in a molecular way the cost of resistance, so as to design realistic cost functions at the cell population level?
- Can we connect stochastic events such as transcription at the single cell level - ruled by genetic regulatory networks and possibly influenced by the cellular environment - with the determination of cell fate (e.g., drug resistance or EMT phenotype) at the cell population level?
- These are qualitative rather than quantitative models. Identification of a continuous trait x with a resistance phenotype will be linked to a given drug and a given cancer cell population studied in Petri dishes; identifying parameters in model functions of phenotype x $(\mu_{[H,C]}(x), r_{[H,C]}(x), d_{[H,C]}(x))$ is another challenge that might be addressed by inverse problems methods if necessary.

Future prospects: develop models of cancer and its therapeutic control according to further considerations

- Structuring the population according to a *multidimensional phenotype*, e.g., survival potential, proliferation potential (and possibly also stem cell-like plasticity, work underway): to be submitted soon
- Adding space (e.g., radial variable in a tumour spheroid) and available resources, accounting for drug diffusion in the tumour: work presently in revision
- Adding local metabolism modifications: oxygen, nutrients, pH... that may influence survival and proliferation
- Energy reallocation: cell population self-assessment in terms of costs (ATP): even with a non-molecular, but rather symbolical identification of energy costs, choice between dormancy, proliferation or death?
- Represent the effects of HDAC inhibitors or other epigenetic drug therapies

Concluding remarks on drug resistance

- Drug resistance in cancer cell populations may be assessed by biological experiments and by mathematical modelling of cell populations structured according to relevant *continuous* traits, amenable to describe phenotype heterogeneity better than discrete traits (e.g., resistance vs. sensitivity)

- Evolutionary bottlenecks may then be represented by convergence of the cell population to a limited number of phenotypes (one for only one drug - Gause's exclusion principle -, 2 for 2 drugs with different mechanisms...)

- Epigenetic or genetic phenomenon? Most likely both, but epigenetic prior to genetic, and reversibility of drug-resistant cell lines produced by drug exposure should always be tested

- Therapeutic consequences of this point of view (epigenetic? reversible? phenomenon) may lead to innovative drug delivery strategies, that may be theoretically optimised by using numerical optimisation algorithms

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