Seminar on mathematical modelling of growth and tissue repair

Cancer as a default of coherence between tissues in metazoans: what mathematical models should be developed to help prediction, prevention and treatment of cancer, *avoiding drug resistance*?

Luís Almeida, Rebecca Chisholm, *Jean Clairambault*[†], Tommaso Lorenzi, Alexander Lorz, Benoît Perthame, Camille Pouchol, Emmanuel Trélat

Mamba INRIA team, Laboratoire Jacques-Louis Lions, Sorbonne Université, Paris

Les Treilles, November 2018

[†]*http : //who.rocq.inria.fr/Jean.Clairambault/Jean_Clairambault_en.html*

SORBONNE UNIVERSITÉ
CRÉATEURS DE FUTURS DEPUIS 1257

IJLL   cnrs   PARIS DIDEROT   *Inria*   *Mamba* MODELLING AND ANALYSIS FOR MEDICAL AND BIOLOGICAL APPLICATIONS

Cancer puzzle: beyond intracellular signalling pathways

- Cancer is a disease of multicellular organisms: it makes little sense (except for monogenic cases: CML, APL,...) to search for its determinants in a single cell

- Cancer as localised loss of coherence between tissues in the same multicellular organism, i.e., localised disruption in the control of cell differentiations?

- Loss of differentiation control *is* cell plasticity and results in phenotype lability

- What is coherence within/between tissues? How is it disrupted in cancer?

- Atavistic hypothesis of cancer: from Boveri to Davies, Lineweaver and Vincent

- Between-species phylostratigraphic analyses of genes of multicellularity and genes altered in cancer: Domazet-Lošo & Tautz 2010

- Perturbed gap junctions (Trosko) and impaired energetic metabolism in cancer

- "Cold genes", extreme cellular stress and *bet hedging* in cancer?

- Epigenetic barriers, "self" (friend-or-foe) recognition and the immune system

- Evolutionary parallelism between the development of multicellularity in different species and the development of the immune system in these different species?

Motivation from and focus on drug resistance in cancer

- Slow genetic mechanisms of 'the great evolution' that has designed multicellular organisms, together with fast reverse evolution on smaller time windows, at the scale of a human disease, may explain transient or established drug resistance.

- Plasticity in cancer cells, i.e., epigenetic (much faster than genetic mutations, and reversible) propension to reversal to a stem-like, de-differentiated status, resulting in fast adaptability of cancer cell populations, makes them amenable to resist abrupt drug insult as response to extreme cellular stress.

- Intra-tumour heterogeneity with respect to drug resistance potential, modelling between-cell phenotypic variability within cancer *cell populations*, is a good setting to represent continuous evolution towards drug resistance in tumours.

- Reversible plasticity is captured by mathematical models that incorporate between-cell heterogeneity by making use of continuous phenotypic variables structuring the population.

- Such models have the advantage of being compatible with optimal control methods for the theoretical design of optimised therapeutic protocols involving combinations of cytotoxic and cytostatic (*and later epigenetic?*) treatments.

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 exposed 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.

- Note that drug resistance (and resistance to radiotherapy) is one of the many forms of fast resistance to cellular stress, possibly coded in 'cold', i.e., strongly preserved throughout evolution, rather than in 'hot', i.e., mutation-prone, genes (Wu et al. PNAS 2015).

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 *molecular* 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 total sensitivity ($x = 0$) towards total resistance ($x = 1$).

- Is such evolution towards drug resistance due to sheer Darwinian selection of the fittest by mutations in differentiation at cell division or, at least partially, due to phenotype adaptation in individual cells? Not clear.

# Drug resistance: evolutionary bottlenecks in cancer

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

- In cancer populations, enhanced heterogeneity with enhanced proliferation results in a high phenotypic or genetic diversity of proliferating clonal subpopulationss

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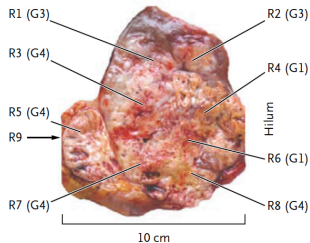

Ding et al. Nature 2012
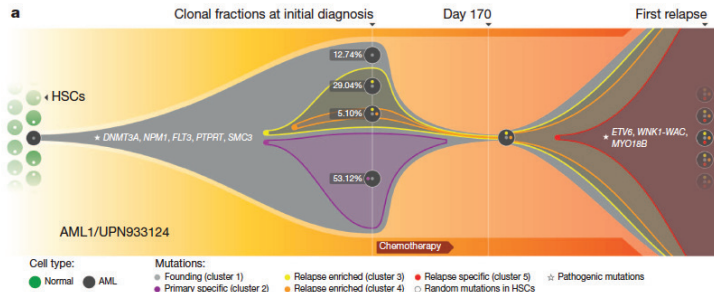
Drug resistance: always mutations and branching?
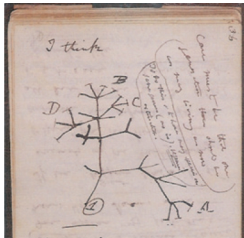
Darwin's notebook 1837
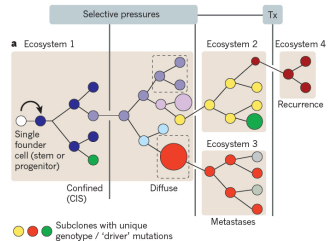
Maley & Greaves Nature 2012
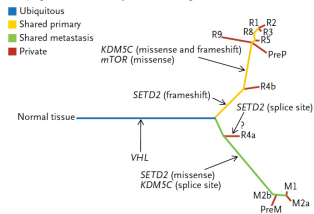
Gerlinger et al. NEJM 2012

Can resistance be assessed by biological experiments? (1)

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

Different Petri dishes, same experimental settings

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

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

Experiment: the answer is always (B): preexistent mutations before selection

However, bacteria are not cancer cells! In particular, they are far from being able of the same plasticity (no differentiation is available for them)

(A) Induced mutation

(B) Spontaneous mutation

(*Luria & Delbrück, Genetics, 1943*)
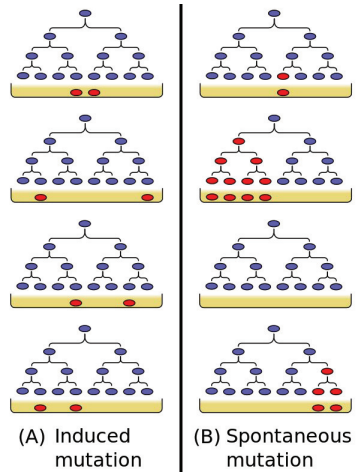
Biological experiment (2): reversible resistance in cancer

- 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:
  Drug Tolerant Persisters, DTPs
- In the same hostile environment, 20% of DTPs resume proliferation:
  Drug Tolerant Expanded Persisters, DTEPs
- Total reversibility to drug sensitivity is obtained by drug withdrawal, occurring after 9 doubling times for DTPs, and 90 doubling times for DTEPs
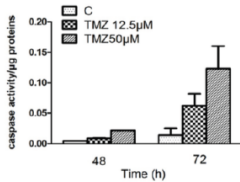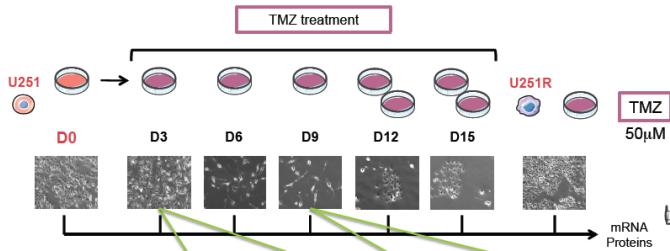- Inhibition of epigenetic enzyme KDM5A blocks emergence of DTPs

PC9s   DTPs   DTEPs
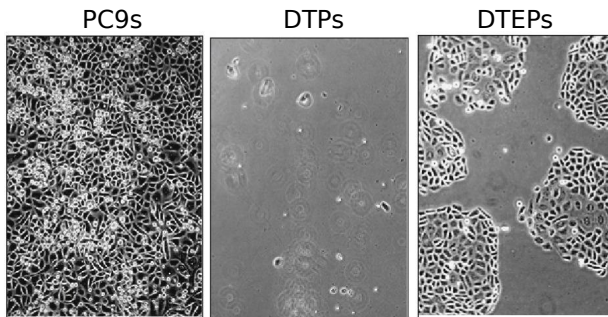
Time (during drug treatment)
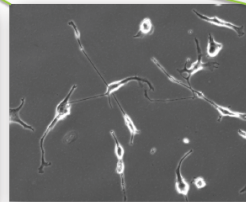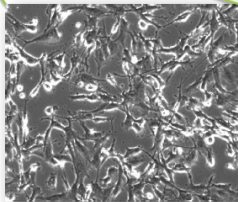
Sharma et al. Cell 2010

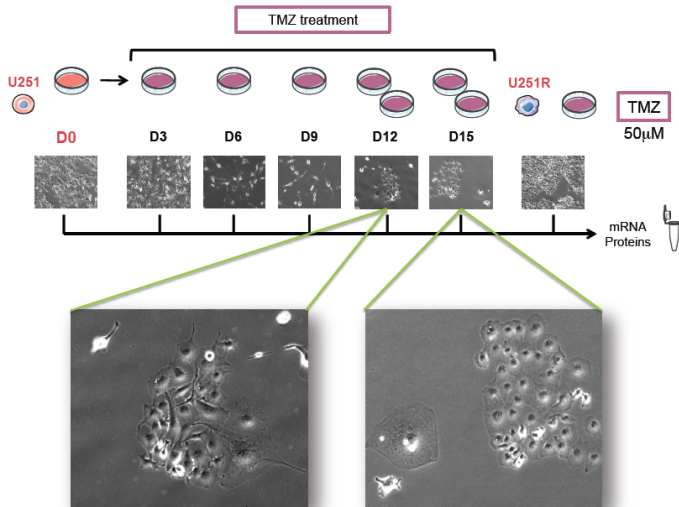# Glioblastoma and temozolomide: as in *Sharma Cell 2010*
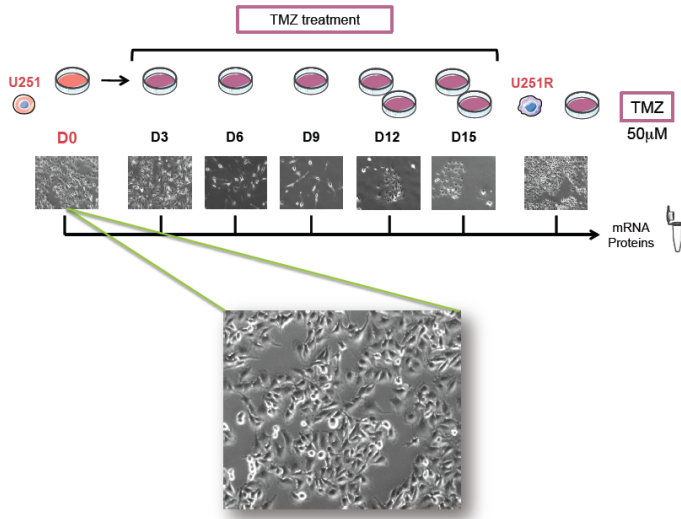
Glioblastoma and temozolomide: as in *Sharma Cell 2010*

Glioblastoma and temozolomide: as in *Sharma Cell 2010*

Glioblastoma and temozolomide: as in *Sharma Cell 2010*
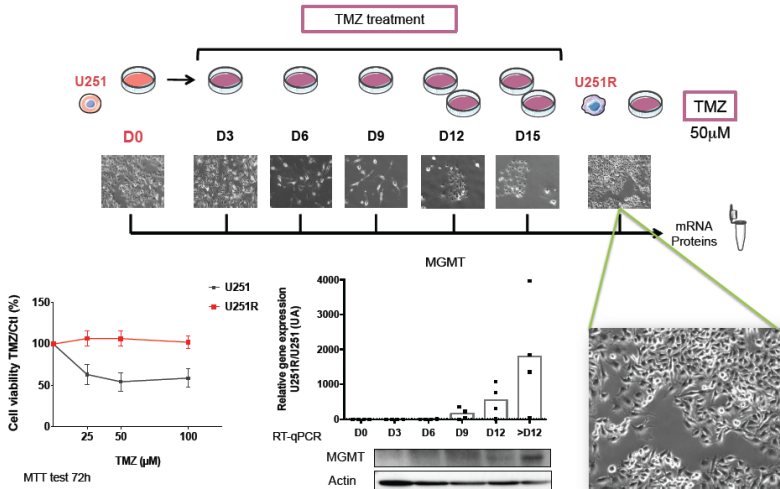
Gene expression followed from D0 to D16 (PCA)
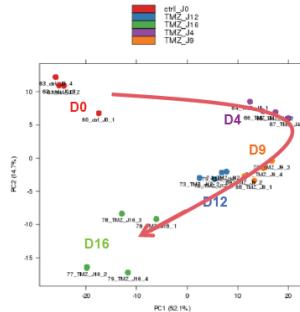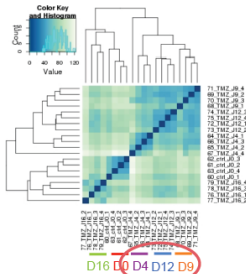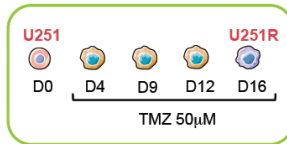
Short time window for combined treatment with an epigenetic drug??

from F. Vallette's INSERM team in Nantes

A possible evolutionary framework (*billion year-term view*): the atavistic hypothesis of cancer (1)

"Nothing in biology makes sense except in the light of evolution" (Th. Dobzhansky, 1973)
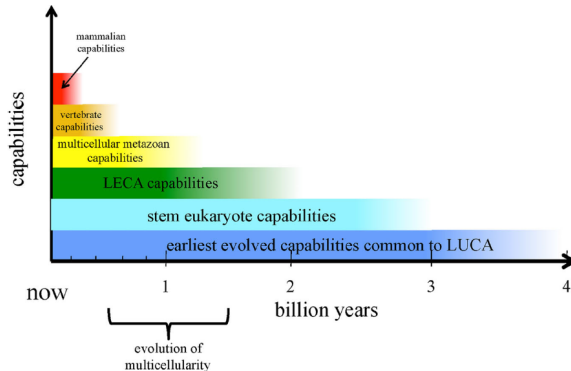
"Cancer: more archeoplasm than neoplasm" (Mark Vincent, 2011) More references: *Boveri 1929, Israel JTB 1996, Davies & Lineweaver Phys Biol 2011, Vincent Bioessays 2011, Lineweaver, Davies & Vincent Bioessays 2014, Chen et al. Nature Comm 2015, Bussey et al. PNAS 2017, Cisneros et al. PLoS One 2017, Trigos et al. PNAS 2017*

A possible evolutionary framework (*billion year-term view*): the atavistic hypothesis of cancer (2)
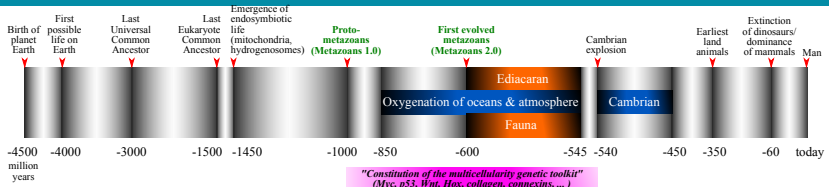
(*see Chisholm et al. 2016, BBA General Subjects DOI:10.1016/j.bbagen.2016.06.009*)

- The genes that have appeared in the process of development to multicellularity are precisely those that are altered in cancer

- In what order in evolution, from 1) proliferation+apoptosis to 2) cell differentiation +division of work, and to 3) *epigenetic control* of differentiation and proliferation?

- Reconstituting the phylogeny of this 'multicellularity toolkit' should shed light on the robustness or fragility of genes that have been altered in cancer

- Attacking cancer on proliferation is precisely attacking its robustness. It would be better to attack its weaknesses (e.g. absence of adaptive immune response)

Why resistance in cancer, not in healthy, cell populations?

- According to the atavistic hypothesis, cancer is a 'backward evolution' from a sophisticated form of multicellularity (us), in which epigenetic processes control gene regulatory networks of transcription factors: differentiation factors, p53, etc., that themselves physiologically control the basis of cellular life: proliferation

- We bear in our genomes many attempts of species evolution since billions of years; dead-end tracks ('unused attractors' in S. Huang and S. Kauffman's version of the Waddington landscape) have been silenced (e.g., by epigenetic enzymes, resulting in evolutionary barriers in this landscape), but are still there

- In cancer, global regulations are lost, differentiation is out of control, so that, without regulation, local proliferations overcome; sophisticated *adaptive epigenetic mechanisms are present, not controlling proliferation, but serving it* (by stochastic expression of so-called *cold* genes? cf. Wu et al. PNAS 2015)

- Primitive forms of cooperation between specialised cells in a locally organised multicellular collection (tumour), with plasticity between them, may be present, exhibiting coherent intratumoral heterogeneity, and escaping external control

- The basic cancer cell is highly plastic and highly capable of adaptation to a hostile environment, as were its ancestors in a remote past of our planet (poor O_2, acidic environment, high UV radiations,...) *and likely presently even more*

Another evolutionary framework (*life-term view*): revisiting the Waddington epigenetic landscape

The classic Waddington landscape (1957) for cell differentiation
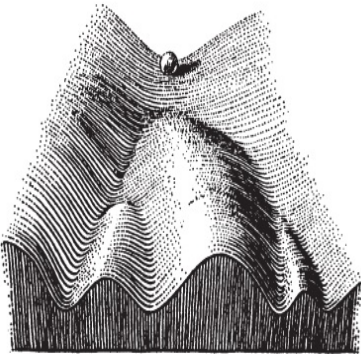
Waddington landscape revisited by S. Huang (2011, 2012, 2013)
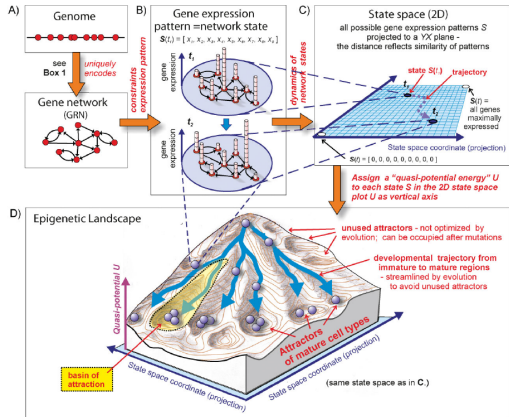
"Nothing in evolution makes sense except in the light of *systems* biology" (S. Huang, 2012)

Genetic and epigenetic: the two landscapes (Sui Huang)

- The epigenetic landscape (a):
  high-dimensional variety (dimensions being given by various states of many gene regulatory networks) endowed with a quasi-potential that governs fast evolution of cells in a genetically homogeneous population, expanded from a point in the fitness landscape (b) of genomes.

- *References: Sui Huang Sem Canc Biol 2011, Bioessays 2012, Canc Metastasis Rev 2013; Zhou Interface 2012; Pisco Br J C 2015...*

- Characterising resistance to a given drug by a phenotypic low-dimensional variable amounts to performing a low-dimensional projection from the global epigenetic landscape (onto a line, a plane, etc.)
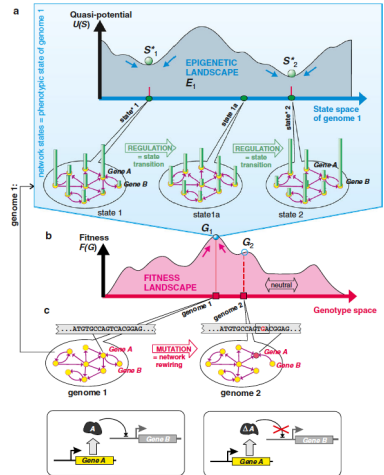
(*Sui Huang, Canc Metastasis Rev 2013*)

The best known case: haematopoiesis
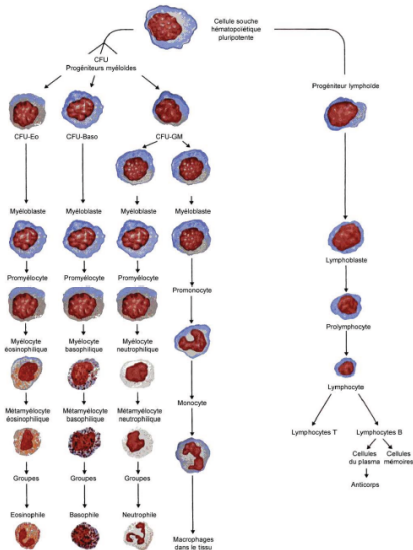
Some milestones to reconstruct the global landscape
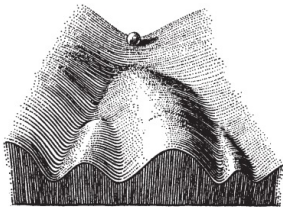
[Classic Waddington landscape]
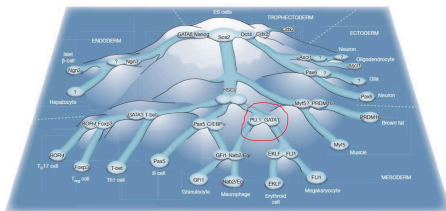
Stem cell fate: modern version by Tariq Enver
(ASH meeting 2011)
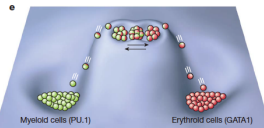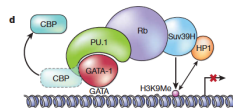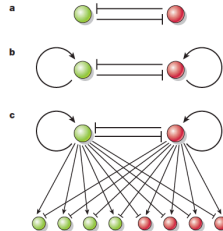
Zoom on the PU.1/GATA1 node (for
equations and bifurcations, see Huang,
Guo, May & Enver *Devel Biol 2007*)

First tentative model of resistance: one drug, cancer cells

- x = level of expression of a drug resistance phenotype (to a given cytotoxic drug)

- $n_H(x, t)$, $n_C(x, t)$ densities of cell populations (H=healthy, C=tumour)

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

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

- $r(x)$ = basic reproduction rate, $d(x)$ = basic death rate; we assume that $r(0) > d(0) > 0$, $\quad r'(\cdot) < 0$, $\quad r(+\infty) = 0$, $\quad d'(\cdot) > 0$

- θ_H and θ_C ($1 > \theta_C >> \theta_H \geq 0$) are the proportions 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)

First tentative model of resistance: one drug, healthy cells

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

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

where the total population is defined as

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

- $\beta > 0$ imposes 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.

First model of resistance, one drug: illustrations (1)

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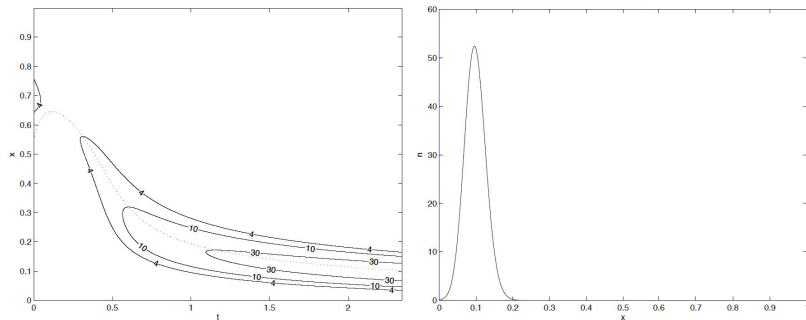
Left panel: starting from a medium phenotype $x = 0.5$, level sets of a drug-sensitive
population in the (t, x) plane. Right panel: asymptotic distribution of this
drug-sensitive population according to the drug resistance phenotype x.

Lorz et al., M2AN 2013

First model of resistance, one drug: illustrations (2)

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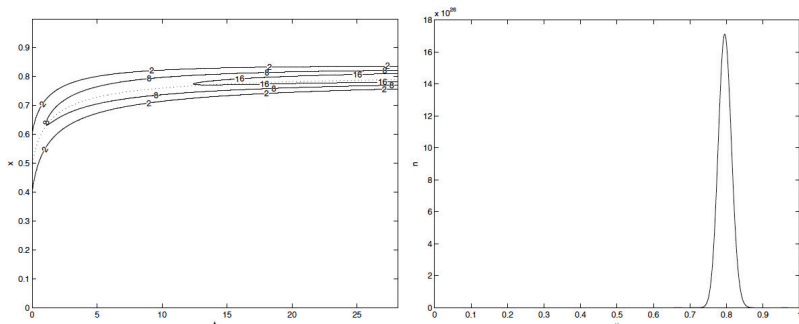
Left panel: starting from a medium phenotype $x = 0.5$, level sets of a *drug- resistant population* in the (t, x) plane. Right panel: asymptotic distribution of this drug-resistant population according to the drug resistance phenotype x.

Lorz et al., M2AN 2013

Simple phenotype-structured population dynamics

- Description of evolution of a population *in time t and in relevant phenotype x*

- 'Structure variable' x: trait chosen as bearing the biological variability at stake

- Variable : $n(t, x)$ population density of individuals bearing trait x at time t

- (1) Evolution in numbers of individuals constituting the population

$$t \mapsto \rho(t) = \int_0^1 n(t, x)\, dx \qquad (\text{if, e.g., } x \in [0, 1])$$

- (2) Asymptotics of distribution of the trait in the population

$$x \mapsto lim_{t \to +\infty} \frac{n(t, x)}{\rho(t)}$$

- Cancer cell populations: (1) tumour growth; (2) asymptotic distribution of trait

- Space is not necessarily a relevant structure variable when studying drug control

Phenotype-structured non-local Lotka-Volterra models

Questions: what is the asymptotic behaviour ($t \rightarrow +\infty$) of

- the total population $\rho(t)$?

- the phenotypes in the population (*i.e.*, possible limits for $\dfrac{n(t, \cdot)}{\rho(t)}$ in $M^1(0, 1)$)?

Nonlocal Lotka-Volterra model: $n(t, x)$ density of cells of trait (phenotype) $x \in [0, 1]$:

$$\frac{\partial n}{\partial t}(t, x) = \big(r(x) - d(x)\rho(t)\big) n(t, x),$$

with

$$\rho(t) := \int_0^1 n(t, x) \, dx \quad \text{and} \quad n(0, x) = n^0(x).$$

We assume reasonable (C^1) hypotheses on r and d, and $n^0 \in L^1([0, 1])$

[More general settings for the growth rate $R(x, \rho(t))$, here $\big(r(x) - d(x)\rho(t)\big)$, have been studied in Benoît Perthame's book Transport equations in biology *(2007)]*

Non-local Lotka-Volterra 1D model: convergence in time

Convergence (one-population case): plot of $t \mapsto \rho(t)$
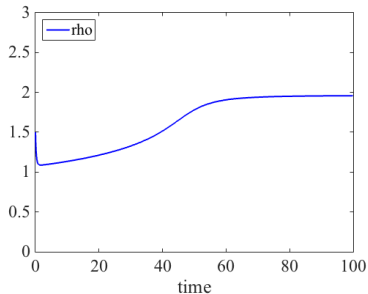
Firstly, it can be shown that: ρ converges to $\rho^\infty = \max_{[0,1]} \dfrac{r}{d}$, i.e., to the smallest value ρ such that $r(x) - d(x)\rho \leq 0$ on $[0,1]$.

[See Camille Pouchol's internship report: *"Modelling interactions between tumour cells and supporting adipocytes in breast cancer"*, UPMC, September 2015, https://hal.inria.fr/hal-01252122]

Non-local Lotka-Volterra 1D model: concentration in x

Concentration (one population): Plot of $x \mapsto n(t, x)$ for different times t
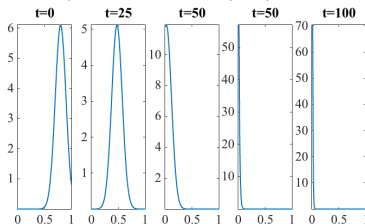
Theorem

- ρ converges to ρ^{∞}, the smallest value ρ such that $r(x) - d(x)\rho \leq 0$ on $[0, 1]$.
- $n(t, \cdot)$ concentrates on the set $\{x \in [0, 1], \ r(x) - d(x)\rho^{\infty} = 0\}$.
- Furthermore, if this set is reduced to a singleton x^{∞}, then

$$n(t, \cdot) \rightharpoonup \rho^{\infty} \delta_{x^{\infty}} \ in \ M^1(0, 1).$$

[See Camille Pouchol's internship report: *"Modelling interactions between tumour cells and supporting adipocytes in breast cancer"*, UPMC, September 2015, https://hal.inria.fr/hal-01252122]

Non-local Lotka-Volterra 1D model: convergence and concentration using a Lyapunov functional

Although in the 1D case a direct proof of convergence based on a BV hypothesis may be obtained, from which concentration easily follows, it is interesting to note, *as this argument can be used in the case of 2 populations*, that a global proof based on the design of a Lyapunov function gives at the same time convergence and concentration: choosing any measure n^∞ on $[0,1]$ such that $\int_0^1 n^\infty(x)\,dx = \rho^\infty = \max_{[0,1]} \dfrac{r}{d}$, and for an appropriate weight $w(x)$ ($= \frac{1}{d(x)}$, P.-E. Jabin & G. Raoul, *J Math Biol 2011*), setting

$$V(t) = \int_0^1 w(x)\left\{n(t,x) - n^\infty(x) - n^\infty(x)\ln n(t,x)\right\}\,dx,$$

one can show that

$$\frac{dV}{dt} = -(\rho(t) - \rho^\infty)^2 + \int_0^1 w(x)\left\{r(x) - d(x)\rho^\infty\right\}n(t,x)\,dx,$$

which is always nonpositive, tends to zero for $t \to \infty$, thus making V a Lyapunov functional, and showing at the same time convergence and concentration. Indeed, in this expression, the two terms are nonpositive and their sum tends to zero; the zero limit of the first one accounts for convergence of $\rho(t)$, and the zero limit of the second one accounts for concentration in x (on a zero-measure set) of $\lim_{t \to +\infty} n(t,x)$.

[See Camille Pouchol's PhD thesis (Sorbonne Université) defended in June 2018, and in a more general case, Pouchol et al., *J Maths Pures Appl* 2018]

Non-local Lotka-Volterra 2D model (2 populations, n_H, n_C) with 2 different drugs and one resistance phenotype x

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

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

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 $\rho_H(t) = \int_0^1 n_H(t,x)\,dx$, $\rho_C(t) = \int_0^1 n_C(t,x)\,dx$, u_1 cytotoxic, u_2 cytostatic drugs.

Simultaneous combinations of the 2 drugs, with increasing equal constant doses
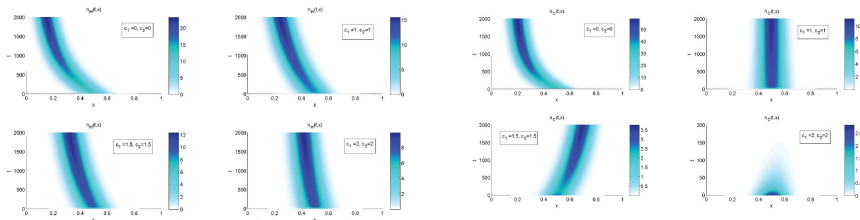
Healthy cells n_H: preserved

Cancer cells n_C: eventually extinct

Proof of concept, or here "Pedestrian's optimisation"   *Lorz et al. M2AN 2013*

Asymptotic behaviour with constant controls

Following an argument by P.-E. Jabin & G. Raoul (*J Math Biol 2011*) we prove at the same time convergence and concentration by using a Lyapunov functional of the form

$$\int w(x) \left\{ n(t,x) - n^\infty(x) - n^\infty(x) \ln n(t,x) \right\} \, dx$$

Theorem

(Asymptotic behaviour theorem, generalising to 2 populations the 1D case)
Assume that u_1 and u_2 are constant: $u_1 \equiv \bar{u}_1$, and $u_2 \equiv \bar{u}_2$. Then, for any positive 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

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

Then $(\rho_H^\infty, \rho_C^\infty)$ is the unique solution of the invertible ($a_{HH}.a_{CC} >> a_{CH}.a_{HC}$) system
$$I_H^\infty = a_{HH}\rho_H^\infty + a_{HC}\rho_C^\infty = a_1,$$
$$I_C^\infty = a_{CH}\rho_H^\infty + a_{CC}\rho_C^\infty = a_2.$$

Let $A_H \subset [0,1]$ (resp., $A_C \subset [0,1]$) be the set of all points $x \in [0,1]$ such that equality hold in one of the inequalities above. Then the supports of the probability measures
$$\nu_H(t) = \frac{n_H(t,x)}{\rho_H(t)} \, dx \quad \text{and} \quad \nu_C(t) = \frac{n_C(t,x)}{\rho_C(t)} \, dx$$
converge respectively to A_H and A_C as t tends to $+\infty$.

Basis of proof (constant controls): a Lyapunov functional

Firstly, the correspondence $(a_1, a_2) \mapsto (\rho_H^\infty, \rho_C^\infty)$ being bijective and controls $\bar{u}_1$, $\bar{u}_2$ being constant and omitted in the sequel, one can write the two inequalities above as

$$\forall x \in [0,1], \quad R_H(x, \rho_H^\infty, \rho_C^\infty) \leq 0 \quad \text{and} \quad \forall x \in [0,1], \quad R_C(x, \rho_C^\infty, \rho_H^\infty) \leq 0$$

with, furthermore

$$\forall x \in A_H, \quad R_H(x, \rho_H^\infty, \rho_C^\infty) = 0 \quad \text{and} \quad \forall x \in A_C, \quad R_C(x, \rho_C^\infty, \rho_H^\infty) = 0$$

Then, for $m_{H,C} := \dfrac{1}{d_{H,C}}$, define the Lyapunov functional $V(t) := V_H(t) + V_C(t)$ where

$$V_{H,C}(t) = \lambda_{H,C} \int_0^1 m_{H,C}(x) \left[n_{H,C}^\infty(x) \ln \left(\frac{1}{n_{H,C}(t,x)} \right) + \left(n_{H,C}(t,x) - n_{H,C}^\infty(x) \right) \right] dx.$$

where $n_{H,C}^\infty(x)$ are measures with support in $A_{H,C}$ such that $\displaystyle\int_0^1 n_{H,C}^\infty(x)\, dx = \rho_{H,C}^\infty$, the positive constants λ_H and λ_C being adequately chosen to make V decreasing along trajectories. The functional V yields simultaneously convergence and concentration.

(Pouchol et al. J. Maths Pures Appl. 2018

How to be deleterious by using constant doses of drugs

[We define the population of sensitive cancer cells by $\rho_{CS}(t) := \int_0^1 (1-x)\, n_C(t,x)\, dx]$
Simulation with $u_1(t) = \mathrm{Cst} = 3.5$ and $u_2(t) = \mathrm{Cst} = 2$, in time $T = 10$
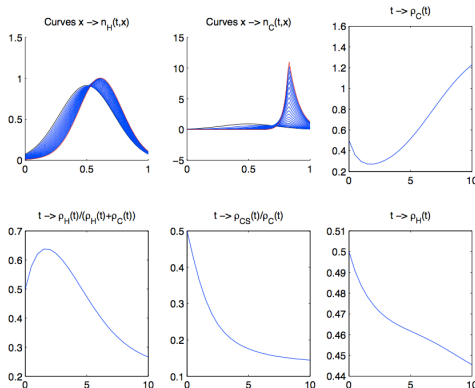
- Quite small effect of the drug pressure on the phenotype of n_H
- n_C quickly concentrates around a resistant phenotype
- Catastrophic effects on ρ_H, ρ_C and ρ_{CS}.

"What does not kill me strengthens me"

- Note that in the representation of the drug targets on cancer cell populations in the integro-differential equation, with the numerical values chosen for the target functions μ_C and r_C standing for the sensitivities to drugs u_1 and u_2,

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

the cytostatic drug u_2 *only slows down proliferation* (softly slowing down velocity in the cell division cycle), but does not arrest it, at least at low doses...

- ...whereas the cytotoxic drug u_1 *kills* the cells by increasing the death term, hence it is actually a direct life threat to the cell population, that 'defends itself' (biological bases under assessment...) by increasing its resistance phenotype x

- This resistance-inducing killing effect should be avoided as long as possible in therapeutics. In summary: limit proliferation but do not try too hard to kill cells, lest the cell population should become resistant, but give cytotoxics only at high doses during a short interval of time (MTD), thus avoiding to trigger resistance.

- An alternative to such use of MTD (maximum tolerated dose) towards the end of the chemotherapy course is *metronomics*, that also prevents developing resistance by giving low doses of cytotoxics... expecting that the population, thwarted in its proliferation, will be kept in check by the immune system. This has not been represented in our optimal control perspective thus far (however, see *Cécile Carrère, J Theor Biol 2017*).

Optimal control problem, phenotype-structured IDE model

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

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

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

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

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

Find controls (u_1, u_2) minimising

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

under the additional constraints

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

(the last constraint, with, e.g., $\theta_H = 0.6$, to limit damage to healthy cells)

Optimal control problem: theoretical results

Theorem
(Optimal control theorem)
Under these conditions, the optimal trajectory in large time $T > 0$ consists of 2 parts:

- a long-time part, with constant controls on $[0, T_1]$, at the end of which populations have almost concentrated in phenotype (for T_1 large)

- a short-time part on $[T_1, T]$ consisting of at most three arcs, for $T - T_1$ small:
    1. a boundary arc, along the constraint $\dfrac{\rho_H(t)}{\rho_H(t) + \rho_C(t)} = \theta_{HC}$,
    2. a free arc (no constraint saturating) with controls $u_1 = u_1^{\max}$ and $u_2 = u_2^{\max}$,
    3. a boundary arc along the constraint $\rho_H(t) \geq \theta_H . \rho_H(0)$ with $u_2 = u_2^{\max}$.

Simulations illustrating this theorem

Simulations with $T = 30$
(optimisation using AMPL-IPOPT)

Simulation with $T = 60$
(optimisation using AMPL-IPOPT)
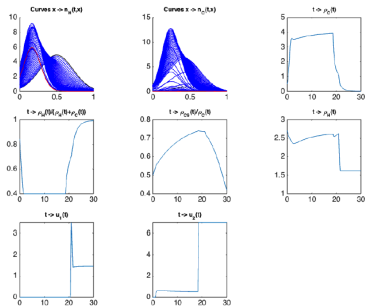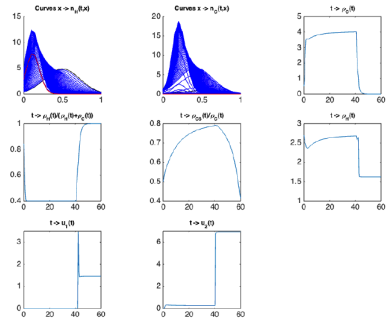

Figure 4: Simulation of (OCP) for $T = 30$.

Figure 5: Simulation of (OCP) for $T = 60$.

Note that this strategy lets the cancer cell population ρ_C grow initially to an equilibrium level, while increasing the ratio $\dfrac{\rho_{CS}}{\rho_C}$ of drug-sensitive cancer cells, before delivering $u_1 = u_1^{max}$; only then is the cytotoxic efficacy maximal.

Interpretation

In a first approximation the optimal trajectory is made of 3 parts, the first one with $u_1 = 0$, the 2nd one with $u_1 = u_1^{\max}$, the 3rd one with u_1 slightly lower than $u_1^{\max}$.

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 more concentrated (hence, as the time T is large).

Comparison with "almost periodic" therapeutic strategies

We mimic actual clinical settings: as long as $\dfrac{\rho_H}{\rho_H + \rho_C} > \theta_{HC}$, we follow the 'drug holiday' strategy by choosing $u_1 = \bar{u}_1 = 0$, $u_2 = \bar{u}_2 = 0.5$. Then, as long as $\rho_H > \theta_H.\rho_H(0)$, we use the maximal amount of drugs. As soon as $\rho_H = \theta_H.\rho_H(0)$, back to the drug holiday strategy. Results (note stabilised ρ_C and increasing ρ_{CS}):
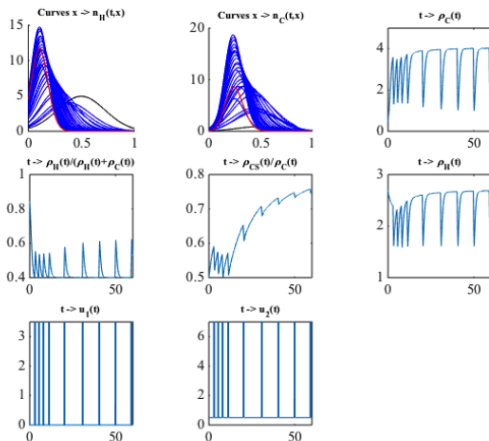
for $T = 60$

Comparison with "almost periodic" therapeutic strategies

1) Mimicking the clinic; 2) the same with saturation of the constraint $\rho_H = \theta_H . \rho_H(0)$
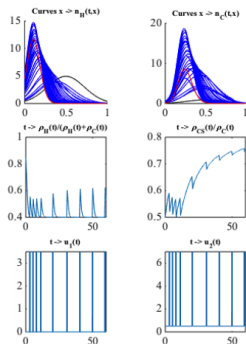
Figure 6: Quasi-periodic strategy, for $T = 60$.
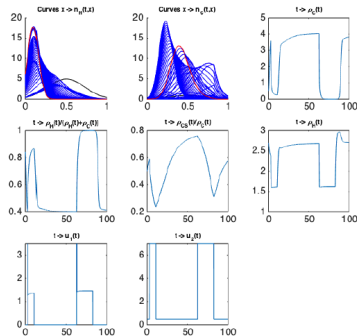
Figure 7: Second quasi-periodic strategy, for $T = 100$.

1) Left: (unsatisfying) periodic strategy: stabilisation of ρ_C only. 2) Right: second strategy, same, but *with added arc following the constraint $\rho_H = \theta_H . \rho_H(0)$, with $u_2 = u_2^{max}$, and control u_1 obtained from* the equality $\dfrac{d\rho_H}{dt} = 0$ (saturation of the constraint) and back to the drug holiday strategy $u_1 = 0$ as ρ_C starts increasing again: we see that ρ_C can be brought arbitrarily close to 0 (tumour eradication?).

Pouchol et al. J. Maths Pures Appl. 2018

Optimising anticancer **drug holiday** *and* **re-challenge** *principles*

Adaptive dynamics of coupled cell populations, healthy and cancer, with a continuous resistance expression level x, relying for each cell population on integro-differential equations of the type

$$\frac{\partial n}{\partial t}(t, x) = \big(r(x) - a\rho(t)\big)\, n(t, x), \text{ with } \rho(t) := \int_0^1 n(t, x)\, dx$$

and combined delivery of cytotoxic (e.g., in AML, anthracyclins) and cytostatic (e.g., in AML, GF receptor antagonist AC220) drugs *targeting term* $r(x)$, to be optimised

Solution of an optimal control problem with unwanted toxicity constraints shows that (after an initial dose of cytotoxic to shrink the tumour burden) the optimal solution should be, qualitatively:

Optimal control: simulation with $T = 60$
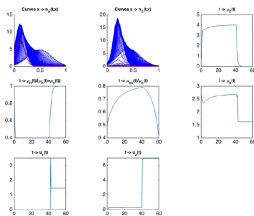
Figure 5: Simulation of (OCP) for $T = 60$.

(*What should absolutely be avoided: high drug doses on a constant mode*)
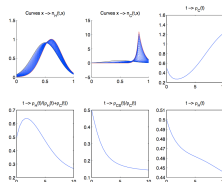
Optimal control solution naturally yields *drug holiday*. Periodised version of the model, using 2 different cytotoxic drugs with distinct molecular targets will justify success of *re-challenge* = re-sensitisation of the cancer cell population to first drug.

(J. Clairambault, C. Pouchol, Inria MAMBA team & E. Trélat, Inria CAGE team)

What about space? Considering both a (1D) resistance phenotype and (1D) space in a tumour spheroid: equations

We assume that the evolution of functions n, s (nutrients), u_1 and u_2 in a 3D radially symmetric tumour spheroid ($r \in [0,1]$) is ruled by the following set of equations:

$$\partial_t n(t,r,x) = \left[\frac{p(x)}{1+\mu_2 u_2(t,r)} s(t,r) - d(x)\varrho(t,r) - \mu_1(x)u_1(t,r)\right] n(t,r,x), \quad (1)$$

$$-\sigma_s \Delta s(t,r) + \left[\gamma_s + \int_0^1 p(x)n(t,r,x)dx\right]s(t,r) = 0, \quad (2)$$

$$-\sigma_u \Delta u_1(t,r) + \left[\gamma_u + \int_0^1 \mu_1(x)n(t,r,x)dx\right]u_1(t,r) = 0, \quad (3)$$

$$-\sigma_u \Delta u_2(t,r) + \left[\gamma_u + \mu_2 \int_0^1 n(t,r,x)dx\right]u_1(t,r) = 0, \quad (4)$$

with zero Neumann conditions at $r = 0$ coming from radial symmetry and Dirichlet boundary conditions at $r = 1$

$$s(t, r = 1) = s_1, \partial_r s(t, r = 0) = 0, u_{1,2}(t, r = 1) = U_{1,2}(t), \partial_r u_{1,2}(t, r = 0) = 0. \quad (5)$$

For each t, we also define $\rho(t,r) = \int_0^1 n(t,r,x)\,dx$ (local density at radius r) and $\rho_T(t) = \int_0^1 \rho(t,r)r^2\,dr$ (global density).

Tumour spheroid: simulations with constant drug doses (1)
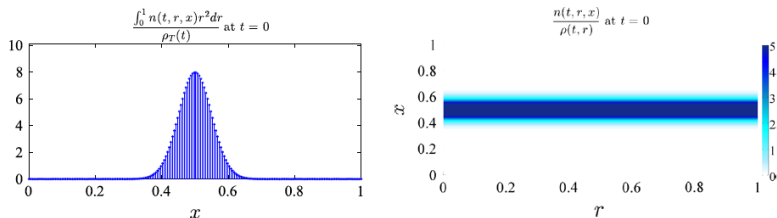
Fig. 1 Initial phenotypic distribution. Plots of $\int_0^1 n(t,r,x)r^2 \mathrm{d}r/\rho_T(t)$ (*left panel*) and $n(t,r,x)/\rho(t,r)$ (*right panel*) at $t = 0$. The initial cell population is almost monomorphic

Evolution without drugs: towards sensitive phenotype ($x \to 0$)
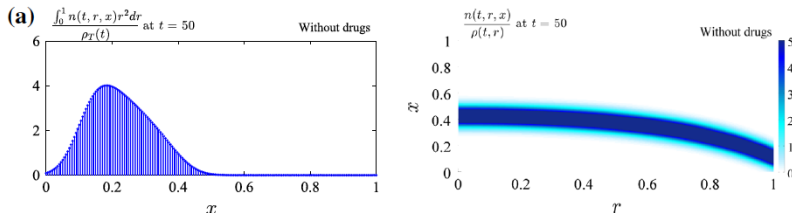
Tumour spheroid: evolution with constant drug doses (2)
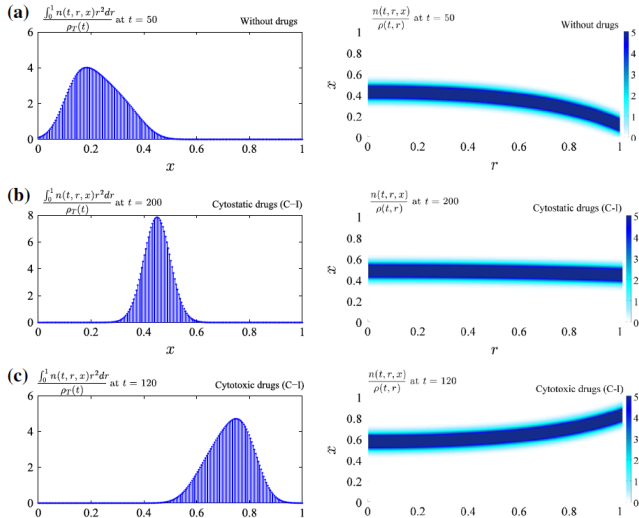
Cytostatic u_2 has only small effects, whereas cytotoxic u_1 clearly induces resistance

Lorz et al. Bull Math Biol 2015

Tumour spheroid (3): constant or bang-bang control?

Therapeutic strategies for (u_1, u_2): (Constant, Bang-bang) vs. (Bang-bang, Constant)

Fig. 11 a Cytotoxic (C-I) and cytostatic (BB-I) drugs. Plots of $\int_0^1 n(t,r,x)r^2 dr$ (*left panel*) and $\rho_T(t)$ (*right panel*). Bang–bang infusion of cytostatic drugs together with constant infusion of cytotoxic drugs weakly affects the dynamics of cancer cells by comparison with the case without therapies, apart from temporary reductions of the global population density. **b** Cytotoxic (BB-I) and cytostatic (C-I) drugs. Plots of $\int_0^1 n(t,r,x)r^2 dr$ (*left panel*) and $\rho_T(t)$ (*right panel*). Bang–bang infusion of 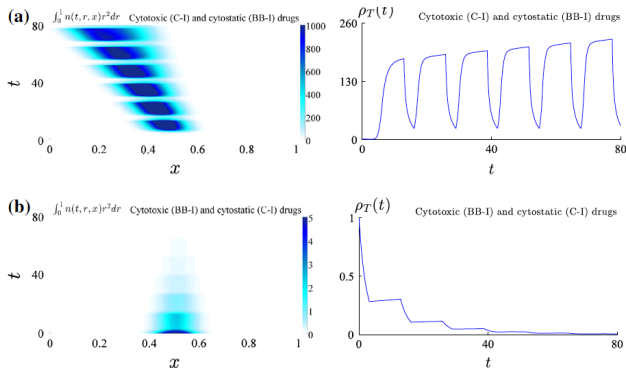cytotoxic drugs together with constant delivery of cytostatic drugs can push cancer cells toward extinction. The unit of time is days. All values are normalized with respect to the initial global population density.

Lorz et al. *Bull Math Biol* 2015

Biological observation: reversible drug resistance in cancer

- 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 (*precisely: provokes rapid death of both DTPs and DTEPs, not affecting PC9s*)

PC9s   DTPs   DTEPs

$\longrightarrow$ Time (during drug treatment) $\longrightarrow$

Sharma et al. Cell 2010
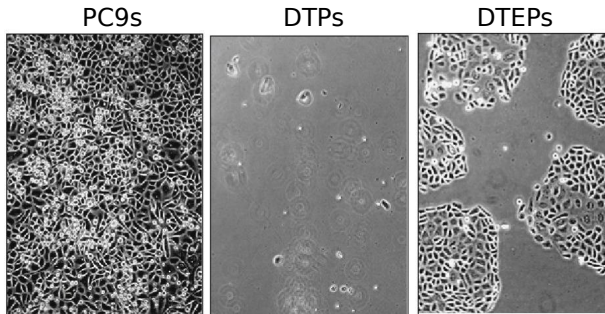
Structured cell population model: *cell-functional* variables

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

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

$$\frac{\partial n}{\partial t}(x, y, t) + \underbrace{\frac{\partial}{\partial y}\Big(v(x, c(t); \bar{v})n(x, y, t)\Big)}_{\substack{\text{Stress-induced adaptation} \\ \text{of the proliferation level}}} =$$

$$\underbrace{\Big[p(x, y, \varrho(t)) - d(x, c(t))\Big]n(x, y, t)}_{\text{Non local Lotka-Volterra selection}} + \underbrace{\beta\Delta n(x, y, t).}_{\substack{\text{Non-genetic} \\ \text{phenotype instability}}}$$

- $\varrho(t) = \int_0^1 \int_0^1 n(x, y, t)\, dx\, dy$, $p(x, y, \varrho(t)) = (a_1 + a_2 y + a_3(1 - x))(1 - \varrho(t)/K)$
  and $d(x, c) = c(b_1 + b_2(1 - x)) + b_3$
- The drift term w.r.t. proliferation potential y represents possible (if $v \neq 0$)
  'Lamarckian-like', epigenetic and reversible, adaptation from PC9s to DTPs
- $v(x, c(t); \bar{v}) = -\bar{v}c(t)H(x^* - x)$ where $t \mapsto c(t)$ is the drug infusion function
- No-flux boundary conditions

Chisholm et al., Cancer Research 2015

Same framework using an agent-based model (ABM)
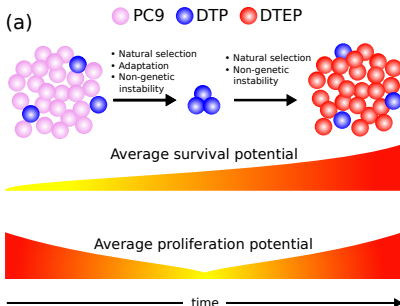
(a) Each cell i undergoes either proliferation, death or remains quiescent:
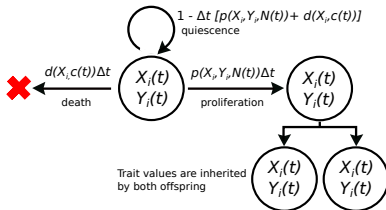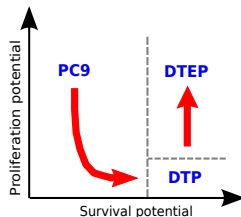
(b) Each cell i updates its trait values according to the discretised SDEs:

$$X_i(t+\Delta t) = X_i(t) + D\sqrt{\Delta t}\, W_i^1$$

$$Y_i(t+\Delta t) = Y_i(t) + D\sqrt{\Delta t}\, W_i^2 + \Delta t\, v(X_i, c(t))$$

Chisholm et al., Cancer Research 2015

Individual cell behaviour (ABM) can be different from the averaged dynamics observed at the population level
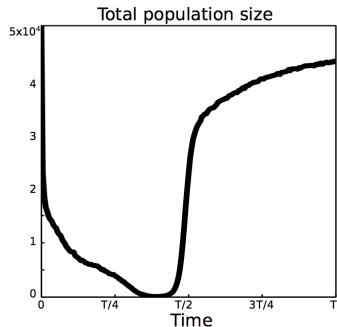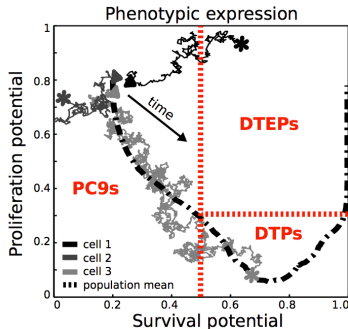
- Evolution in the I-B model (here no DTPs initially present, adaptation on): heterogeneity of behaviours in the population of PC9 cells.
- Left: Trajectories of the phenotypic expression of 3 individual cells and mean phenotypic expression of the cell population (dashed line). Triangles: initial phenotype of cells; asterisks: last phenotype expressed by cells before death
- Right: Corresponding global population density as a function of time.

Chisholm et al., Cancer Research 2015

AB model and PDE model recover phenotype dynamics

During drug exposure and after drug withdrawal: total recovery of drug sensitivity
(either high or low drug dose)
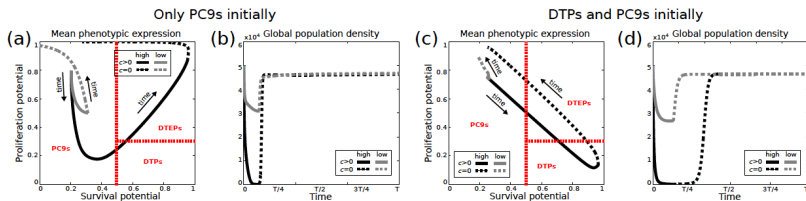
(a), (b) Only PC9s initially, adaptation on $v \neq 0$: 'Lamarckian' scenario, or
Luria-Delbrück scenario (A)

(c), (d) PC9s and DTPs initially, no adaptation $v = 0$: 'Darwinian' scenario, or
Luria-Delbrück scenario (B)

Chisholm et al., Cancer Research 2015

Resensitisation after drug washout is in the model

During drug exposure and after drug withdrawal: total recovery of drug sensitivity (either high or low drug dose)

Two scenarios: Lamarckian adaptation, or sheer Darwinian selection of the fittest
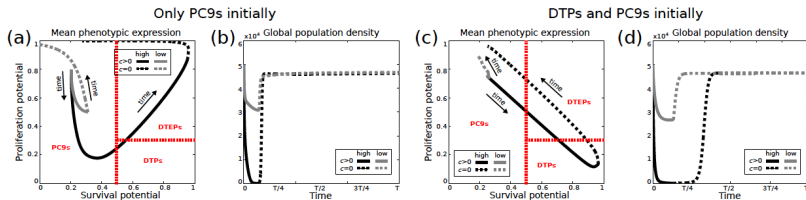
(a), (b) Only PC9s (no DTPs initially), adaptation on ($v \neq 0$): 'Lamarckian' scenario

(c), (d) PC9s and DTPs initially, no adaptation ($v = 0$): 'Darwinian' scenario
(sheer selection of the fittest = DTPs, supposed to be present in the initial population)

Phenotype heterogeneity in the cancer cell population
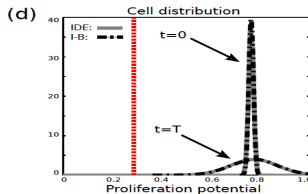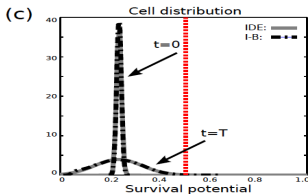
The PC9 cell population becomes more heterogeneous when it is left to evolve in the absence of drug treatment: starting from an initial concentrated phenotype (x_0, y_0), the phenotype (x, y) diffuses in the population according to a Gaussian-like curve. (c) Projection onto the x phenotype axis; (d) Projection onto the y phenotype axis.
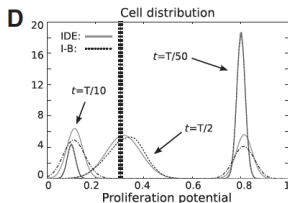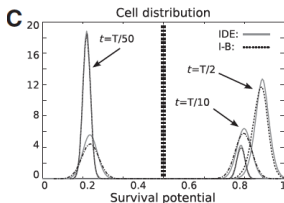
C, **D**: Under drug treatment, heterogeneity persists when phenotypes evolve (here, Darwinian scenario: DTPs are initially present)

Chisholm et al., Cancer Research 2015

Use PDE (or AB) model to address 3 questions

Q1. Is non-genetic instability (Laplacian term) crucial for the emergence of DTEPs?

Q2. What can we expect if the drug dose is low?

Q3. Could genetic mutations, i.e., an integral term involving a kernel with small support, to replace both adapted drift (advection) and non-genetic instability (diffusion), generate similar dynamics?

Consider $c(\cdot) = constant$ and two scenarios:
  (i) (*'Darwinian' scenario (B)*: the dogma) PC9s and few DTPs initially, no adaptation ($v = 0$)

  (ii) (*'Lamarckian' scenario (A)*: the outlaw) Only PC9s initially, adaptation present ($v \neq 0$)

To make a long story short, **Q1**. Always yes! Whatever the scenario
                          Q2. Low drug doses result in DTEPs, but no DTPs
                          Q3. Never! Whatever the scenario

A1. Non-genetic instability is crucial for the emergence of DTEPs

[*Scenario (B)* PC9s and few DTPs initially present]

DTPs and PC9s initially
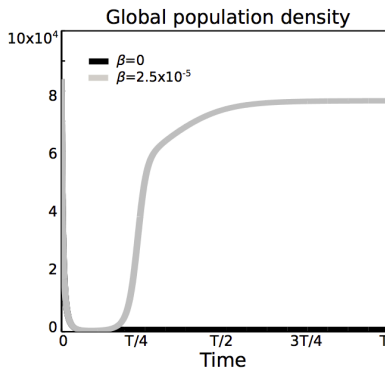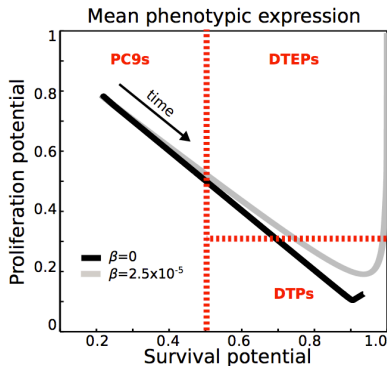
Extinction when $\beta = 0$ (here, adaptation is absent $v = 0$)

A1. Non-genetic instability is crucial for the emergence of DTEPs

[*Scenario (A) Only PC9s initially present*]

Extinction when $\beta = 0$ (here, adaptation is present $v \neq 0$)

Q2. What can we expect if the drug dose is low?

Definition (LC$_\gamma$ 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

A2. High dose of cytotoxic drugs is necessary for the transient dominance of DTPs

[*Scenario (B)* PC9s and DTPs initially present]

DTPs and PC9s initially

Mean phenotypic expression

Global population density

Low drug dose does not let appear DTPs (here, adaptation is absent $v = 0$)

A2. High dose of cytotoxic drugs is necessary for the transient dominance of DTPs

[*Scenario (A)* Only PC9s initially present]

Only PC9s initially
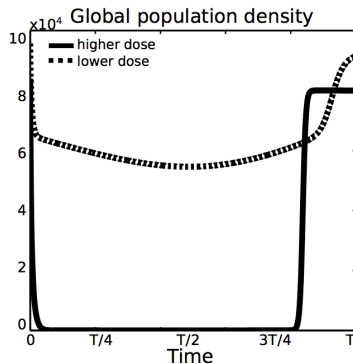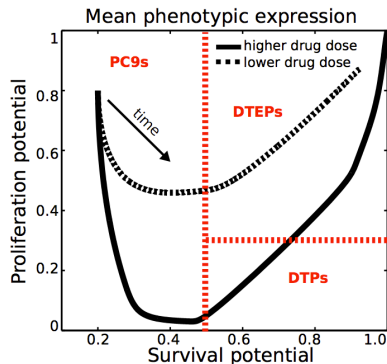
Low drug dose does not let appear DTPs (here, adaptation is present $v \neq 0$)

Chisholm et al., Cancer Research 2015

Q3. Could genetic mutations generate similar dynamics?

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

$$\frac{\partial n}{\partial t}(x, y, t) = \underbrace{\left[(1-\alpha)p(x, y, \varrho(t)) - d(x, c(t))\right]n(x, y, t)}_{\text{birth and death term due to sheer selection}}$$

$$+ \underbrace{\alpha \int_0^1 \int_0^1 p(\xi, \eta, \varrho(t))M(x, y|\xi, \eta; \sigma)n(\xi, \eta, t)d\xi \, d\eta,}_{\text{birth term due to genetic mutations}}$$

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

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

A3. Genetic mutations cannot generate similar dynamics

[*Scenario (B)* Initially there are DTPs and PC9s]

- G: only mutations and selection, vs.

- NG: non-genetic phenotype instability and selection
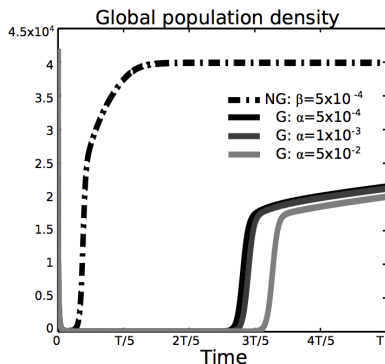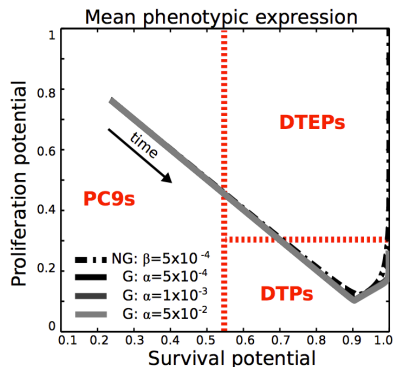
G: mutations do not let occur total recovery (NG: here, adaptation is absent $v = 0$)

Chisholm et al., Cancer Research 2015

A3. Genetic mutations cannot generate similar dynamics

[*Scenario (A)* Initially there are only PC9s]

- G: only mutations and selection, vs.

- NG: non-genetic phenotype instability, adaptation and selection
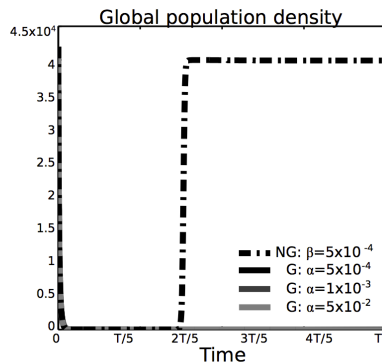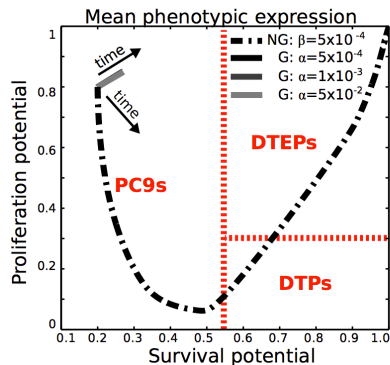
G: total extinction (NG: here, adaptation is present $v \neq 0$)

Chisholm et al., Cancer Research 2015

An experiment to decide between scenarios A and B

According to this model, it should be possible to decide between scenarios, by designing a biological experiment *using a low dose exposure*: Simulations show that:

In the presence of a low drug dose, if Scenario A [$\bar{v} > 0$: no DTPs present initially, Lamarckian adaptation present] is true, then the mitotic rate should show a sharp decrease for a long time, to increase again after that time, then yielding DTEPs, (Figure below: central and right panels, grey and convex lines only)

whereas if Scenario B [$\bar{v} = 0$: no Lamarckian instruction, DTPs present initially, and only Darwinian selection] prevails, then the mitotic rate should slowly increase at first, to secondarily decrease and finally increase again, yielding DTEPs. (Figure below: initially concave lines : left panel, all lines; central and right panels, black line only)

(Chisholm et al., Cancer Research 2015)
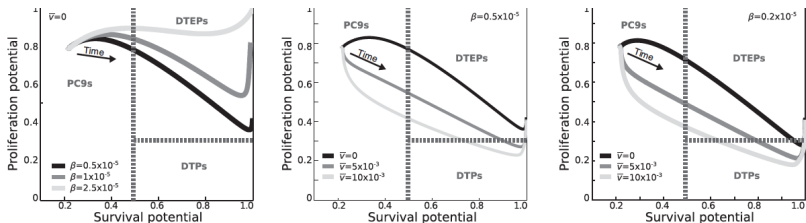
Summary of simulation results on the *Sharma et al.* paper

- Both mathematical models (AB, IDE) reproduce the main experimental observations

- To see the transient appearance of the DTPs during high-dose drug therapy:

  - If there are **some DTPs** present initially, model explanation requires only
    - non-genetic instability
    - selection

  - If **no DTPs** are present initially, model explanation requires interplay between
    - stress-induced adaptation
    - non-genetic instability
    - selection

- Therapeutic consequences? Not clear yet. Epigenetic drugs? Not many of them exist (in particular no KDM5A inhibitor). Acting on epigenetics by modifying metabolism? Combining cytotoxic (inducing drug resistance) drugs and cytostatic drugs at low doses (not inducing drug resistance)? To be assessed using this model?

Modelling *bet hedging* using 3 cell-functional variables?

- What is more relevant for stress response of a cell population (adaptable, as in the case of a tumour): maintain a subpopulation of all-stress resistant cells, or maintain a subpopulation of cells expressing 'cold genes' and able to launch different resistance mechanisms in different cells? (... stochastically chosen?)

- *Bet hedging* as a 'tumour strategy' to diversify its responses to deadly stress (as high doses of cytotoxic drugs) by launching different stress response mechanisms in different cells? (ABC transporters, detoxication enzymes, DNA repair...)

- Stress response through derepression of *cold genes*? Wu et al. PNAS 2015: existence of very ancient genes, constituted in a remote past of our planet, able to put at work survival programs in a state of emergency, with *bet hedging*, in a cancer cell population?

- Does *bet hedging* shuffle phenotypes, setting favorable bases for the emergence of specialisation (Michod et al. JTB 2006) and cooperativity in tumours (Tabassum & Polyak Nature Rev. Cancer 2015, Polyak & Marusyk Nature 2014), making them viable?

- Bet hedging setting for $n(x, y, \theta, t)$, with x=fecundity, y=viability, θ=plasticity:

$$n_t + \nabla \cdot \{V(x, y, \theta, D)\, n\} = \alpha(\theta)n_{xx} + \beta(\theta)n_{yy} + n\left\{r(x, y, \theta) - \frac{\rho(t)}{C(x, y)} - \mu(x, y, \theta, D)\right\}$$

(underway with C. Carrère, T. Lorenzi, G. Nadin)

References

- Lorz, A, Lorenzi, T, Hochberg, ME, *JC*, Perthame, B. Populational adaptive evolution, chemotherapeutic resistance and multiple anticancer therapies. *ESAIM: Mathematical Modelling and Numerical Analysis (M2AN)*, 47(02):377-399, 2013.

- Chisholm, RH, Lorenzi, T, Lorz, A, Larsen, AK, Almeida, L, Escargueil, A, *JC*. Emergence of drug tolerance in cancer cell populations: an evolutionary outcome of selection, non-genetic instability and stress-induced adaptation. *Cancer Research*, 75(6):930-939, 2015.

- 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. *Bull. Math. Biol.*, 77(1):1-22, 2015.

- Lorenzi, T., Chisholm, RH, Desvillettes, L, Hughes, BD. Dissecting the dynamics of epigenetic changes in phenotype-structured populations exposed to fluctuating environments. *Journal of Theoretical Biology*, 386:166-176, 2015.

- Chisholm, RH, Lorenzi, T, Lorz, A. Effects of an advection term in nonlocal Lotka-Volterra equations. *Comm. Math. Sciences*, 14:1181–1188, 2016.

- Chisholm, RH, Lorenzi, T., *JC*. Cell population heterogeneity and evolution towards drug resistance in cancer: biological and mathematical assessment, theoretical treatment optimisation. *BBA General Subjects*, 1860(11):2627-2645, 2016.

- Lorenzi, T., Chisholm, RH, *JC*. Tracking the evolution of cancer cell populations through the mathematical lens of phenotype-structured equations. *Biology Direct*, 11:43, 2016.

- Pouchol, C., Trélat, E, Lorz, A, *JC*. Asymptotic analysis and optimal control of an integro-differential system modelling healthy and cancer cells exposed to chemotherapy. *JMPA*, 2018.

- See also https://who.rocq.inria.fr/Jean.Clairambault/JCarticles.html

Why is evolution important in cancer? Basic questions on *multicellularity and cancer*

- **Cancer is a disease of multicellular organisms**, that has been evidenced, including in fossils, in the whole animal kingdom
- Cancer *is* the failure of maintenance of a coherent (=founded on stable cellular differentiations) multicellularity, or else:
- **Cancer may be defined as a loss of cohesion of tissues and organs of a same organism following failures in differentiation**
- Does there exist in the construction of multicellularity a qualitative succession of emergences of families of genes responsible for 1. proliferation and apoptosis 2. differentiation (transcription factors?); 3. epigenetic control of differentiations ? Phylogenetic scenarios of evolution of mutations in AML go in the opposite direction with increasing malignancy (Hirsch et al. Nature Comm. 2016)
- Some gene mutations predispose subjects to well-identified organ cancers: do these genes play a role in the anatomic constitution of multicellularity?
- Evolution proceeds by *tinkering* (*François Jacob*, 'Evolution and tinkering', *Science* 1977), using every possible avaible material: what in such a succession of tinkerings makes an organism viable but fragile?
- **The genes that are altered in cancers are the same that serve multicellularity design** (*Domazet-Lošo & Tautz 2010, Davies & Lineweaver 2011*): can we methodically collect these genes?

Questions (continued)

- What defines *a same organism* ? A 'self' that would be conserved during the sequences of differentiations that in Man lead from the first embryonic cell to the '200 terminally differentiated cell types' ?

- What holds together, normally without conflict, the cell types (interferon??), and what does the immune system recognise as non-self (foe rather than friend) in a cancer cell? Is there a *duality* between immune control and epigenetic barriers?

- Is there a relationship of such coherence with the major histocompatibility complex (MHC)? What is its primary function, if not to ensure organism cohesion (of tissues), and how does such coherence (of signals) operate?

- Can we parallel evolution of species and evolution of their immune system? Some enlightenment to collect genes active at multicellularity constitution?

- Loss of control of differentiations: do all cancers have in their evolution an epigenetic origin or an epigenetic mandatory step?

- Some is known of mutations in genes that control epigenetics (e.g., DNMT3A, TET2) in early leukaemogenesis, and of genes of cell metabolism (IDH1, IDH2) in cancers (AML, glioblastoma): can we propose and exemplify a standard scenario linking perturbations of metabolism / perturbations of epigenetic control of differentiations / cancers?

Questions (continued)

- Energetic metabolism of the cell, intercellular communications and cancer: appearance of gap junctions in multicellularity and perturbations of physiological gap junctions, essential to multicellularity, in solid tumours? (*James Trosko*)

- Glycolytic vs. mitochondrial respiratory phenotypes: do cancer cells shift easily from one to the other (in other words, does a tumour practice a form of metabolic *bet hedging*?) Gravenmier et al. Bull. Math. Biol. 2017)

- What are the advantages and drawbacks of these 2 phenotypes? (efficiency of the TCA [=Krebs] cycle vs. rapidity of anaerobic glycolysis) When did appear the mitochondrial respiratory chain as a necessary condition for the physiological establishment of reliable intercellular communications?

Questions (continued)

- What is more relevant for stress response of a cell population (adaptable, as in the case of a tumour): maintain a subpopulation of all-stress resistant cells, or maintain a subpopulation of cells expressing 'cold genes' and able to launch different resistance mechanisms in different cells? (... stochastically chosen?)

- *Bet hedging* as a 'tumour strategy' to diversify its responses to deadly stress (as high doses of cytotoxic drugs) by launching different stress response mechanisms in different cells? (ABC transporters, detoxication enzymes, blocking influx, DNA repair)

- Stress response through derepression of *cold genes*? Wu et al. PNAS 2015: existence of very ancient genes, constituted in a remote past of our planet, able to put at work des survival programs in a state of emergency, with *bet hedging*, in a cancer cell population?

- Does *bet hedging* shuffle phenotypes, setting favorable bases for the emergence of specialisation (Michod et al.) and cooperativity in tumours (Tabassum & Polyak, Polyak & Marusyk), making them viable?

- Bet hedging setting for $n(x, y, \theta, t)$, with x=fecundity, y=viability, θ=plasticity:

$$n_t + \nabla \cdot \{V(x, y, \theta, D)\, n\} = \alpha(\theta) n_{xx} + \beta(\theta) n_{yy} + n \left\{ r(x, y, \theta) - \frac{\rho(t)}{C(x, y)} - \mu(x, y, \theta, D) \right\}$$

Questions (continued)

- Phenotypic heterogeneity of cancer cell populations in a same tumour in the case of stress response: result of primary massive de-differentiation?

- "Maintenance of phenotypic heterogeneity within cell populations is an evolutionarily conserved mechanism that underlies population survival upon stressful exposures." (Guler et al. Cancer Cell 2017) Chromatin regulators as 'cold genes' aiming at maintaining a subpopopulation of resistant cells in case of extreme, life-threatening, stress?

- Role of transposable elements in the maintenance of such heterogeneity? "In the context of evolution, activation, and propagation of transposable elements enables organisms to adapt to changing conditions by generating genomic diversity (...), but can also result in reduced fitness." (Guler et al. Cancer Cell 2017)

A few articles that open ways and challenges

1. *The atavistic theory of cancer*
   - A.S. Trigos, R.B. Pearson, A.T. Papenfuss, D.L. Goode, Altered interactions between unicellular and multicellular genes drive hallmarks of transformation in a diverse range of solid tumors. PNAS 114 (24):6406-6411, 2017

   - K.J. Bussey, L.H. Cisneros, C.H. Lineweaver, P.C.W. Davies, Ancestral gene regulatory networks drive cancer. PNAS 114(24):6160-6162, 2017

   - L.H. Cisneros, K.J. Bussey, A.J. Orr, M. Miločević, C.H. Lineweaver, P.C.W. Davies, Ancient genes establish stress-induced mutation as a hallmark of cancer. PLoS One 12(4):e0176258, 2017

   - Wu, A. *et al.* Ancient hot and cold genes and chemotherapy resistance emergence. PNAS 112(33):10467-10472, 2015

   - C.H. Lineweaver, P.C.W. Davies, M.D. Vincent, Targeting cancer's weaknesses (not its strengths): therapeutic strategies suggested by the atavistic model, Bioessays 36(9):827-835, 2014

   - P.C.W. Davies, C.H. Lineweaver, Cancer tumors as metazoa 1.0: tapping genes of ancient ancestors, Phys. Biol. 8(1):015001, 2011

   - M.D. Vincent, Cancer: a de-repression of a default survival program common to all cells?: a life-history perspective on the nature of cancer, Bioessays 34(1):72-82, 2011

   - M.D. Vincent, Cancer: beyond speciation, Advances in Cancer Research 112:283-350, 2011

   - L. Israel, Tumour progression: random mutations or an integrated survival response to cellular stress conserved from unicellular organisms? J. Theor. Biol. 178(4):375-380, 1996

   - T. Boveri, The origins of malignant tumors, Williams & Wilkins, Baltimore, 1929

A few articles that open ways and challenges

2. *Genes of multicellularity and genes that are altered in cancer*

- T. Domazet-Lošo, D. Tautz, An ancient evolutionary origin of genes associated with human genetic diseases, Mol. Biol. Evol. 25(12):2699-2707 (2008)

- T. Domazet-Lošo, D. Tautz, Phylostratigraphic tracking of cancer genes suggests a link to the emergence of multicellularity in metazoa, BMC Biol. 8(1):66 (2010)

- M. Srivastava, O. Simakov, J. Chapman, B. Fahey, M.E.A. Gauthier, T. Mitros, G.S. Richards, C. Conaco, M. Dacre, U. Hellsten, et al., The Amphimedon queenslandica genome and the evolution of animal complexity, Nature 466(7307):720-727 (2010)

- ... and others cited by Davies et Lineweaver, Phys. Biol. (2011)

2bis. *Cohesion of tissues, coherence between functions and organs*

- M. Plankar, I. Jerman, R. Krašovec, On the origin of cancer: Can we ignore coherence? Progr. Biophys. Mol. Biol. 106:380-390, 2011

A few articles that open ways and challenges

3. *Sui Huang: the Waddington epigenetic landscape revisited*

- S. Huang, Cell lineage determination in state space: a systems view brings flexibility to dogmatic canonical rules, PLoS Biol. 8 (5) (May 2010) e1000380.

- S. Huang, On the intrinsic inevitability of cancer: from foetal to fatal attraction, Semin. Cancer Biol. 21 (3) (Jun 2011) 183-199.

- S. Huang, The molecular and mathematical basis of Waddington's epigenetic landscape: a framework for post-Darwinian biology? Bioessays 34(2):149-157 (2012)

- S. Huang, Tumor progression: chance and necessity in Darwinian and Lamarckian somatic (mutationless) evolution, Prog. Biophys. Mol. Biol. 110(1):69-86 (2012)

- S. Huang, Genetic and non-genetic instability in tumor progression: link between the fitness landscape and the epigenetic landscape of cancer cells, Cancer Metastasis Rev. 32(3-4):423-448 (2013)

- S. Huang, G. Eichler, Y. Bar-Yam, D.E. Ingber, Cell fates as high-dimensional attractor states of a complex gene regulatory network, Phys. Rev. Lett. 94(12):128701 (2005)

- S. Huang, I. Ernberg, S. Kauffman, Cancer attractors: a systems view of tumors from a gene network dynamics and developmental perspective, Semin. Cell Dev. Biol. 20(7):869-876 (2009)

- S. Huang, Y.-P. Guo, G.May, T. Enver, Bifurcation dynamics in lineage- commitment in bipotent progenitor cells, Dev. Biol. 305 (2):695-713 (2007)

- A.O. Pisco, S. Huang, Non-genetic cancer cell plasticity and therapy-induced stemness in tumour relapse: 'what does not kill me strengthens me', Br. J. Cancer 112(11):1725-1732 (2015)

- A.O. Pisco, A. Brock, J. Zhou, A. Moor, M. Mojtahedi, D. Jackson, S. Huang, Non- Darwinian dynamics in therapy-induced cancer drug resistance, Nat. Commun. 4:2467 (2013)

- A.O. Pisco, A. Fouquier d'Hérouël, S. Huang, Conceptual Confusion: the case of Epigenetics, BioRxiv 053009 (2016)

- J.X. Zhou, M.D.S. Aliyu, E. Aurell, S. Huang, Quasi-potential landscape in complex multi-stable systems, J. R. Soc. Interface 9(77):3539-3553 (2012)

A few articles that open ways and challenges

4. *James Trosko and gap junctions*

- J.E. Trosko, Mechanisms of tumor promotion: possible role of inhibited intercellular communication, Eur. J. Cancer Clin. Oncol. 23 (6) (Jun 1987) 599-601

- J.E. Trosko, Gap Junctional Intercellular Communication as a Biological "Rosetta Stone" in Understanding, in a Systems Biological Manner, Stem Cell Behavior, Mechanisms of Epigenetic Toxicology, Chemoprevention and Chemotherapy, J. Membrane Biol. 218:93-100 (2007)

- J.E. Trosko, A conceptual integration of extra-, intra- and gap junctional-intercellular communication in the evolution of multi-cellularity and stem cells: how disrupted cell-cell communication during development can affect diseases later in life, Int. J. Stem Cell Res. Ther. 3(1):021 (2016)

A few articles that open ways and challenges

5. *Bet hedging, emergence of specialisation and multicellularity, tumoral cooperativity*

- B. Brutovsky, D. Horvath, Structure of Intratumor Heterogeneity: Is Cancer Hedging Its Bets? arXiv 1307.0607, 2013

- R.E. Michod, D. Roze, Cooperation and conflict in the evolution of multicellularity, Heredity 36:1-7, 2001

- R.E. Michod et al., Life-history evolution and the origin of multicellularity, J. Theor. Biol. 239:257-272, 2006

- K. Polyak, A. Marusyk, Clonal evolution, Nature, 508:52-53, 2014

- D.P. Tabassum, K. Polyak, Tumorigenesis: it takes a village, Nature Rev. Cancer, 15:473-483, 2015

A few articles that open ways and challenges

6. *Epigenetic bases of differentiation, epigenetic causes of cancer, epigenetics of DTPs*

- H.D. Madhani From a to α: Yeast as a Model for Cellular Differentiation, Cold Spring Harbor Laboratory Press, New York (2007)

- F. Nicol-Benoit, P. Le Goff, D. Michel, Drawing a Waddington landscape to capture dynamic epigenetics, Biol. Cell. 105(12):576-584 (2013)

- P.A. Jones, J.-P. Issa, S. Baylin. Targeting the cancer epigenome for therapy, Nat. Rev. Genet. 17:630-641 (2016)

- A.P. Feinberg, M.A. Koldobskiy, A. Göndör, Epigenetic modulators, modifiers and mediators in cancer aetiology and progression, Nat. Rev. Genet. 17:284-299 (2016)

- A.P. Feinberg, Phenotypic plasticity and the epigenetics of human disease, Nature 447 (7143):433-440 (May 2007)

- A.P. Feinberg, H. Cui, R. Ohlsson, DNA methylation and genomic imprinting: insights from cancer into epigenetic mechanisms, Semin. Cancer Biol. 12 (5):389-398 (Oct. 2002)

- A.P. Feinberg, R. Ohlsson, S. Henikoff, The epigenetic progenitor origin of human cancer, Nat. Rev. Genet. 7 (1):21-33 (Jan. 2006)

- A.P. Feinberg, H. Cui, R. Ohlsson, DNA methylation and genomic imprinting: insights from cancer into epigenetic mechanisms, Semin. Cancer Biol. 12 (5):389-398 (Oct. 2002)

- T. Mourier, L.P. Nielsen, A.J. Hansen, E. Willerslev, Transposable elements in cancer as a by-product of stress-induced evolvability, Front. Genet. 5, 156 (2014)

- G.D. Guler et al., Repression of Stress-Induced LINE-1 Expression Protects Cancer Cell Subpopulations from Lethal Drug Exposure, Cancer Cell 32:221-237 (Aug. 2017)

- M.A. Reid, ZW Dai, J.W. Locasale, The impact of cellular metabolism on chromatin dynamics and epigenetics, Nat. Cell Biol. (Publ. online, Oct. 2017)

A few articles that open ways and challenges

One may also consult a few personal articles

- Goldman, A., Kohandel, M., Clairambault, J. Integrating Biological and Mathematical Models to Explain and Overcome Drug Resistance in Cancer, Part 1: Biological Facts and Studies in Drug Resistance, Current Stem Cell Reports, 3:253-259 (August 2017)

- Goldman, A., Kohandel, M., Clairambault, J. Integrating Biological and Mathematical Models to Explain and Overcome Drug Resistance in Cancer, Part 2: From Theoretical Biology to Mathematical Models, Current Stem Cell Reports, 3:260-268 (August 2017)

- Chisholm, R.H., Lorenzi, T., Clairambault, J. Cell population heterogeneity and evolution towards drug resistance in cancer: biological and mathematical assessment, theoretical treatment optimisation. BBA General Subjects, special issue on system genetics, 1860:2627-2645 (June 2016)

- Almeida, A., Chisholm, R.H., Clairambault, J., Lorenzi, T., Lorz, A., Pouchol, C., Trélat, E. Why is evolution important in cancer and what mathematics should be used to treat cancer? Focus on drug resistance. pp. 107-120 in *Trends in Biomathematics: Modeling, Optimization and Computational Problems*, Springer 2018 (17th BIOMAT conference, Moscow, October 2017)

- *See also* https://who.rocq.inria.fr/Jean.Clairambault/JCarticles.html