Mathematical Biology *on the Mediterranean* Conference 2019

Modelling plasticity of cancer cells and emergence of drug-induced drug resistance using adaptive cell population dynamics: a therapeutic perspective

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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 ($x = 1$).

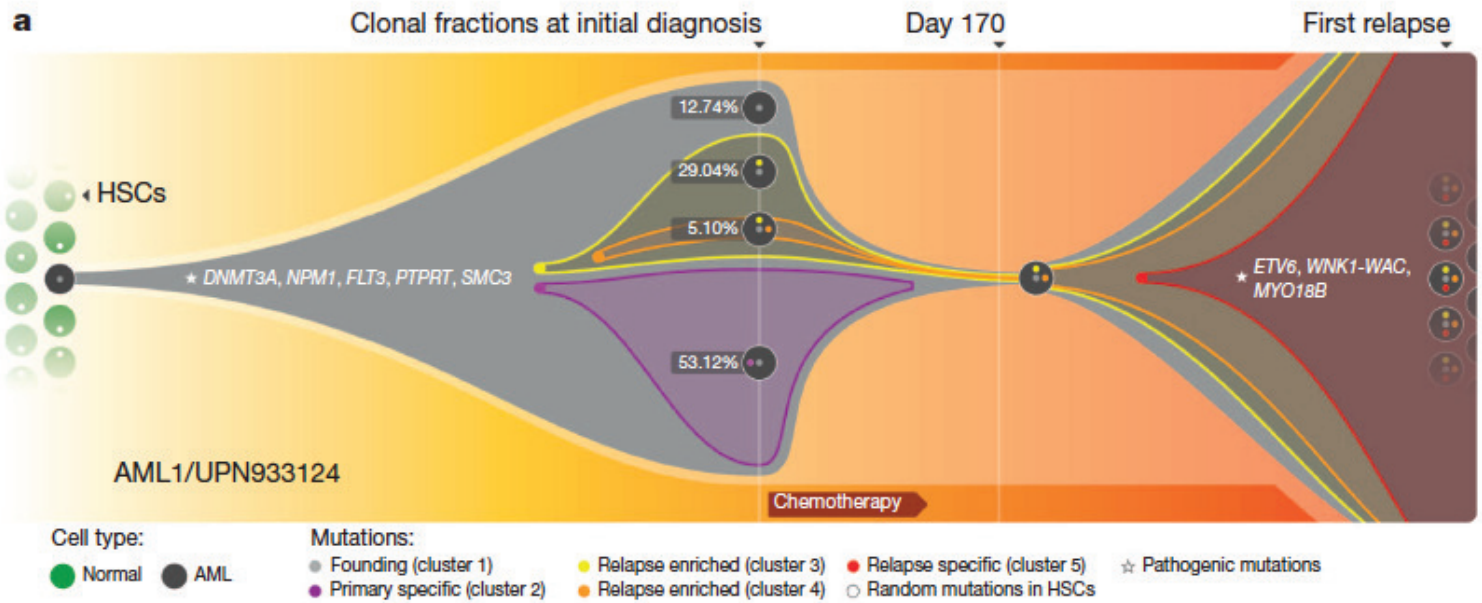
- Is such evolution towards drug resistance due to sheer Darwinian selection of the fittest by mutations in differentiation at cell division or, at least partially, due to phenotype adaptation in individual cells? Not clear.

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*).

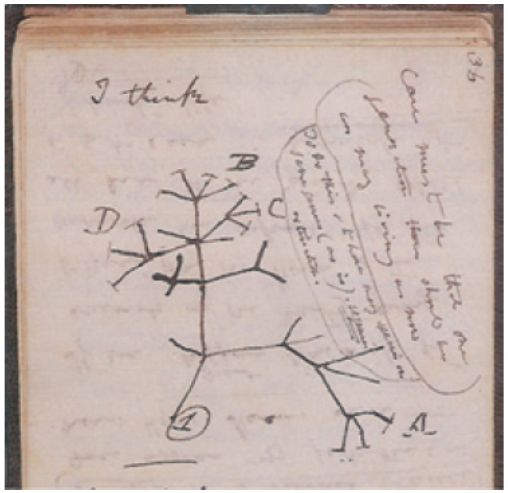
- In cancer populations, enhanced heterogeneity with enhanced proliferation results in a high phenotypic or genetic diversity of proliferating clonal subpopulations

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

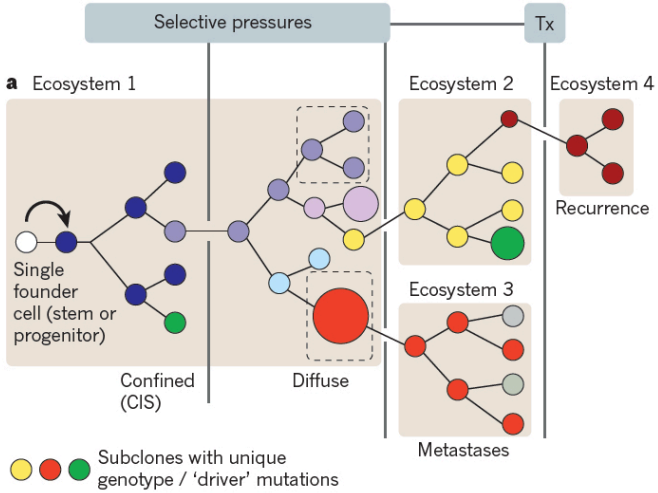


Ding et al. Nature 2012

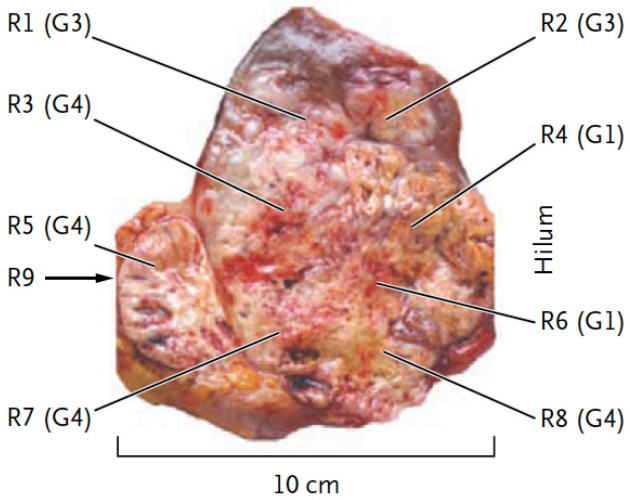
Drug resistance: always mutations and branching?

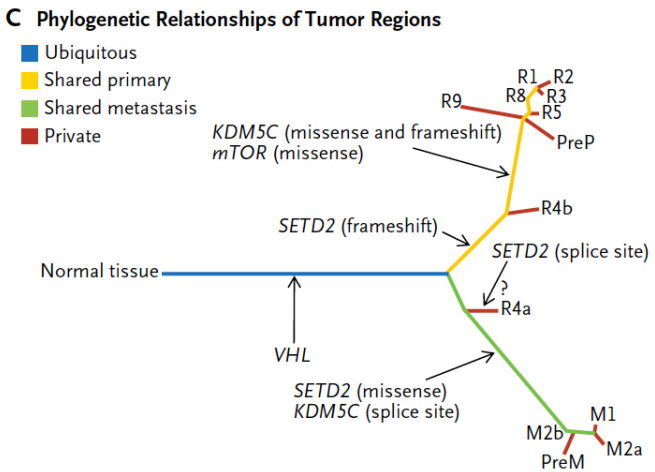


Darwin's notebook 1837



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)

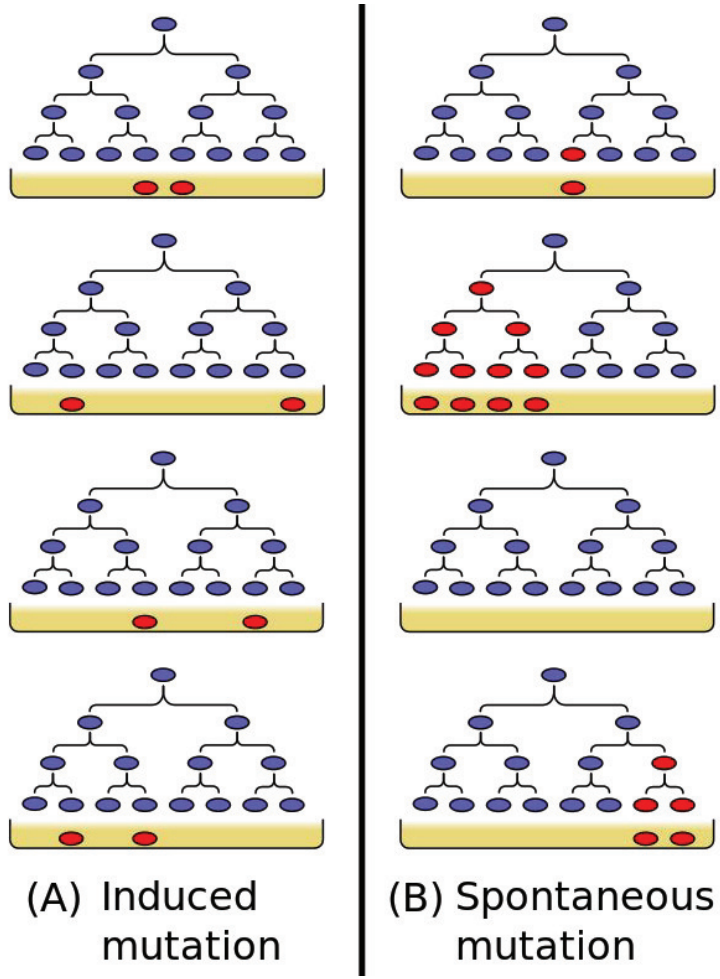
Different Petri dishes, same experimental settings

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

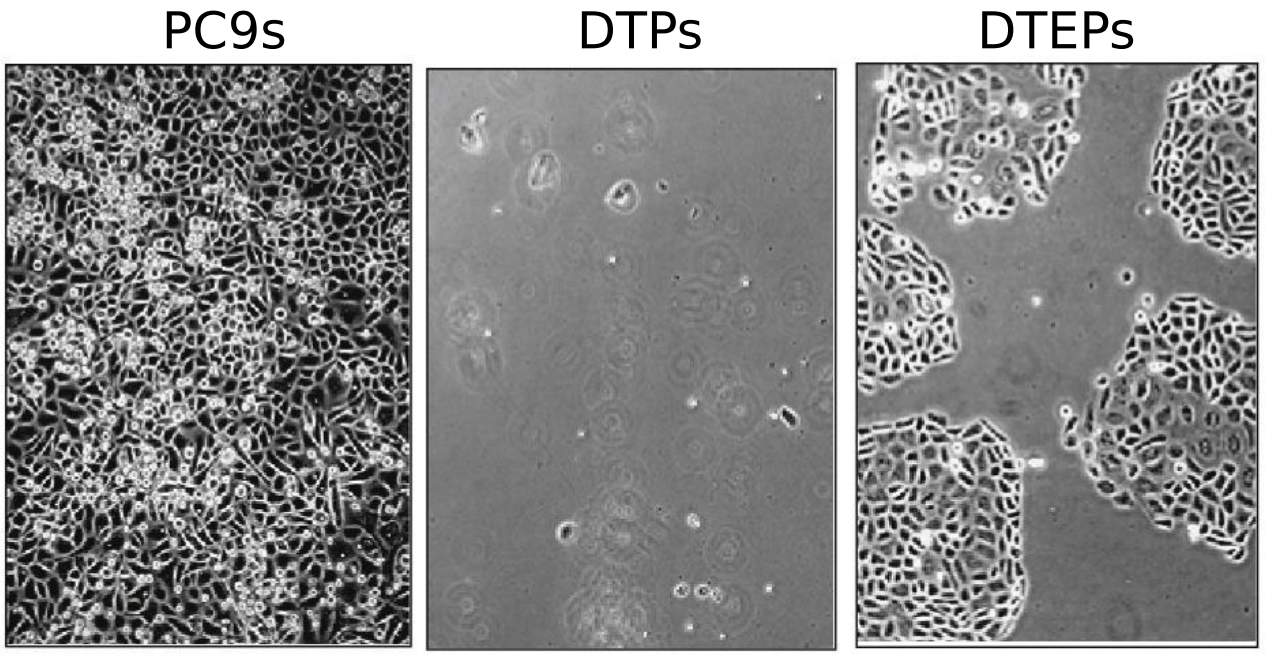
However, bacteria are not cancer cells! In particular, they are far from being able of the same plasticity (no differentiation is available for them)



(*Luria & Delbrück, Genetics, 1943*)

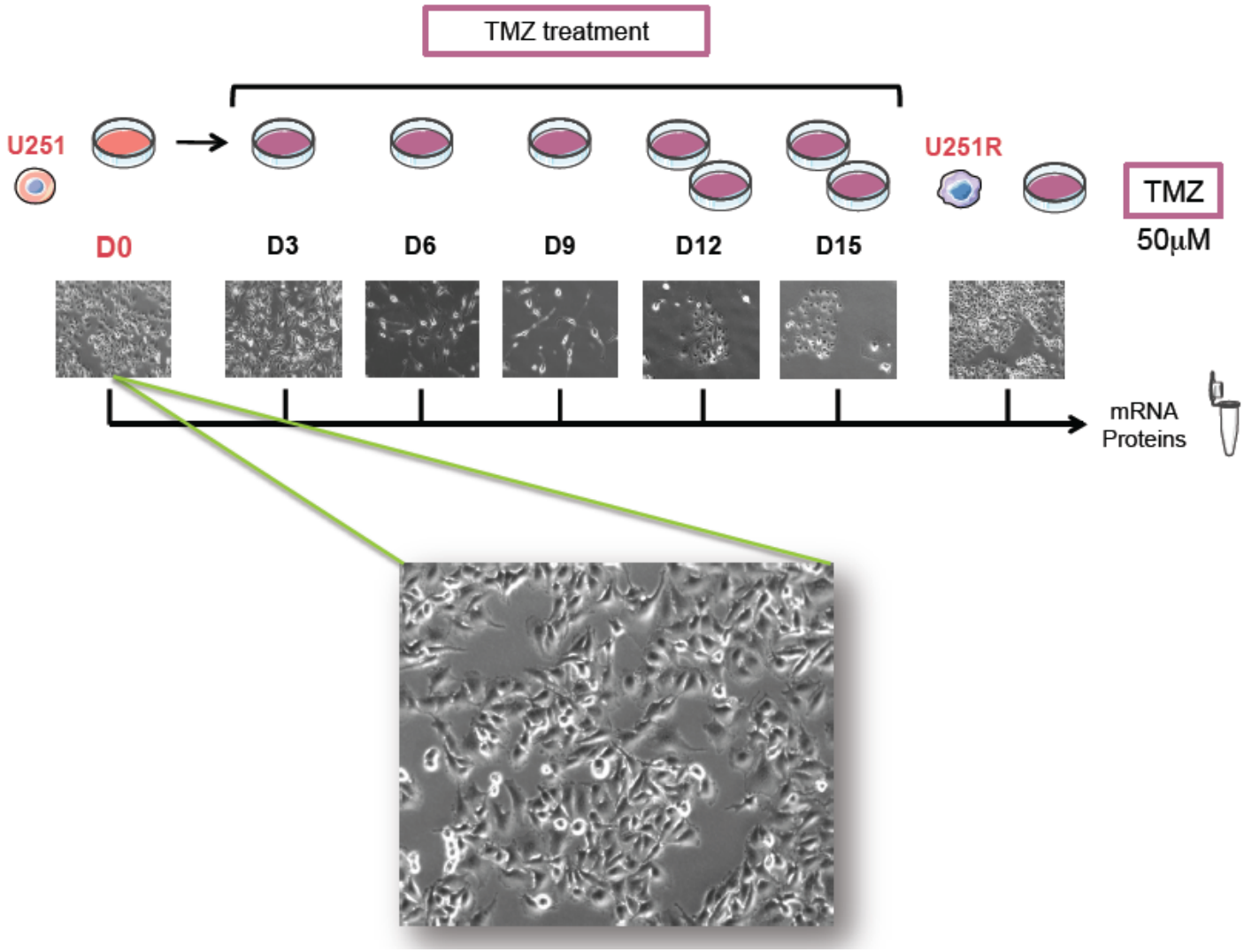
Biological experiment (2): reversible resistance in cancer

- 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



Sharma et al. Cell 2010

Glioblastoma and temozolomide: as in *Sharma Cell 2010*



from F. Vallette's INSERM team in Nantes

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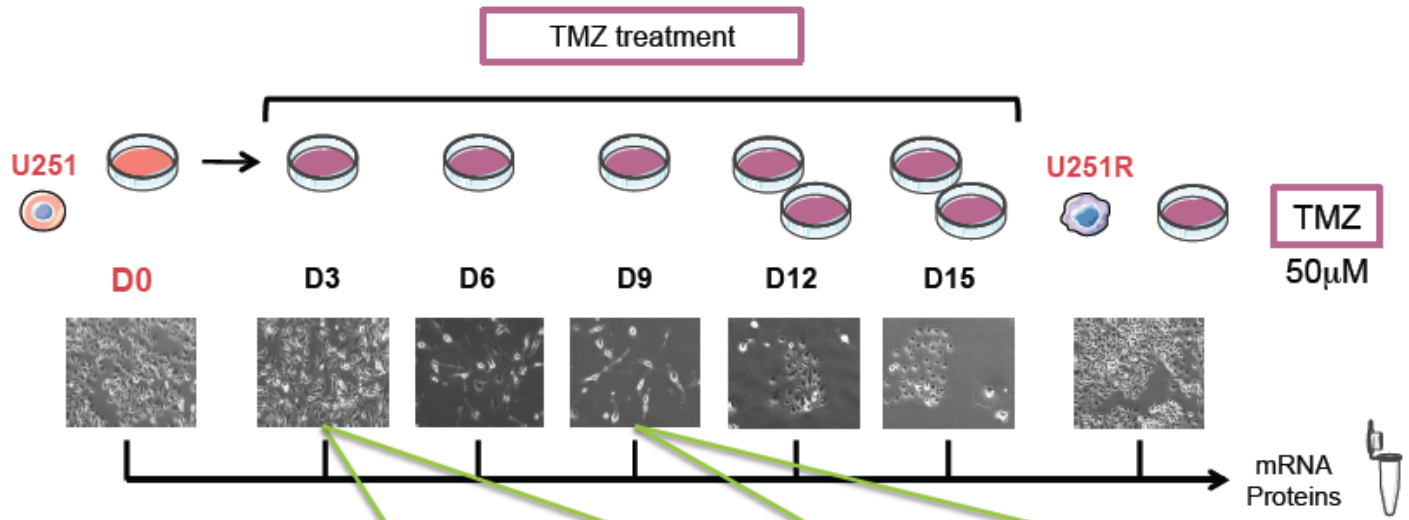
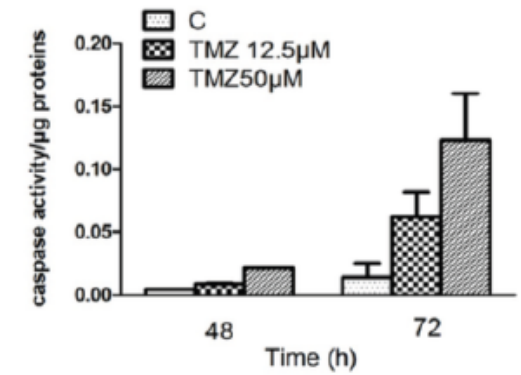
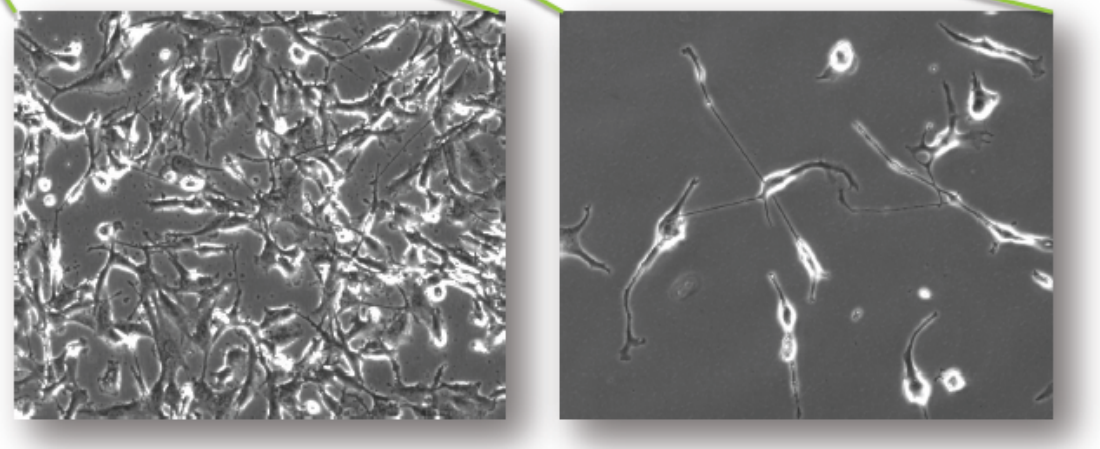




Gratas *et al*, 2014, Oncotarget



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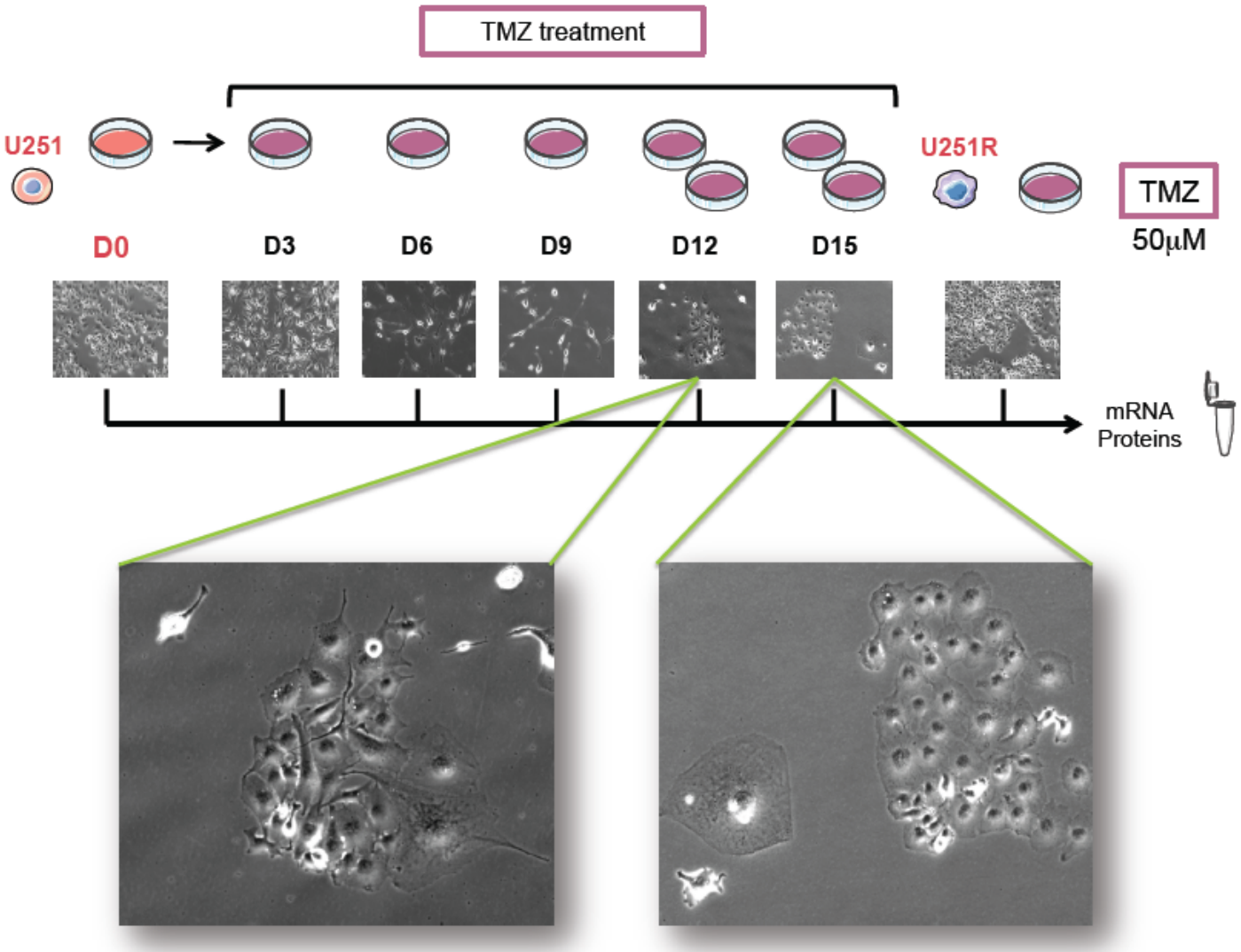

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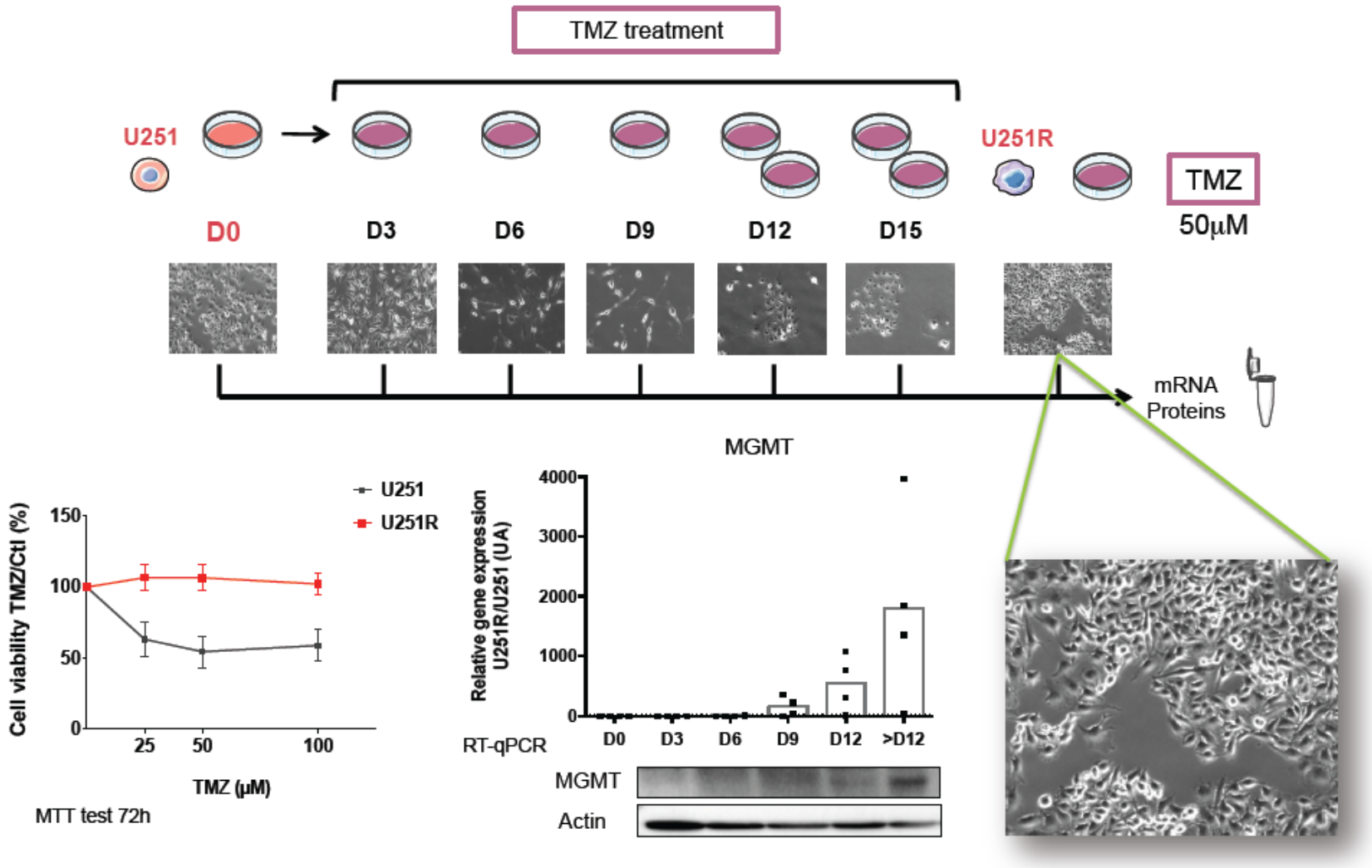
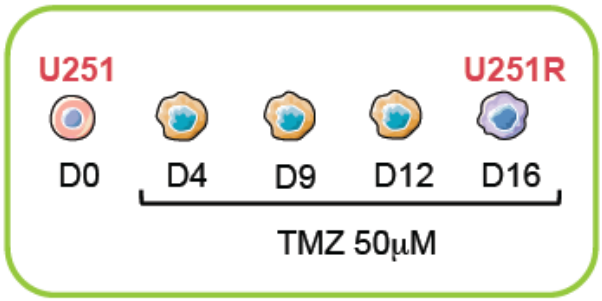
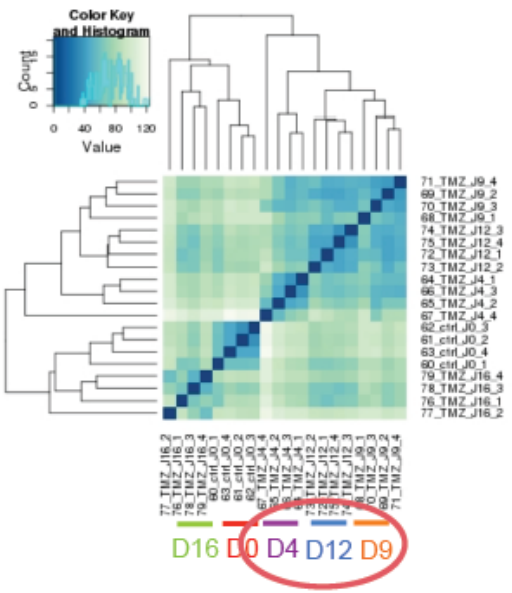


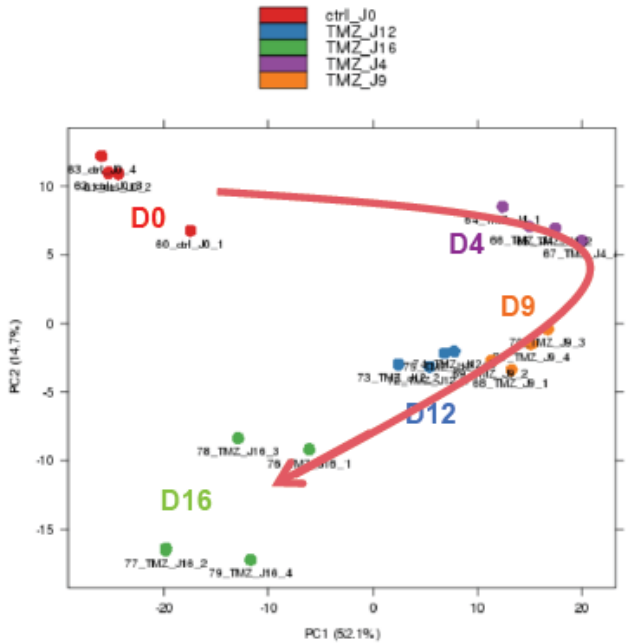
Gene expression followed from D0 to D16 (PCA)

Whole transcriptome sequencing

RNA-Seq







Short time window for combined treatment with an epigenetic drug??

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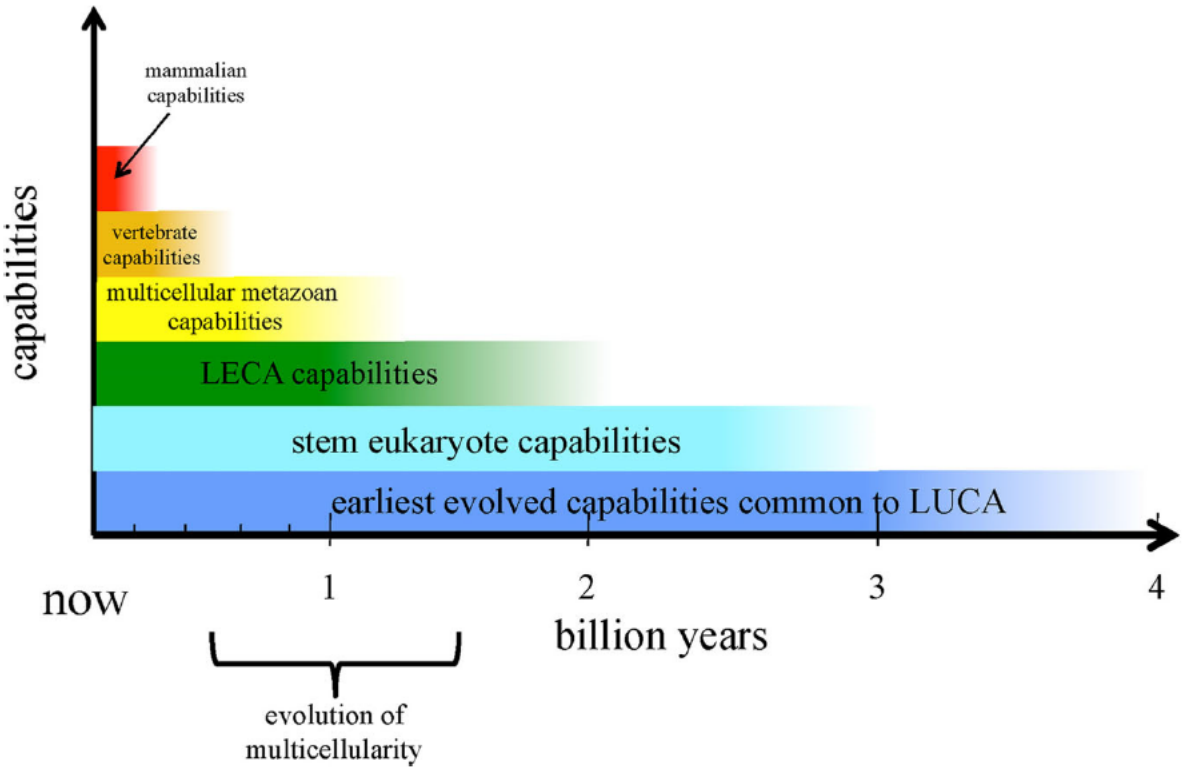
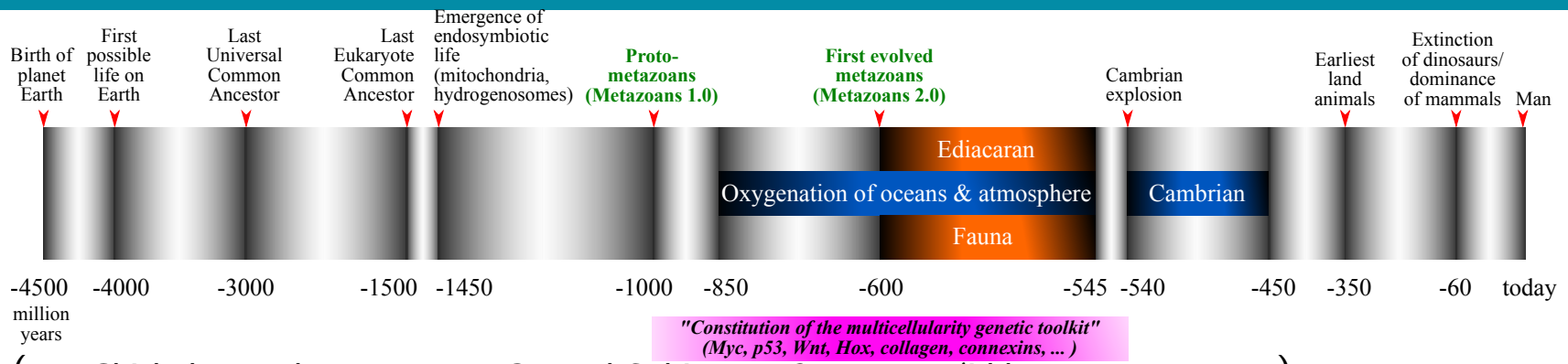
A possible evolutionary framework (*billion year-term view*): the atavistic hypothesis of cancer (1)

"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*)

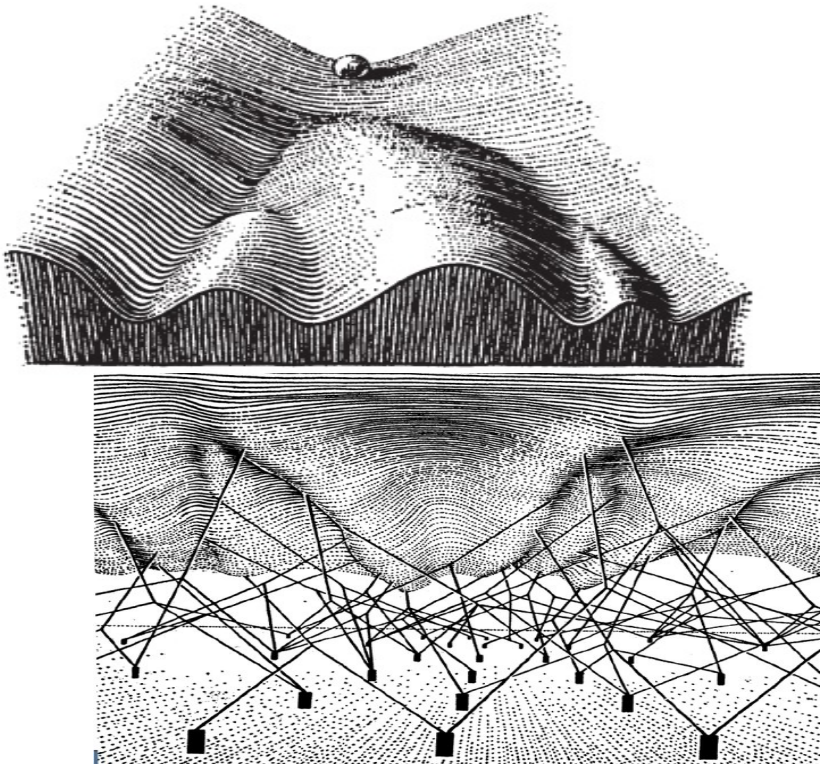
- The genes that have appeared in the process of development to multicellularity are precisely those that are altered in cancer
- In what order in evolution, from 1) proliferation+apoptosis to 2) cell differentiation +division of work, and to 3) *epigenetic control* of differentiation and proliferation?
- 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?

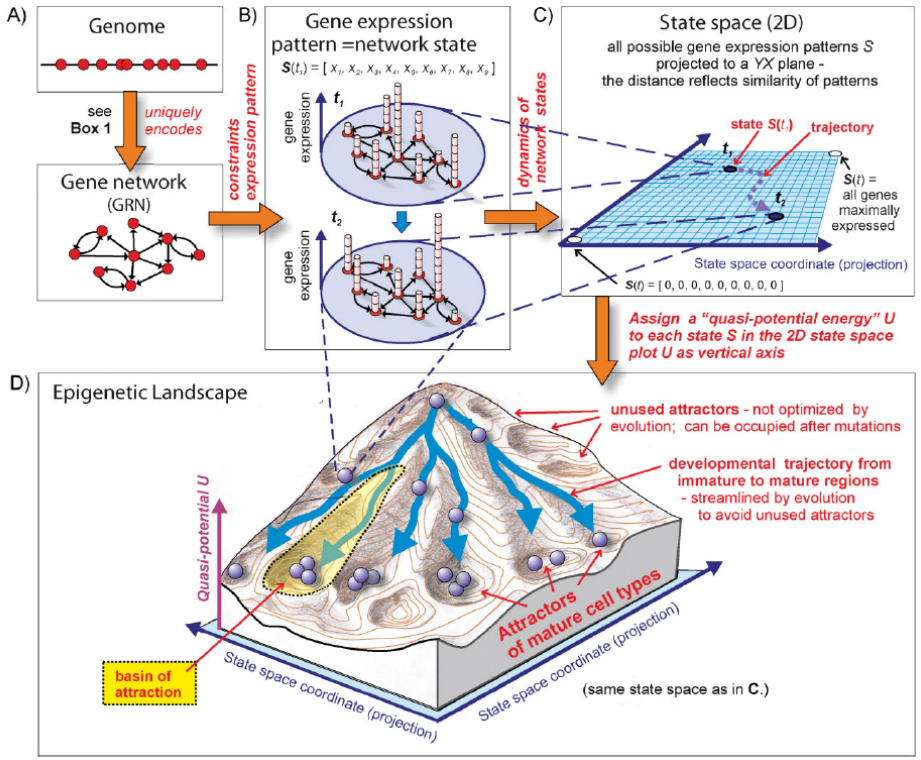
- According to the atavistic hypothesis, cancer is a 'backward evolution' from a sophisticated form of multicellularity (us), in which epigenetic processes control gene regulatory networks of transcription factors: differentiation factors, p53, etc., that themselves physiologically control the basis 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 ("The strategy of genes", 1957) for cell differentiation



Waddington landscape revisited by S. Huang (2011, 2012, 2013)



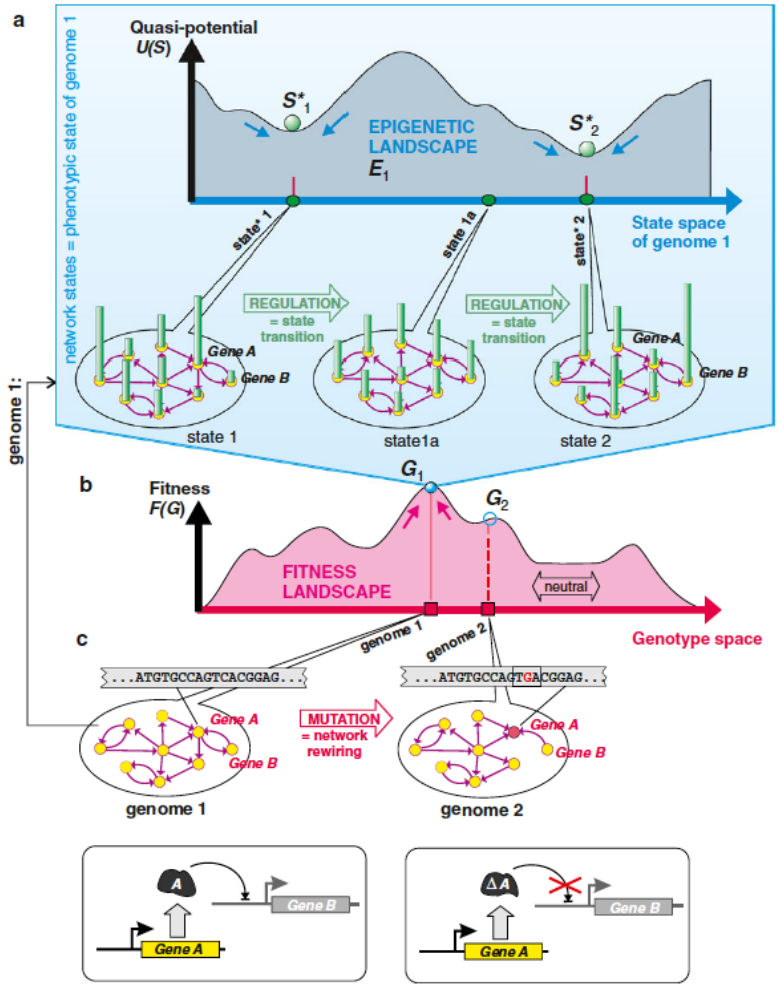
"Nothing in evolution makes sense except in the light of *systems* biology" (S. Huang, 2012)

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.

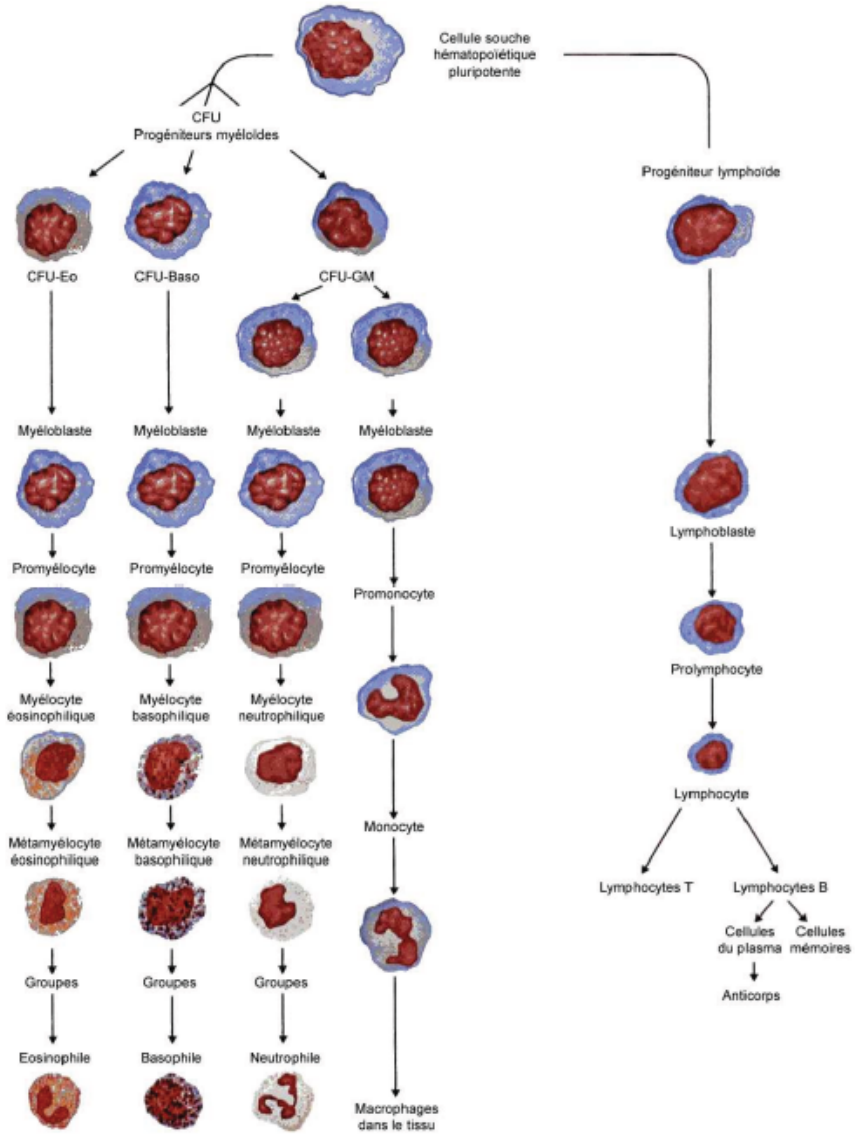
- *References: Sui Huang Sem Canc Biol 2011, Bioessays 2012, Canc Metastasis Rev 2013; Zhou Interface 2012; Pisco Br J C 2015...*

- 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.)

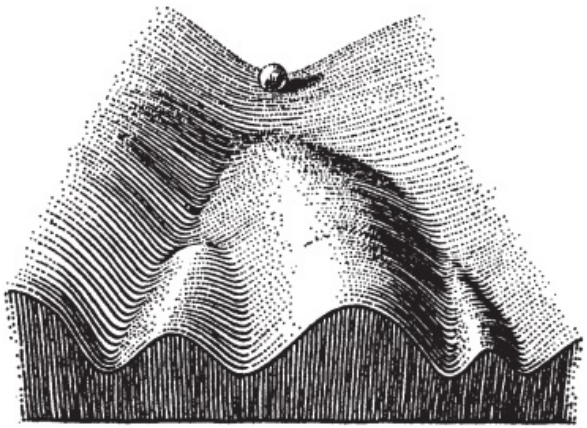


(*Sui Huang, Canc Metastasis Rev 2013*)

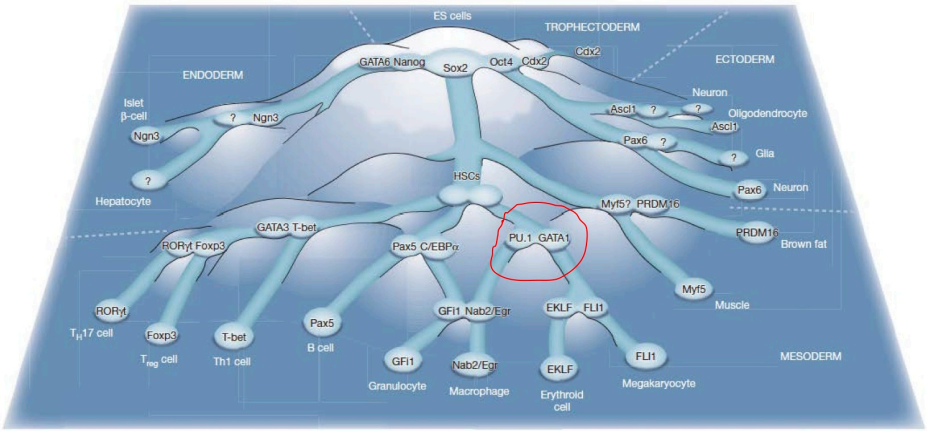
The best known case: haematopoiesis



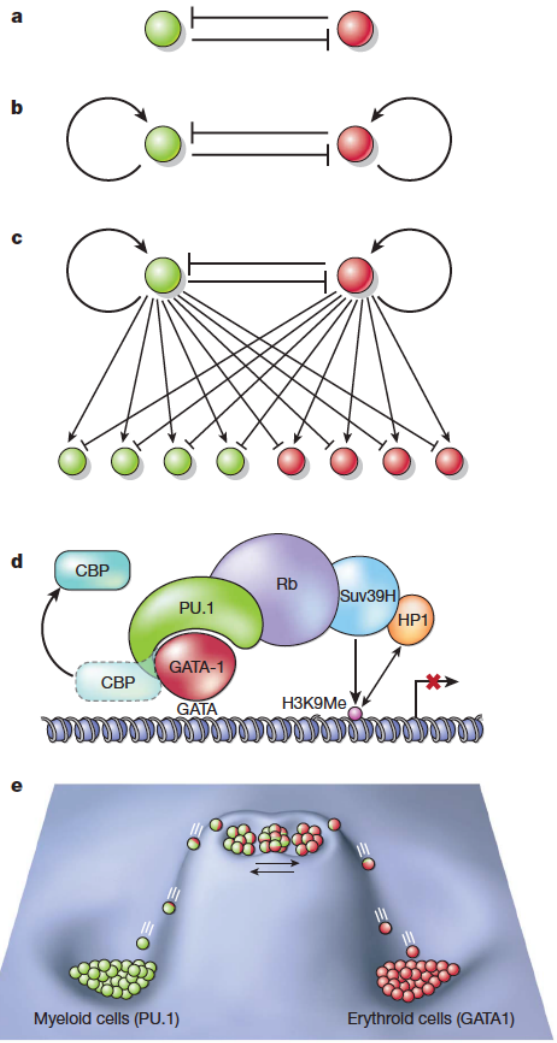
Some milestones to reconstruct the global landscape



[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)

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

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

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

- 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

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

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

where the total population is defined as

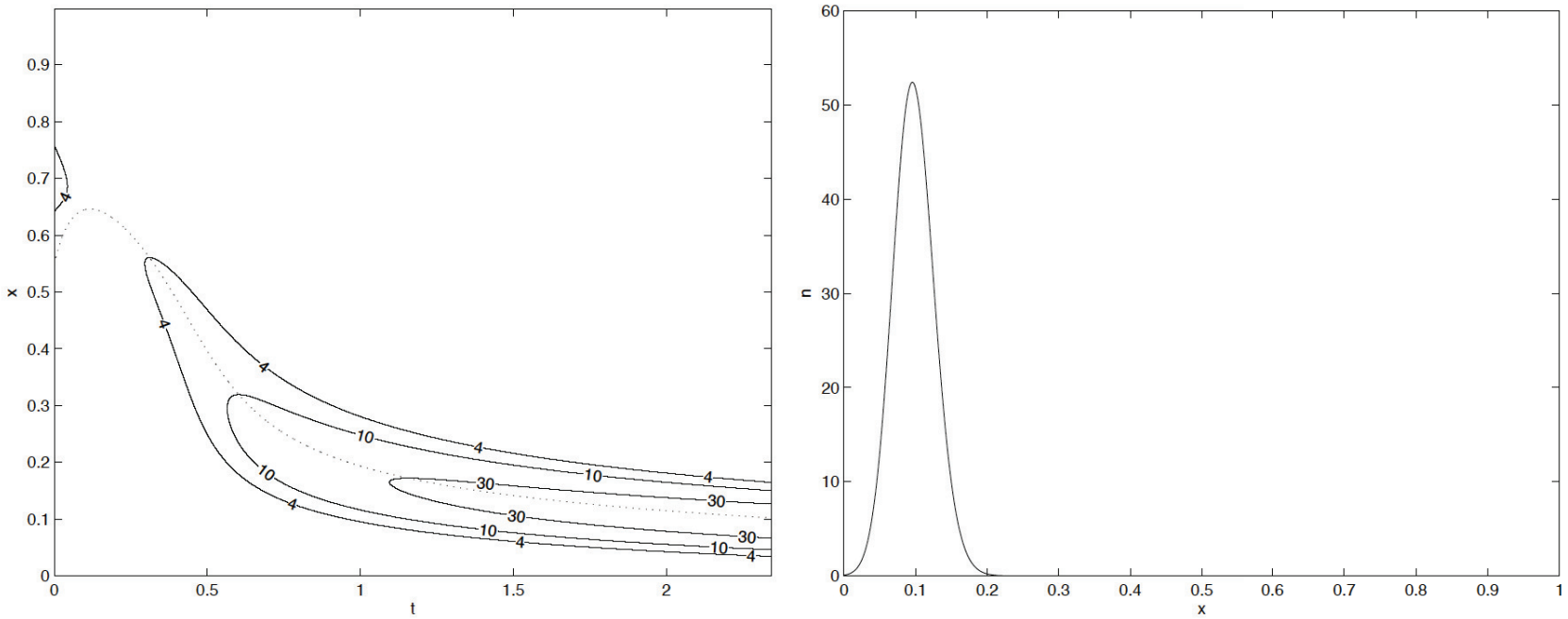
$$\rho(t) = \rho_H(t) + \rho_C(t); \rho_H(t) = \int_{x=0}^{\infty} n_H(x,t)dx; \rho_C(t) = \int_{x=0}^{\infty} n_C(x,t)dx.$$

- $\beta > 0$ 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.

Lorz et al., M2AN 2013

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)*

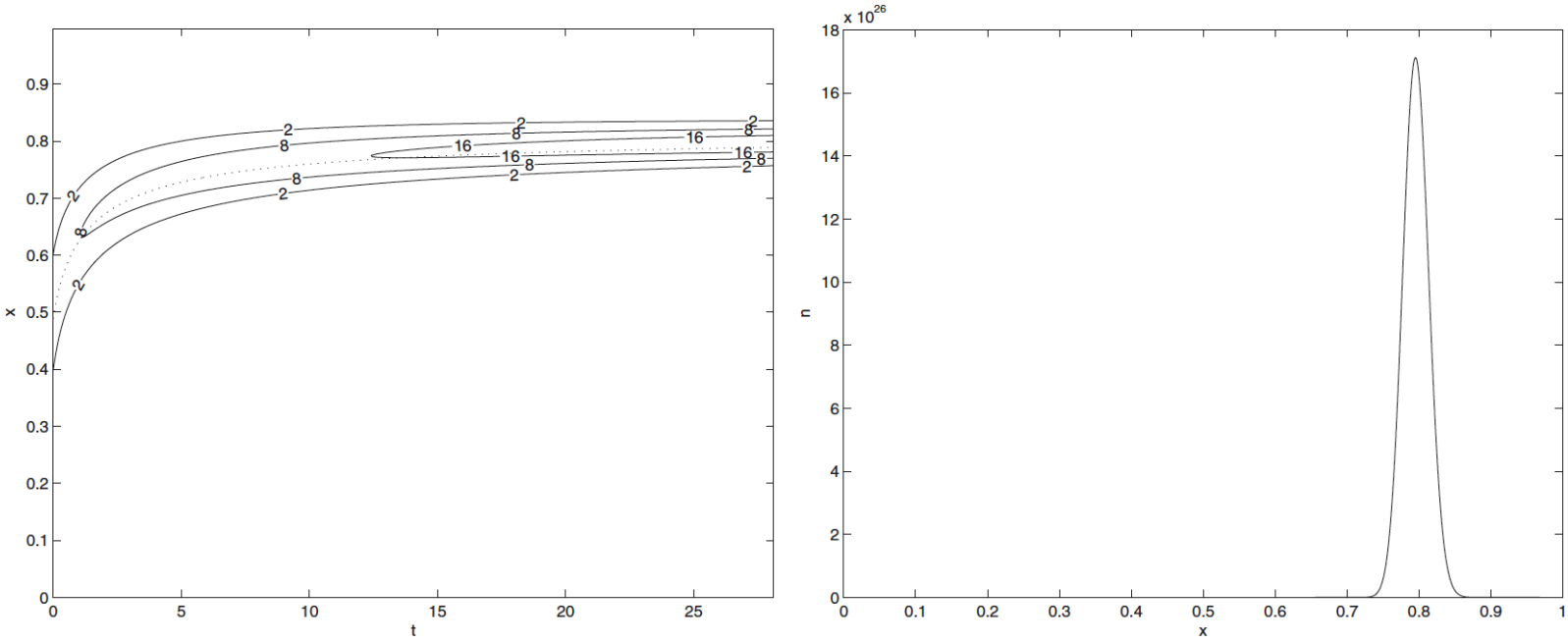


Left panel: starting from a medium phenotype $x = 0.5$, *level sets of a drug-sensitive population in the* (t, x) *plane. Right panel: asymptotic distribution of this drug-sensitive population according to the drug resistance phenotype* x.

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*.*

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

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

- (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 ($t \to +\infty$) 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

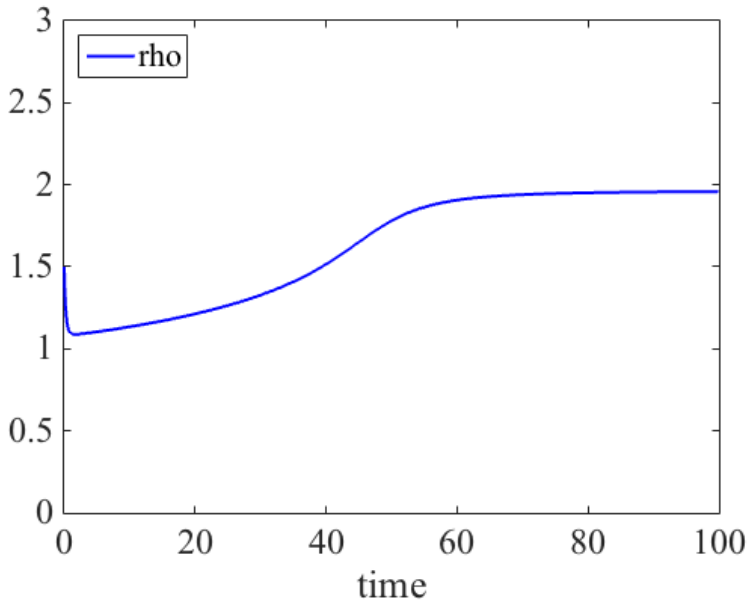
$$\rho(t) := \int_0^1 n(t,x)\, dx \quad \text{and} \quad n(0,x) = n^0(x).$$

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 $t \mapsto \rho(t)$

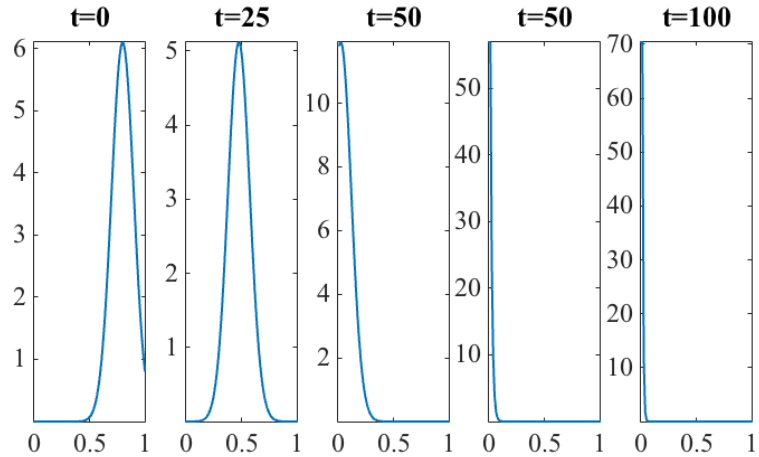


Firstly, it can be shown that: ρ converges to $\rho^\infty = \max_{[0,1]} \frac{r}{d}$, i.e., to the smallest value ρ such that $r(x) - d(x)\rho \leq 0$ on $[0, 1]$.

[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*

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

[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 $\int_0^1 n^\infty(x)\,dx = \rho^\infty = \max_{[0,1]} \frac{r}{d}$, 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]

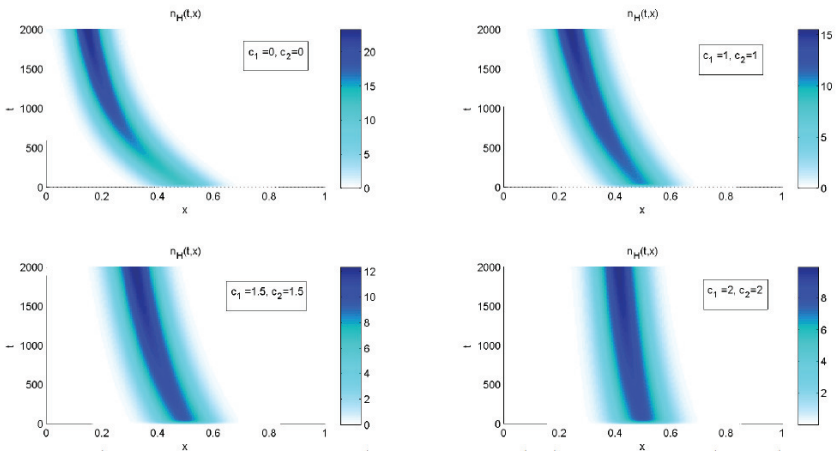
Non-local Lotka-Volterra 2D model (2 populations, n_H, n_C) with 2 different drugs and one resistance phenotype x

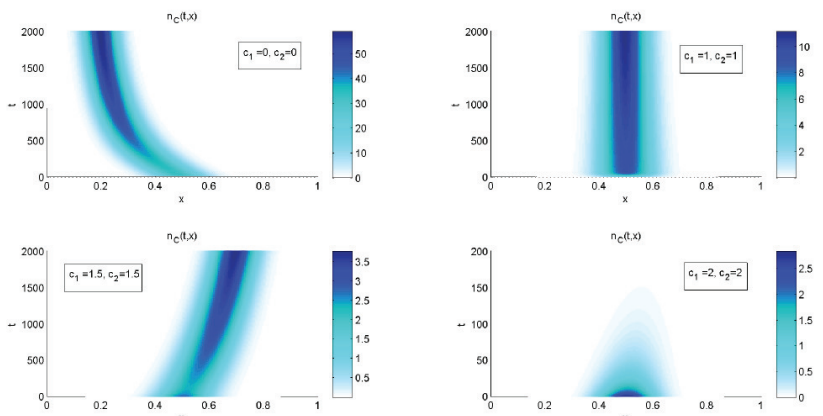
$$\frac{\partial}{\partial t} n_H(t,x) = \left[\frac{r_H(x)}{1 + k_H u_2(t)} - d_H(x) I_H(t) - u_1(t) \mu_H(x) \right] n_H(t,x)$$

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

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, by definition

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

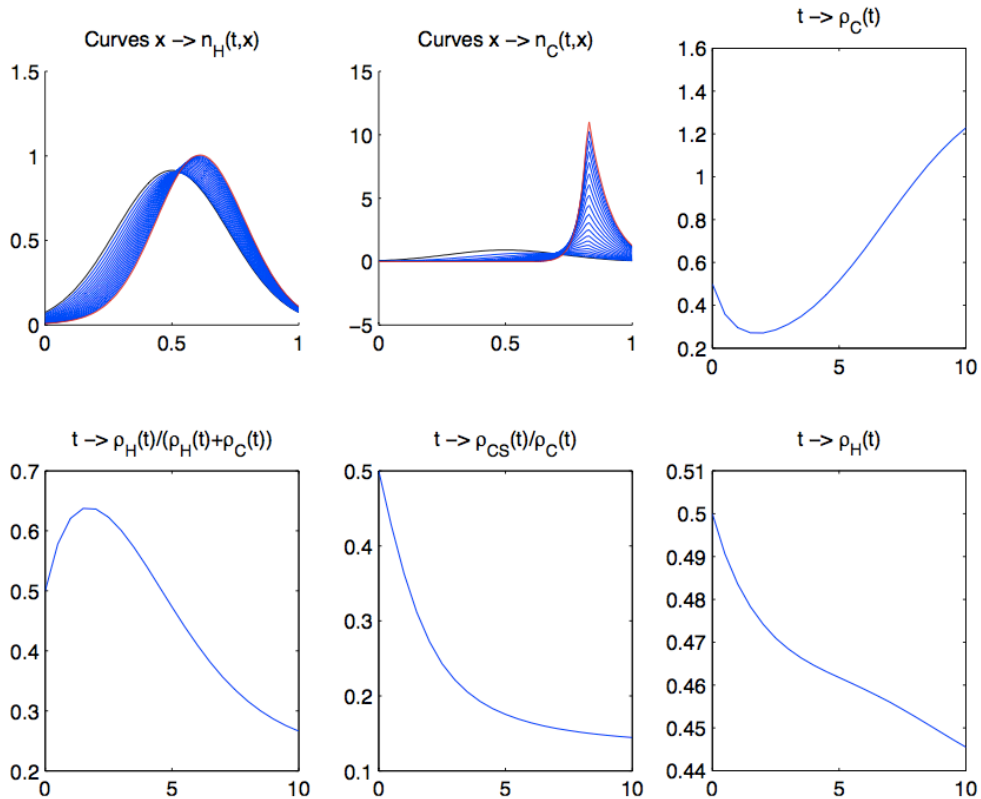
Then, for $m_{H,C} := \dfrac{1}{d_{H,C}}$, define the Lyapunov functional $V(t) := V_H(t) + V_C(t)$ where

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

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.

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)

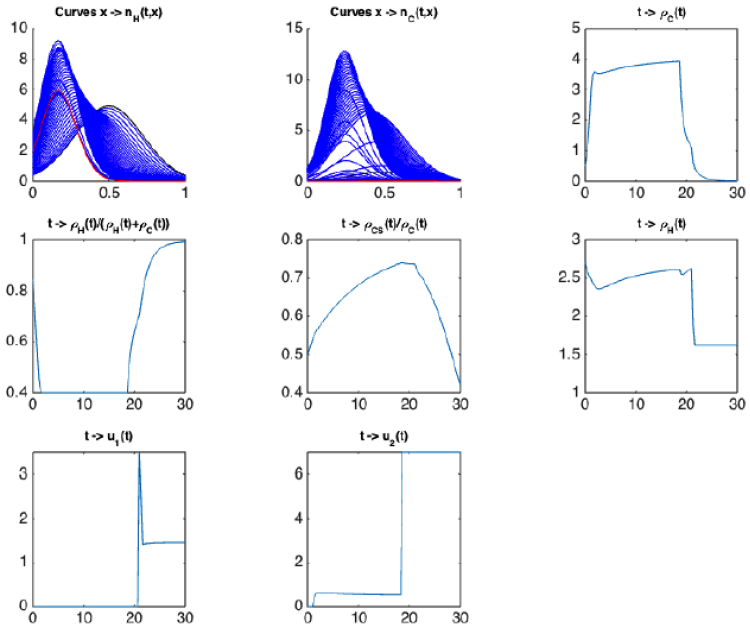


Figure 4: Simulation of (**OCP**) for $T = 30$.

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

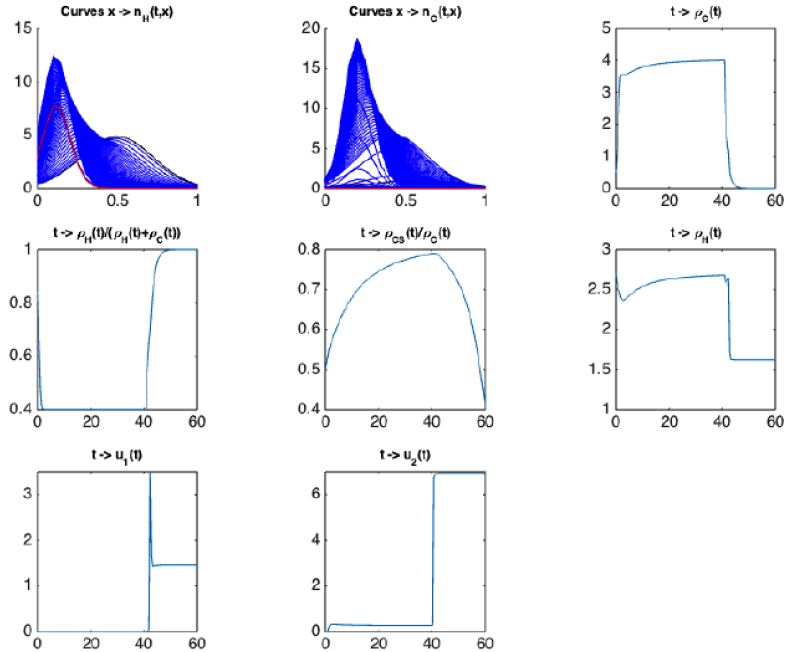


Figure 5: Simulation of (**OCP**) for $T = 60$.

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 $u_1^{\max}$.

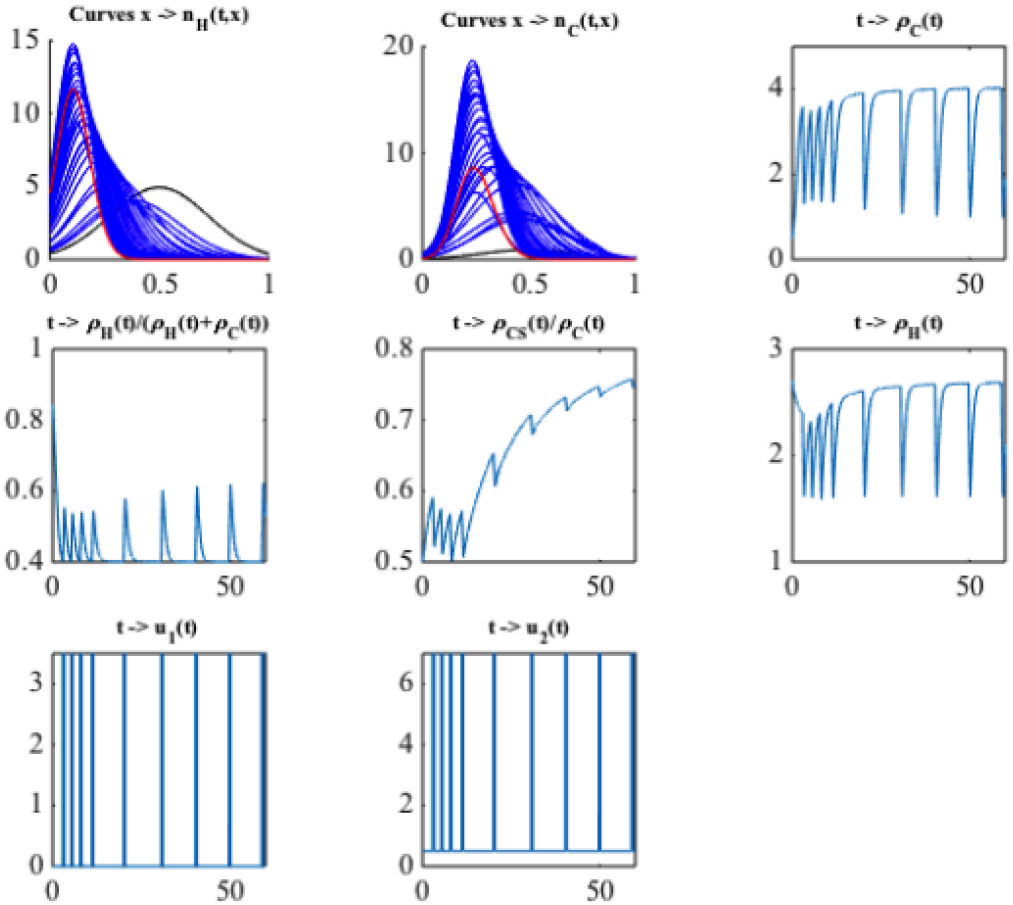
Main idea:

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 $\frac{\rho_H}{\rho_H + \rho_C} > \theta_{HC}$, 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 $\rho_H = \theta_H.\rho_H(0)$

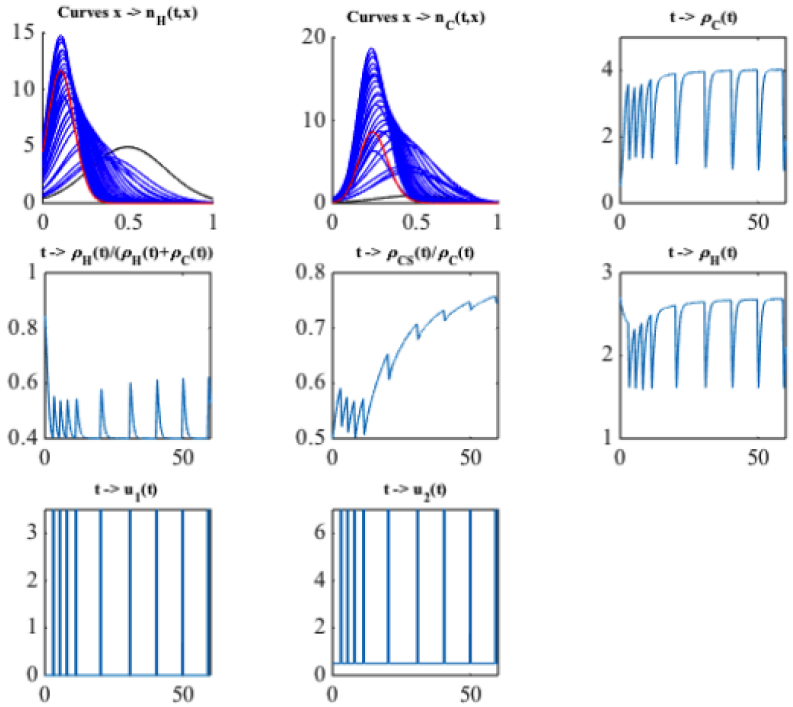


Figure 6: Quasi-periodic strategy, for $T = 60$.

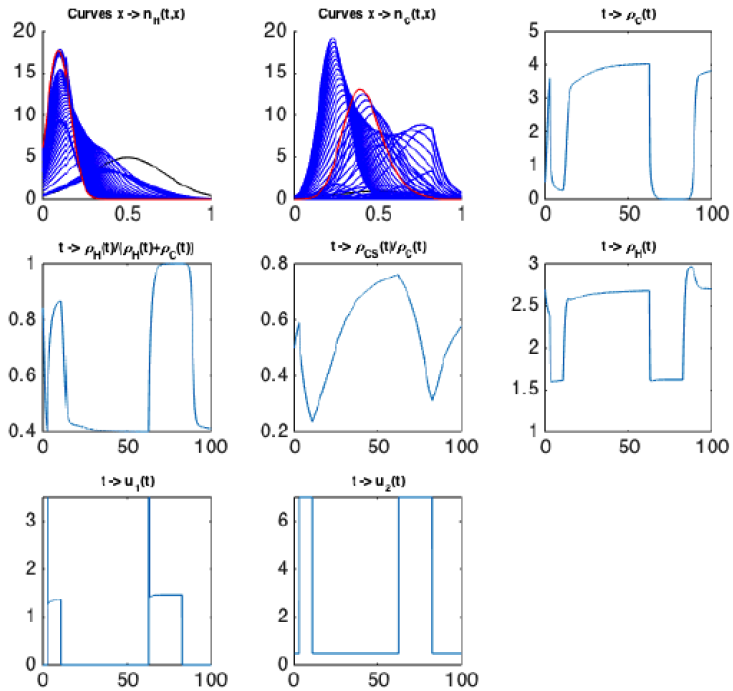
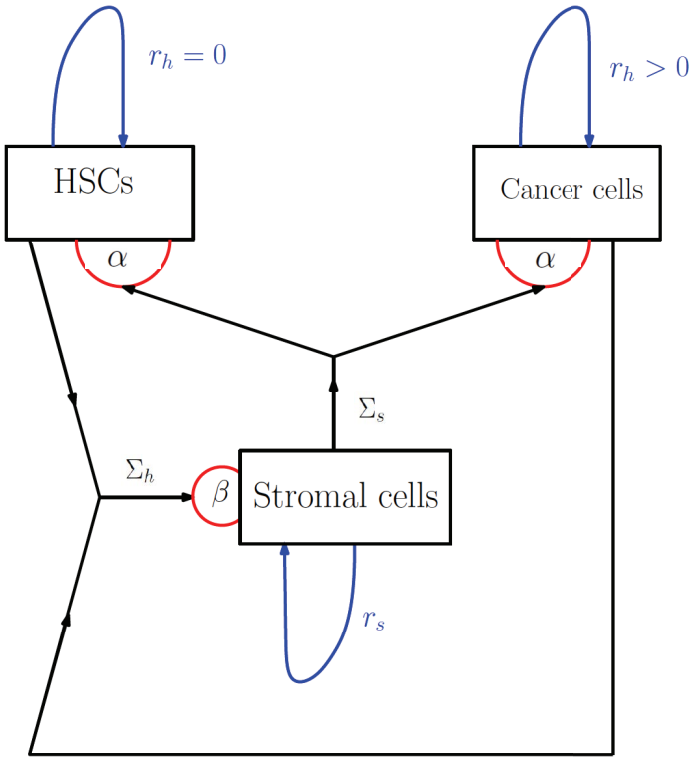


Figure 7: Second quasi-periodic strategy, for $T = 100$.

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 $\frac{d\rho_H}{dt} = 0$ (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?).

Another concentration problem with two symbiotic cell populations, haematopoietic stem cells and stromal cells



where

$$\rho_h(t) = \int_a^b n_h(t,x)\,dx, \rho_s(t) = \int_c^d n_s(t,x)\,dx$$

with bidirectional cross-talk functions (trophic Σ_s and 'call for support' Σ_h)

$$\Sigma_h(t) := \int_a^b \psi_h(x) n_h(t,x)\,dx,$$

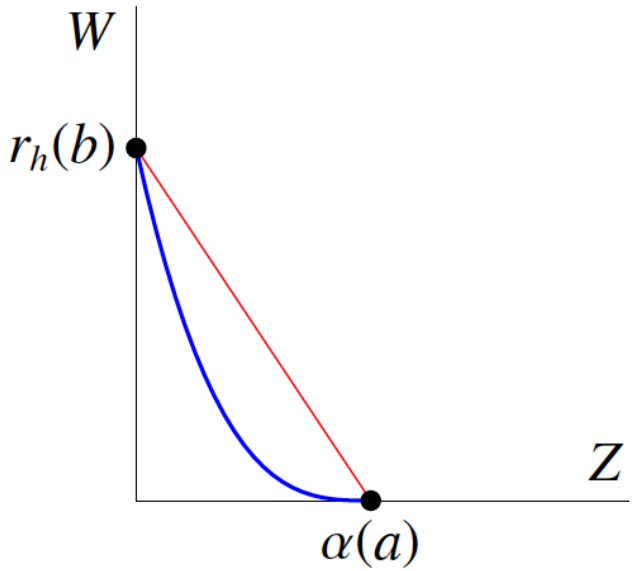
$$\Sigma_s(t) := \int_c^d \psi_s(y) n_s(t,y)\,dy$$

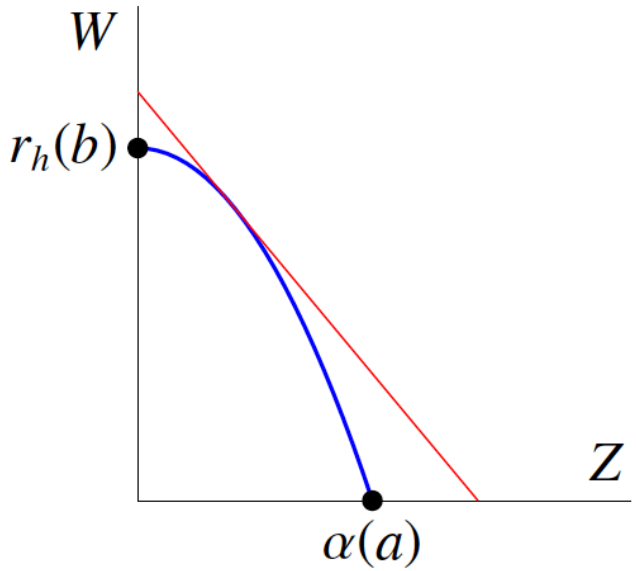
$$\begin{cases} \partial_t n_h(t,x) = \Big[r_h(x) - \rho_h(t) - \rho_s(t) + \alpha(x)\Sigma_s(t)\Big] n_h, & x \in (a,b), t > 0, \\ \partial_t n_s(t,y) = \Big[r_s(y) - \rho_h(t) - \rho_s(t) + \beta(y)\Sigma_h(t)\Big] n_s, & y \in (c,d), t > 0, \end{cases}$$

with initial data $n_h(0,x) = n_{h0}(x) \geq 0, \quad n_s(0,y) = n_{s0}(y) \geq 0.$

(Nguyen et al. MBE 2019)

A convexity condition to obtain (at most) dimorphism with respect to malignancy trait x, i.e., coexistence between healthy ($x = a$) and leukaemic ($x = b$) stem cells $n_h(t, x)$





(Blue curve $x \in [a, b] \mapsto (Z = \alpha(x), W = r_h(x))$*; red straight line* $Z\hat{\Sigma}_s + W = \hat{\rho}_h + \hat{\rho}_s$*)*

- Assume that $(\hat{n}_h, \hat{n}_s)$ is any evolutionary stationary distribution (ESD) that does not vanish. Then $\hat{n}_h$ is monomorphic if one of the following hypotheses is fulfilled:

either (i) α is strictly monotone and $r_h(\alpha^{-1})$ is concave on $[0, \alpha(a)]$, or (ii) r_h is strictly monotone and $\alpha(r_h^{-1}))$ is concave on $[0, r_h(b)]$, or (iii) r_h, α are strictly concave.

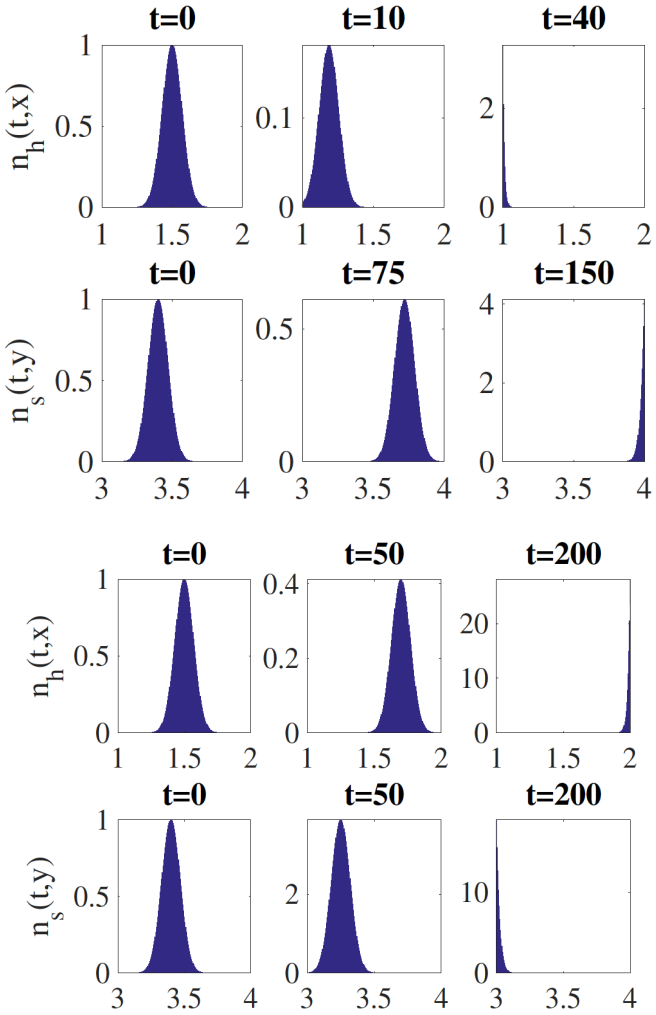
- Also $\hat{n}_h$ is at most dimorphic if one of the following hypotheses is fulfilled:

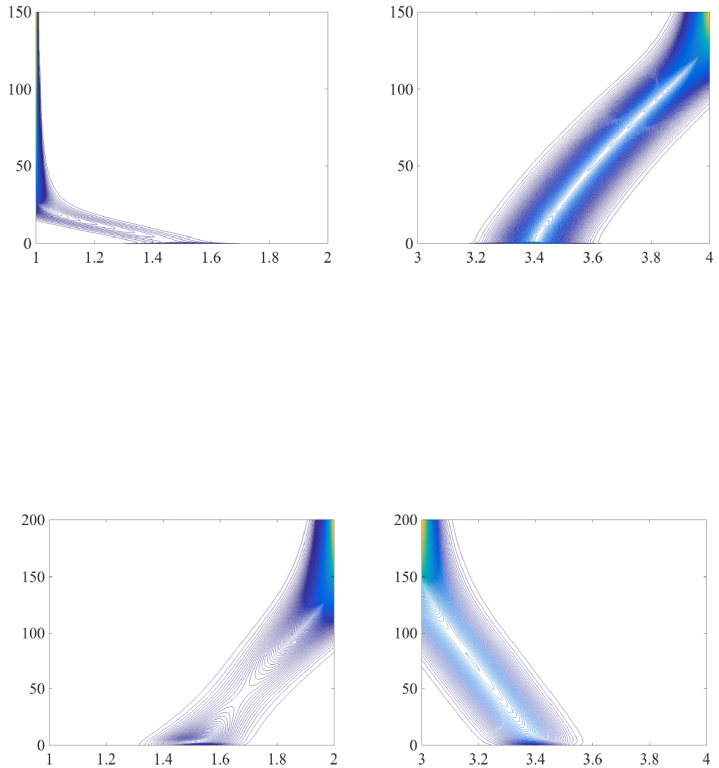
either (i) α is strictly monotone and $r_h(\alpha^{-1})$ is convex on $[0, \alpha(a)]$, or (ii) r_h is strictly monotone and $\alpha(r_h^{-1}))$ is convex on $[0, r_h(b)]$, or (iii) r_h, α are strictly convex.

- The same conclusions as above hold for $\hat{n}_s$ provided that similar assumptions on r_s, β are supposed.

(Nguyen et al. MBE 2019)

Two different monomorphic cases of ESDs for HSCs

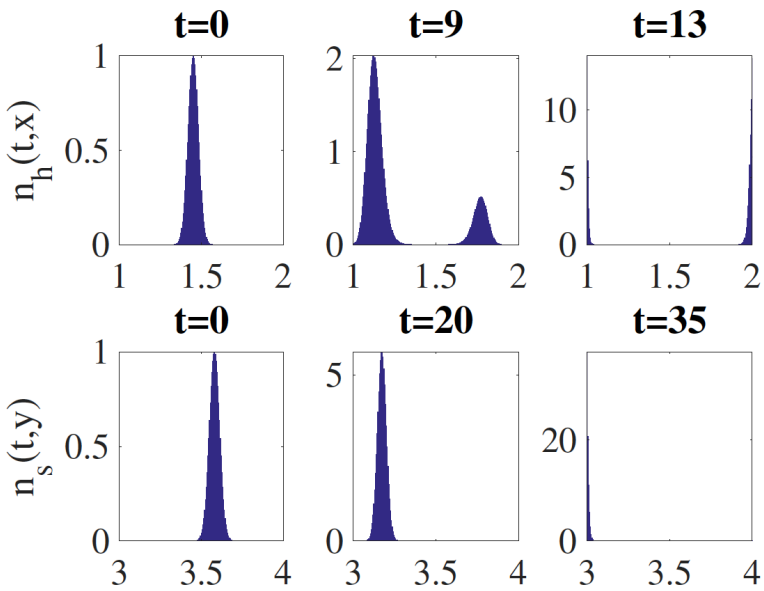


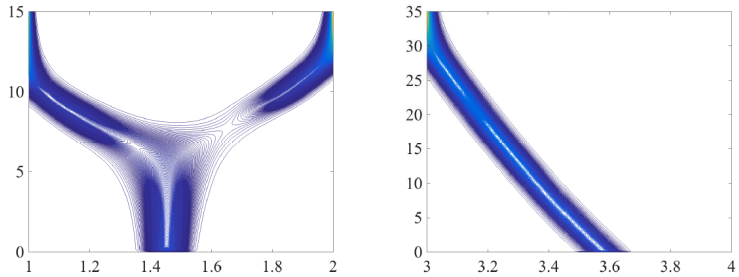


Left: snapshots of trait distributions. Right: continuous evolution of n_h and n_s populations with time, where traits $x \in (1, 2)$ (malignancy) and $y \in (3, 4)$ (support capacity) are in abscissae, time in ordinates. 1st case (upper panels): strong stromal support trtait y and extinction of the leukaemic clone; 2nd case (lower panels) : weak stromal support trait y and extinction of the healthy clone.

(Nguyen et al. MBE 2019)

A case of dimorphic (healthy/leukaemic) ESD for HSCs





Left: snapshots of trait distributions. Right: continuous evolution of n_h and n_s populations with time, where traits $x \in (1,2)$ (malignancy) and $y \in (3,4)$ (support capacity) are in abscissae, time in ordinates. Weak support trait y, but existence of lots of stromal cells $n_s(t,y)$, and coexistence of the two clones.

In fact (a remark by Benoît Perthame), to obtain such dimorphism, one must have diffusion. Even though the equations are nonlocal Lotka-Volterra-like (quasi-ODEs) without diffusion, their numerical simulation introduces diffusion, and this might be the right explanation for such dimorphism!

(Nguyen et al. MBE 2019)

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)

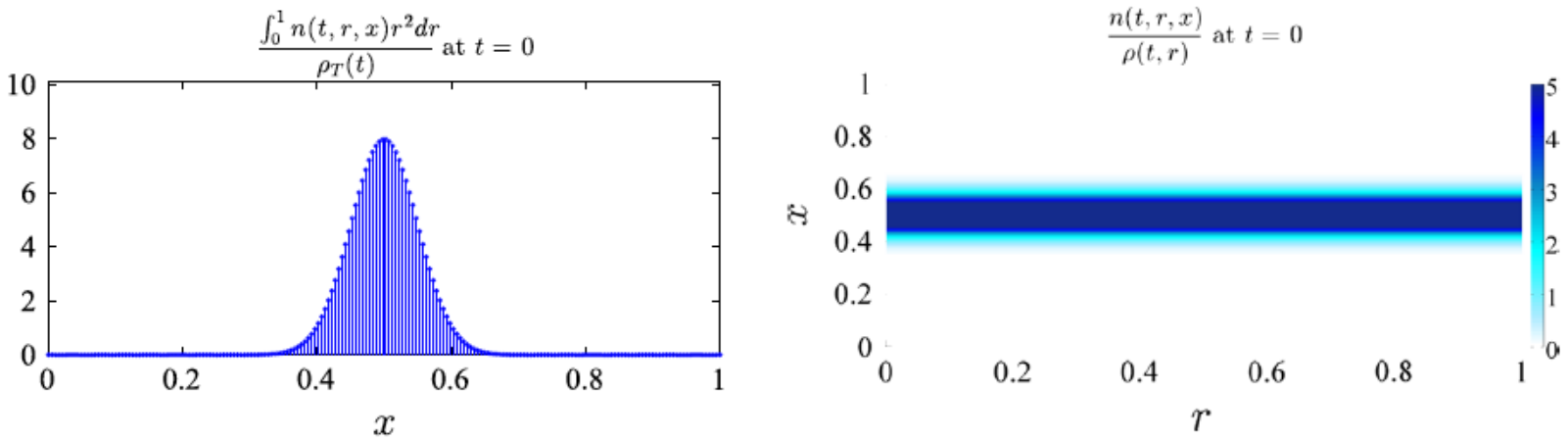
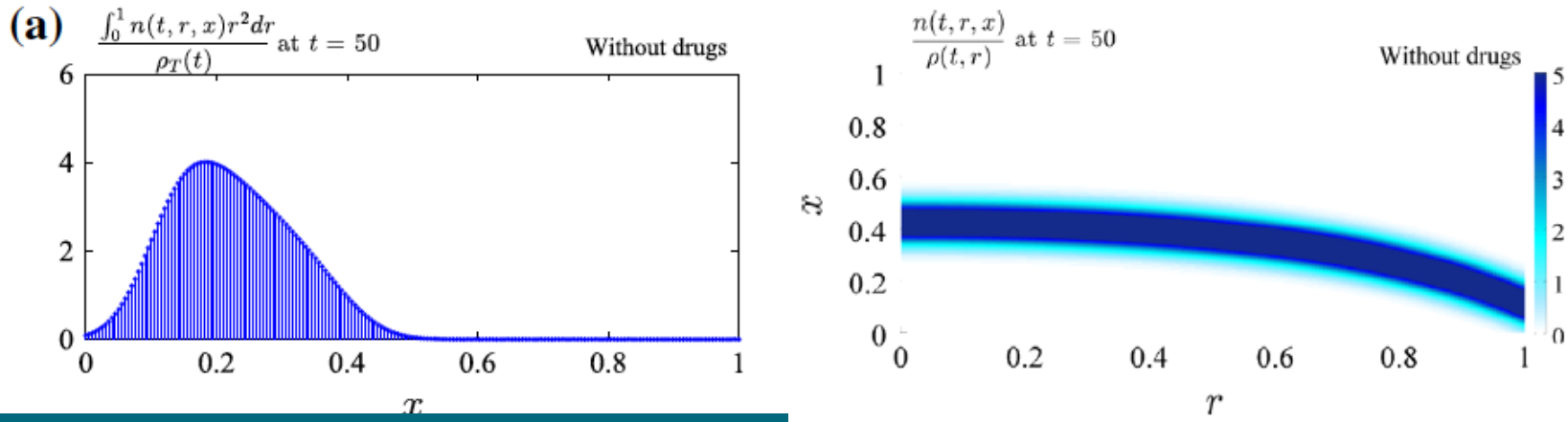
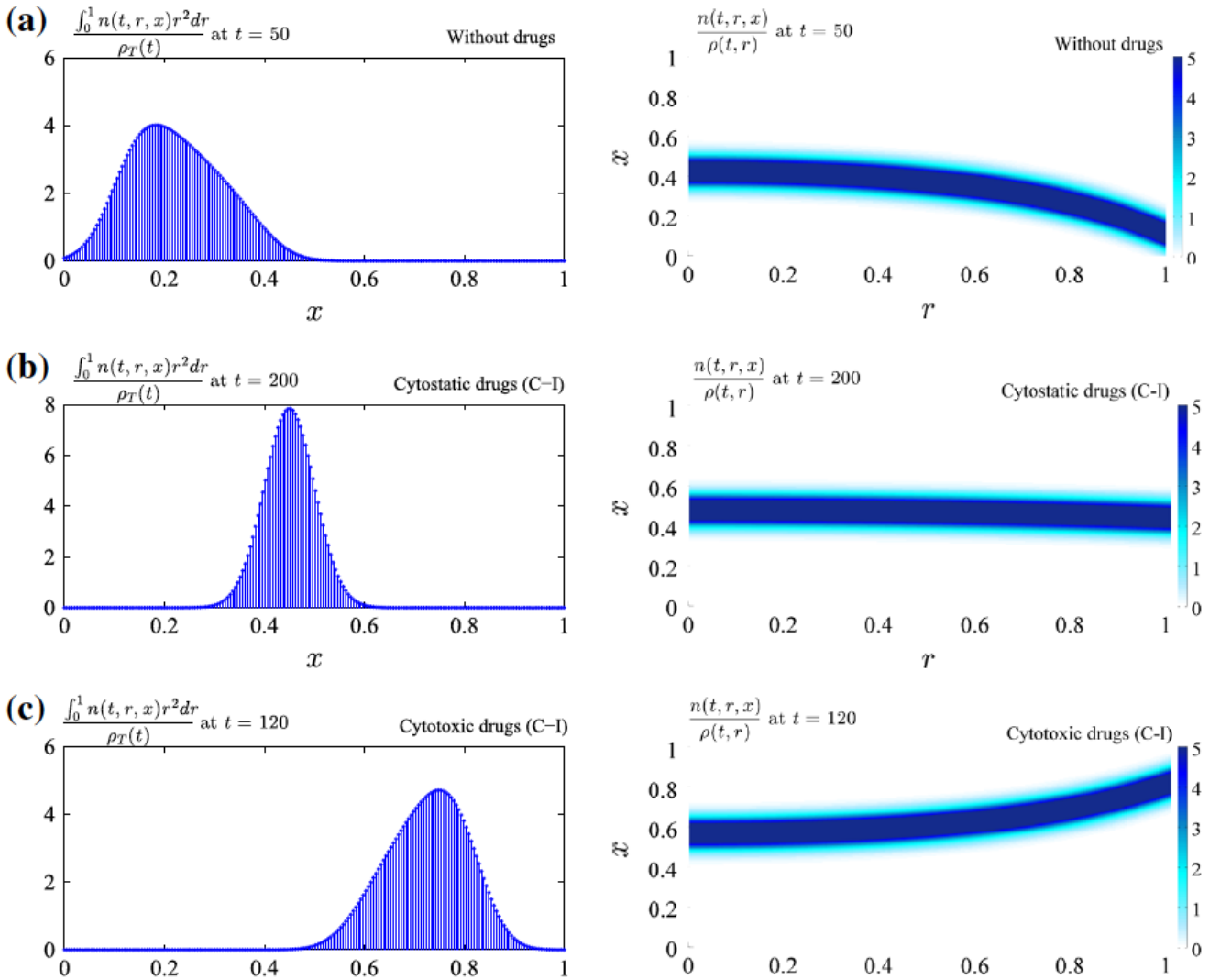


Fig. 1 Initial phenotypic distribution. Plots of $\int_0^1 n(t,r,x)r^2\mathrm{d}r/\rho_T(t)$ (*left panel*) and $n(t,r,x)/\rho(t,r)$ (*right panel*) at $t=0$. The initial cell population is almost monomorphic

Evolution without drugs: towards sensitive phenotype ($x \to 0$)



Tumour spheroid: evolution with constant drug doses (2)



Cytotostatic 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)

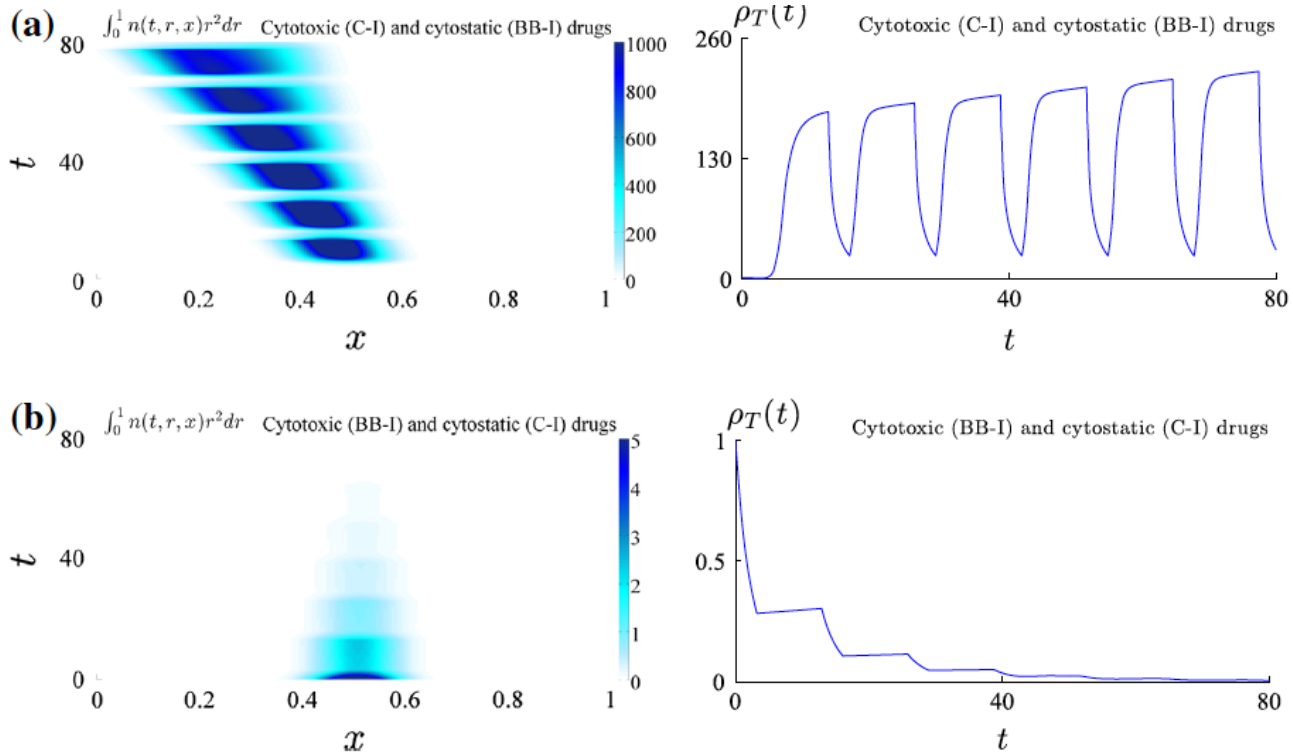
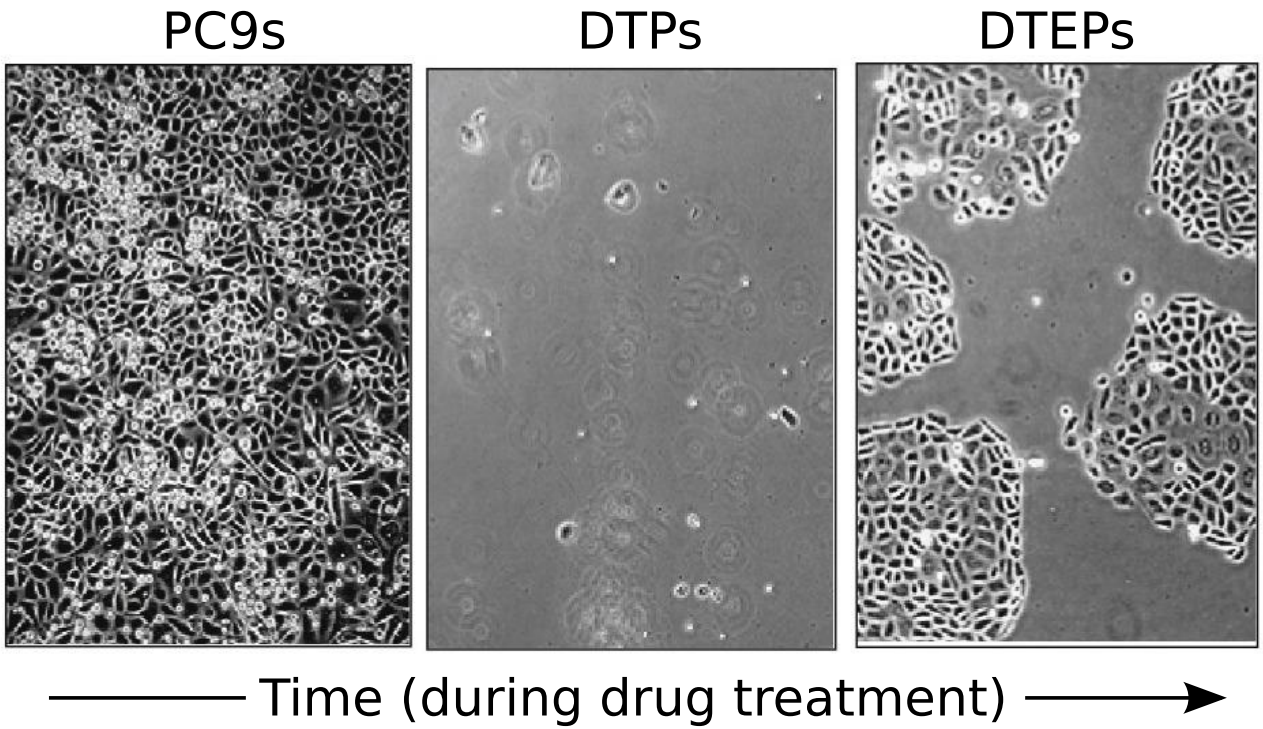


Fig. 11 **a** Cytotoxic (C-I) and cytostatic (BB-I) drugs. Plots of $\int_0^1 n(t, r, x)r^2 \mathrm{d}r$ (*left panel*) and $\rho_T(t)$ (*right panel*). Bang–bang infusion of cytostatic drugs together with constant infusion of cytotoxic drugs weakly affects the dynamics of cancer cells by comparison 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 $\int_0^1 n(t, r, x)r^2 \mathrm{d}r$ (*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*)



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

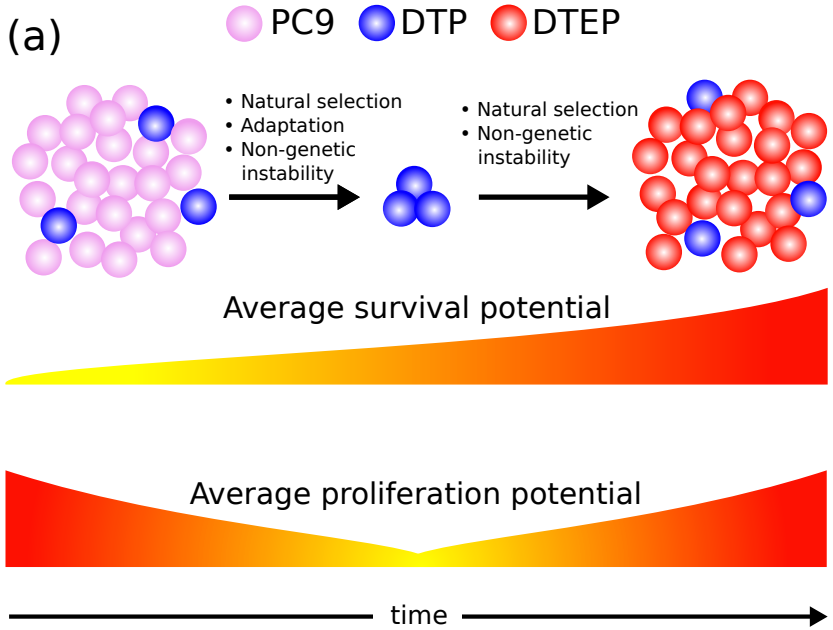
$$\frac{\partial n}{\partial t}(x, y, t) + \underbrace{\frac{\partial}{\partial y}\Big(v(x, c(t); \bar{v})n(x, y, t)\Big)}_{\text{Stress-induced adaptation of the proliferation level}} =$$

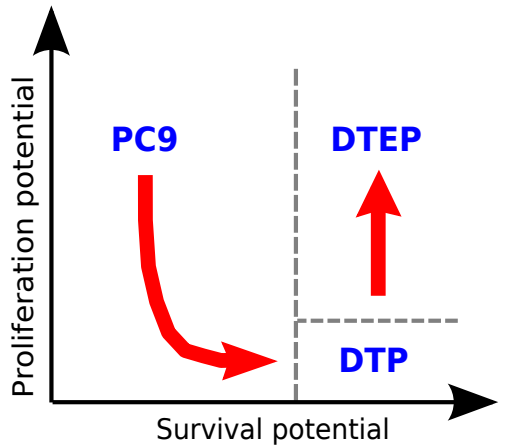
$$\underbrace{\Big[p(x, y, \varrho(t)) - d(x, c(t))\Big]n(x, y, t)}_{\text{Non local Lotka-Volterra selection}} + \underbrace{\beta\Delta n(x, y, t).}_{\text{Non-genetic phenotype instability}}$$

- $\varrho(t) = \int_0^1 \int_0^1 n(x, y, t)\, dx\, dy$, $p(x, y, \varrho(t)) = (a_1 + a_2 y + a_3(1 - x))(1 - \varrho(t)/K)$ 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

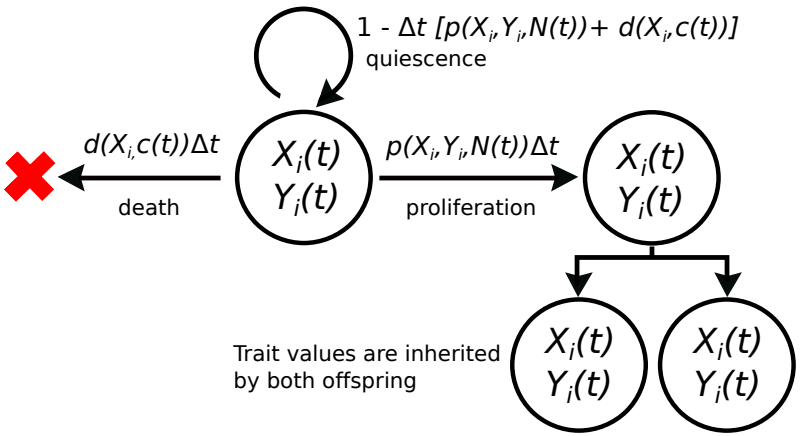
Chisholm et al., Cancer Research 2015

Same framework using an agent-based model (ABM)





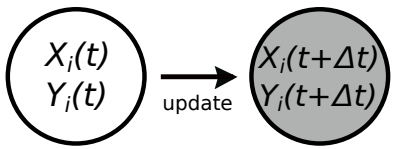
(a) Each cell i undergoes either proliferation, death or remains quiescent:



(b) Each cell i updates its trait values according to the discretised SDEs:

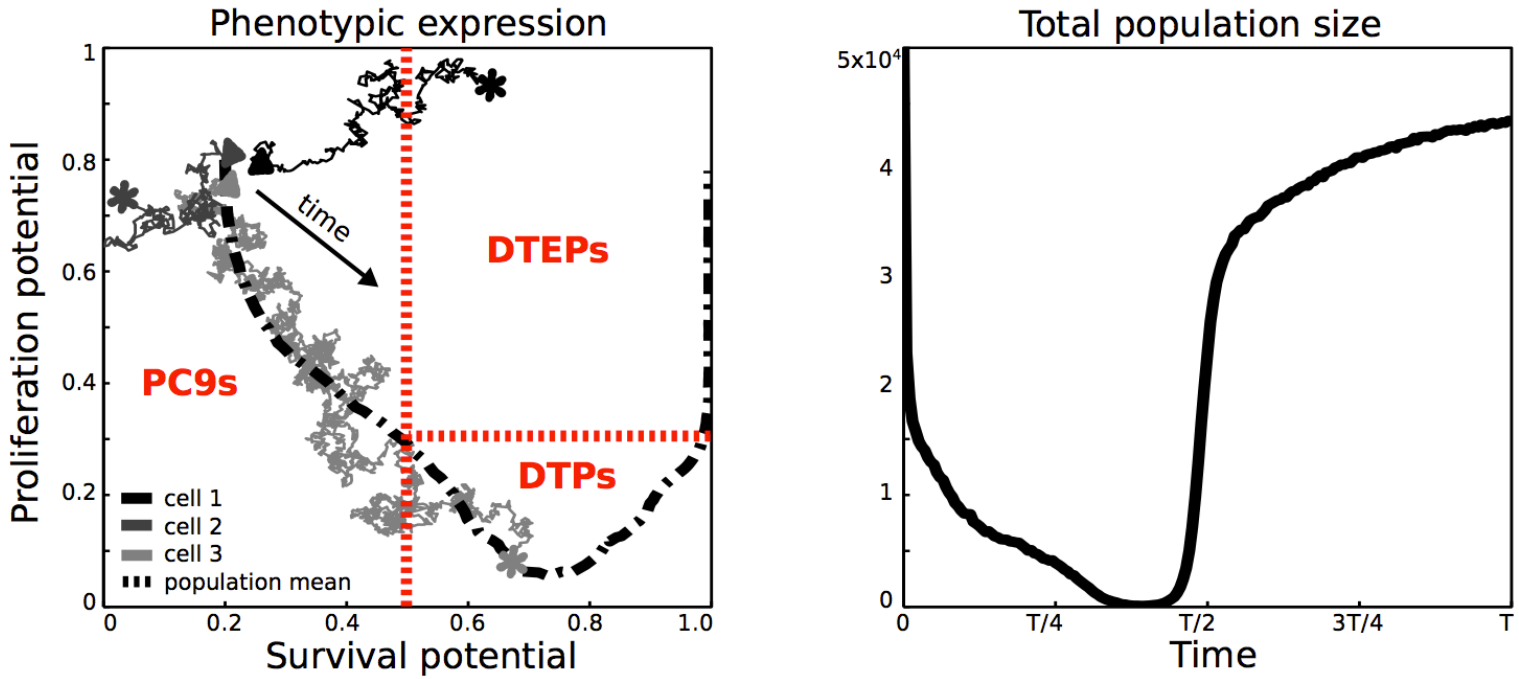
$$X_i(t+\Delta t) = X_i(t) + D\sqrt{\Delta t}\, W_i^1$$

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



Chisholm et al., Cancer Research 2015

Individual cell behaviour (ABM) can be different from the averaged dynamics observed at the population level

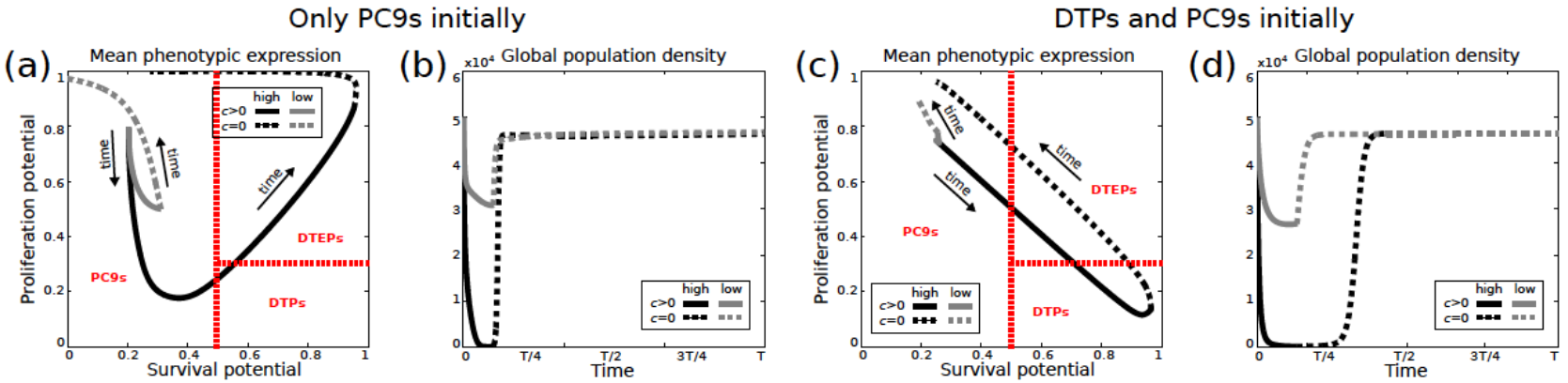


- Evolution in the I-B model (here no DTPs initially present, adaptation on): heterogeneity of behaviours in the population of PC9 cells.
- Left: Trajectories of the phenotypic expression of 3 individual cells and mean phenotypic expression of the cell population (dashed line). Triangles: initial phenotype 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)

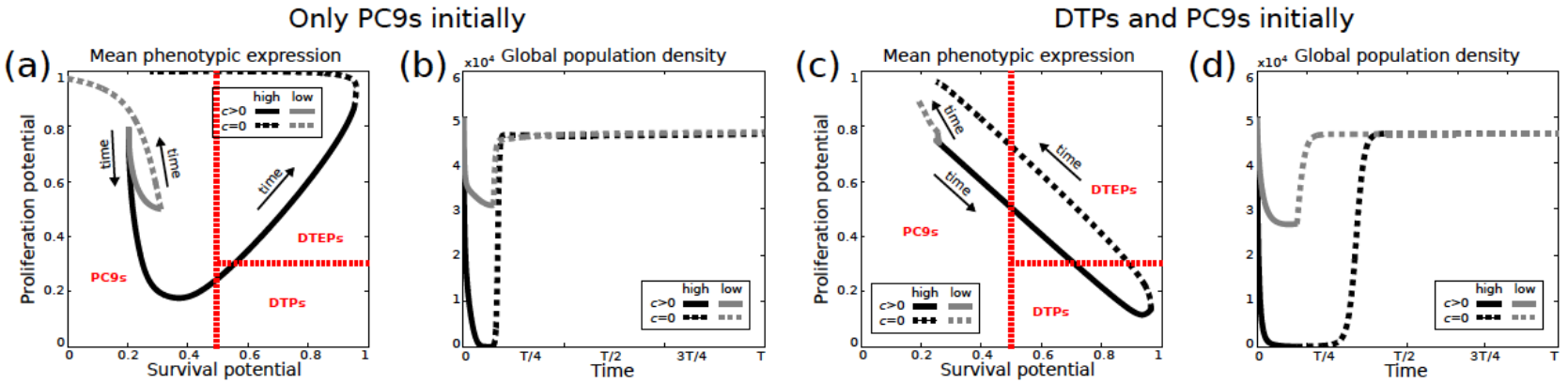
(c), (d) PC9s and DTPs initially, no adaptation $v = 0$: *'Darwinian' scenario*, or Luria-Delbrück scenario (B)

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

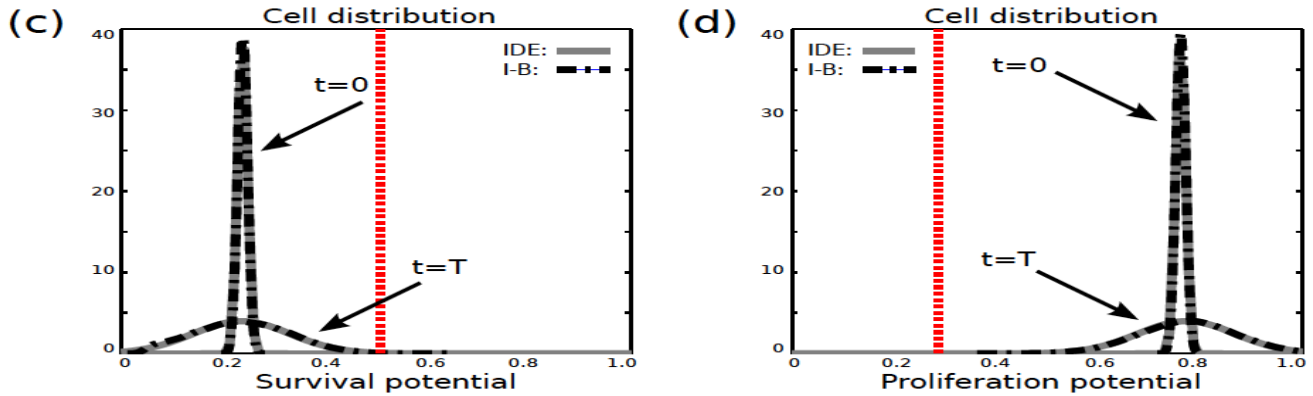


(a), (b) Only PC9s (no DTPs initially), adaptation on ($v \neq 0$): *'Lamarckian' scenario*

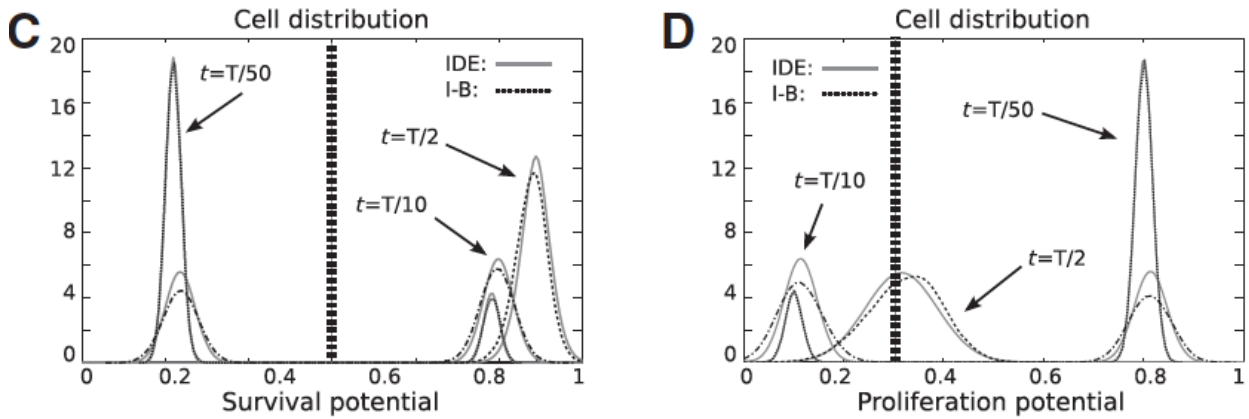
(c), (d) PC9s and DTPs initially, no adaptation ($v = 0$): *'Darwinian' scenario*
(sheer selection of the fittest = DTPs, supposed to be present in the initial population)

Chisholm et al., Cancer Research 2015

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 ($v \neq 0$)

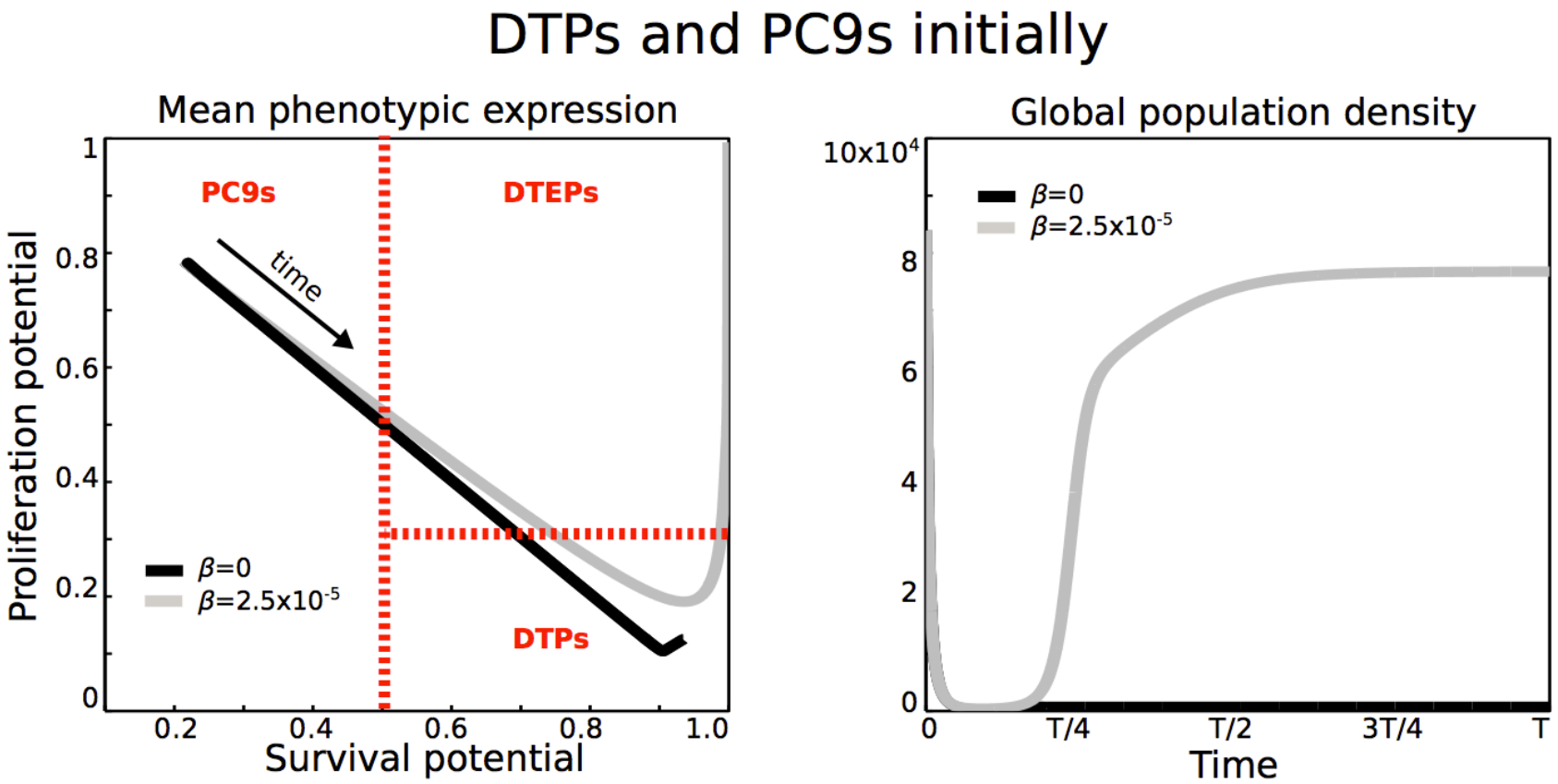
To make a long story short, **Q1**. Always yes! Whatever the scenario

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]

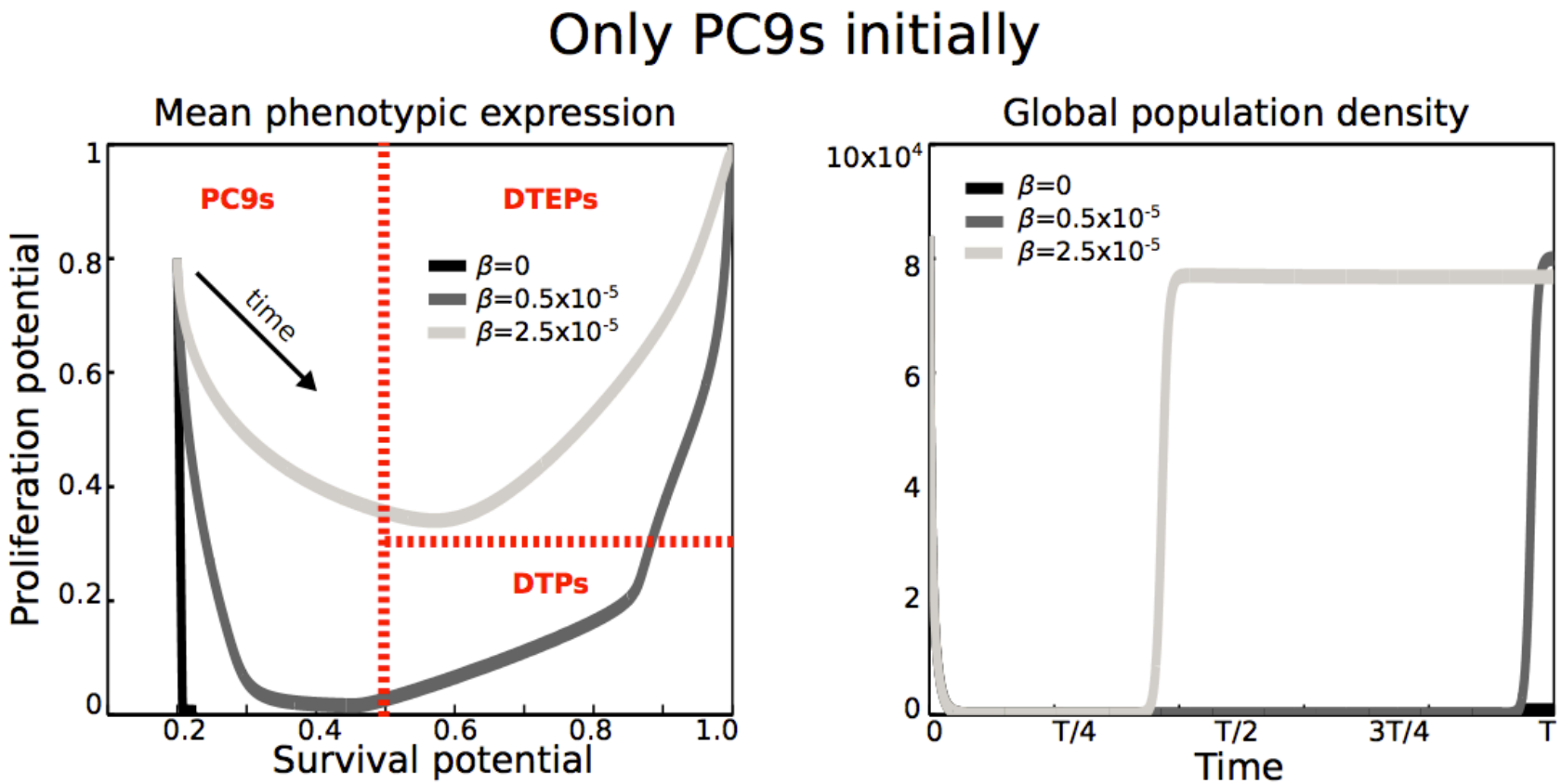


Extinction when $\beta = 0$ (here, adaptation is absent $v = 0$)

Chisholm et al., Cancer Research 2015

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 $v \neq 0$)

Chisholm et al., Cancer Research 2015

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

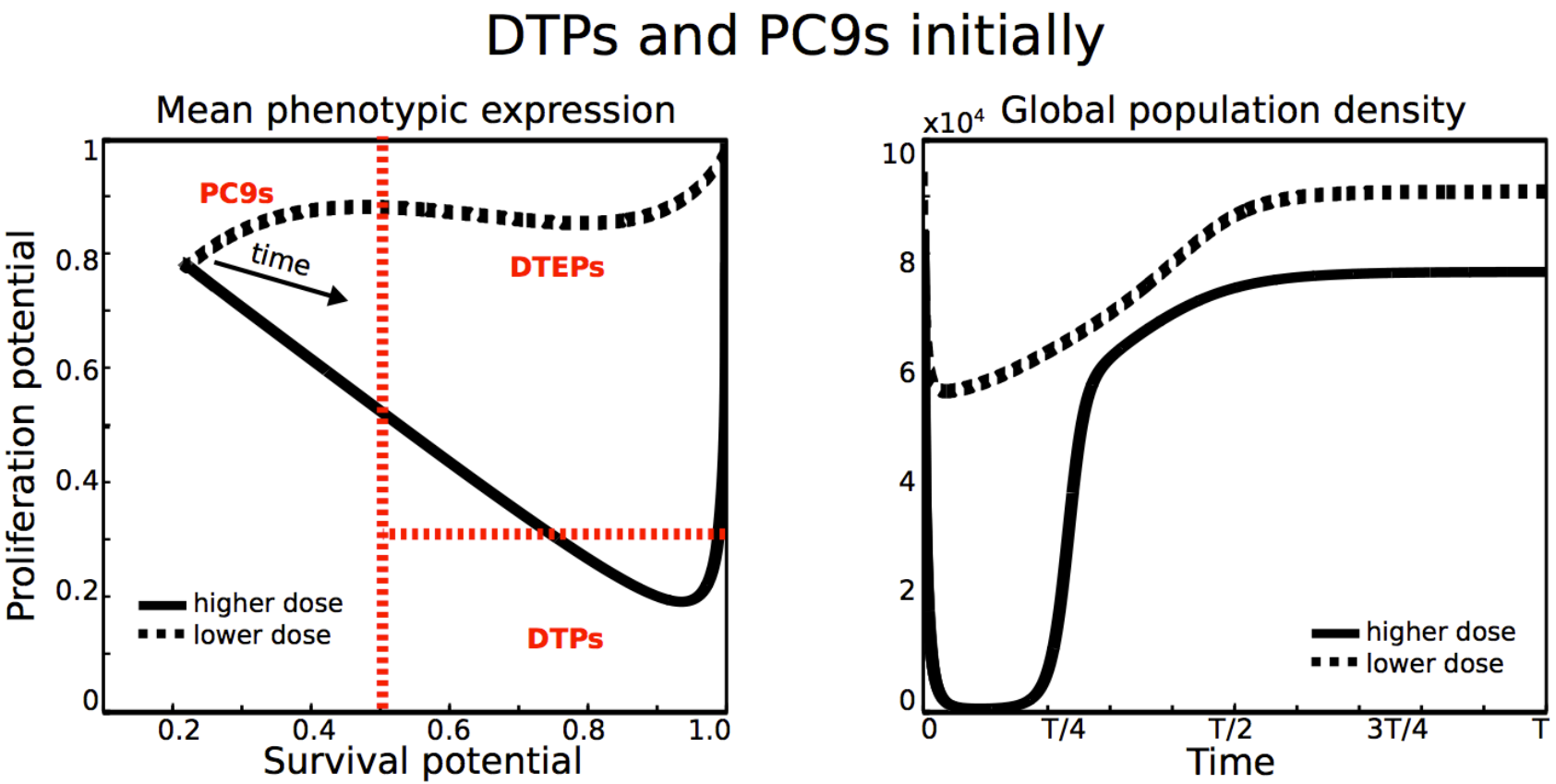
Definition (LC_γ dose)

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

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

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

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

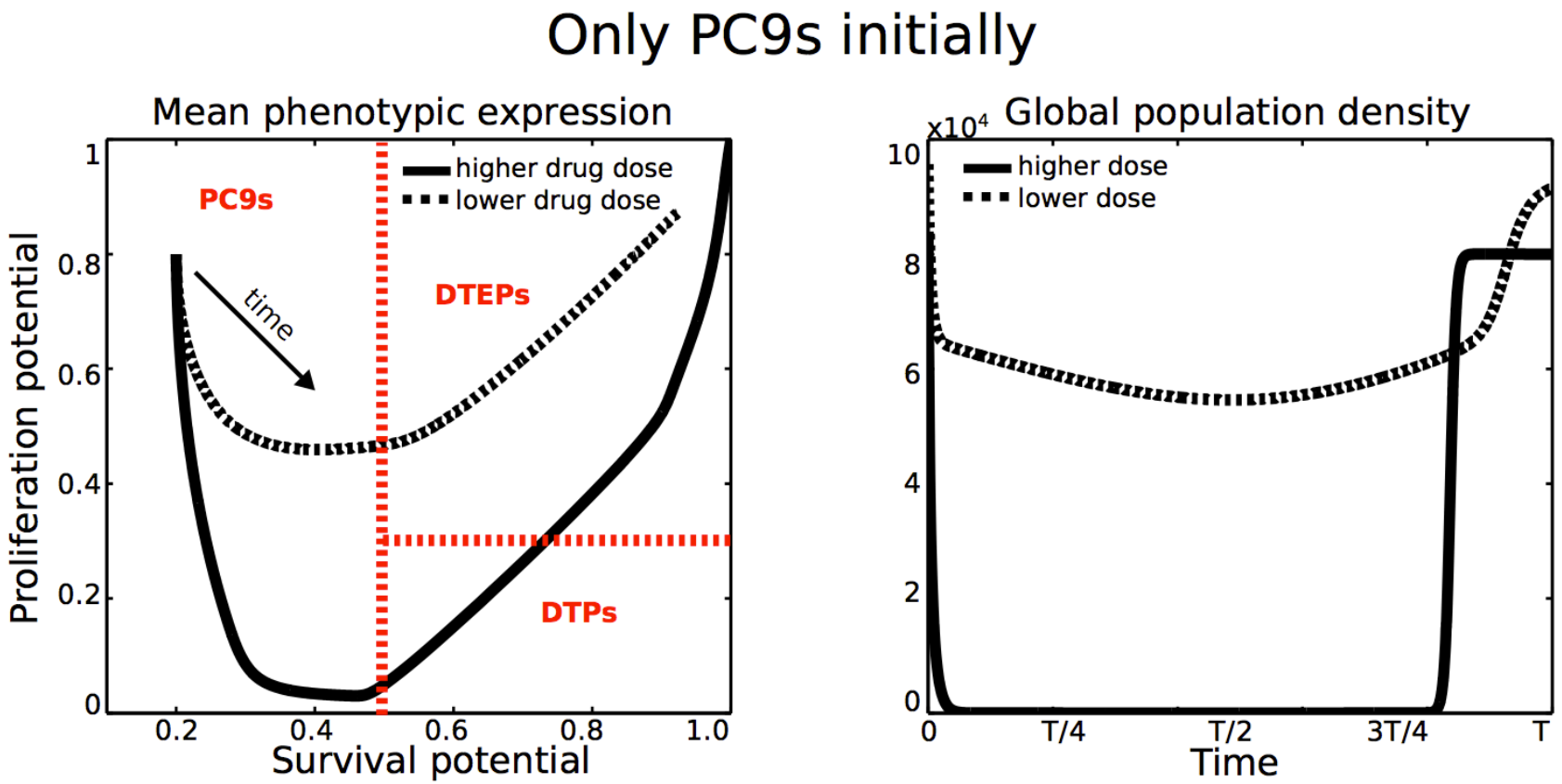


Low drug dose does not let appear DTPs (here, adaptation is absent $v = 0$)

Chisholm et al., Cancer Research 2015

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

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



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*)

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

where the mutation kernel is defined as,

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

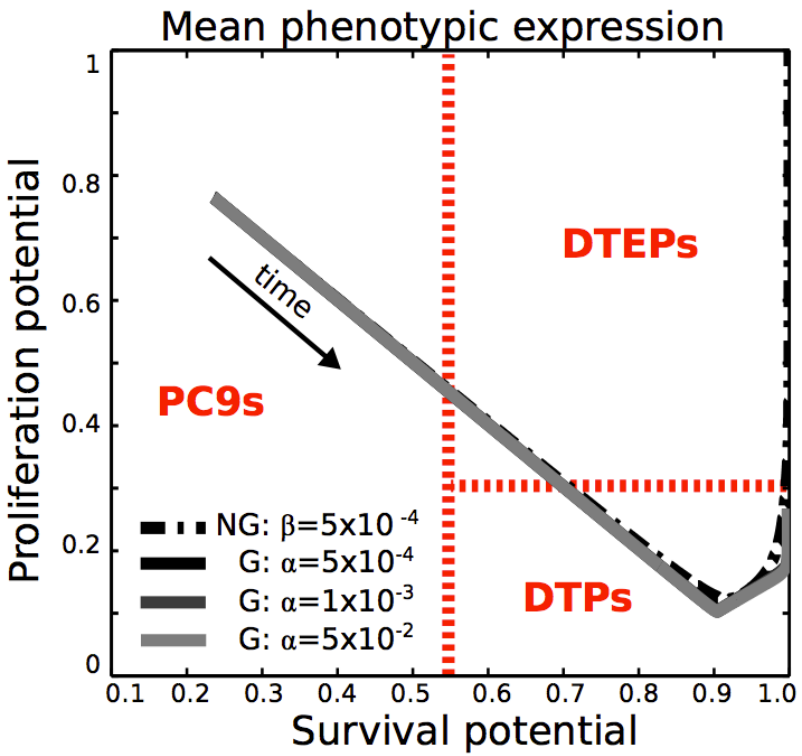
and C_M is a normalisation constant such that

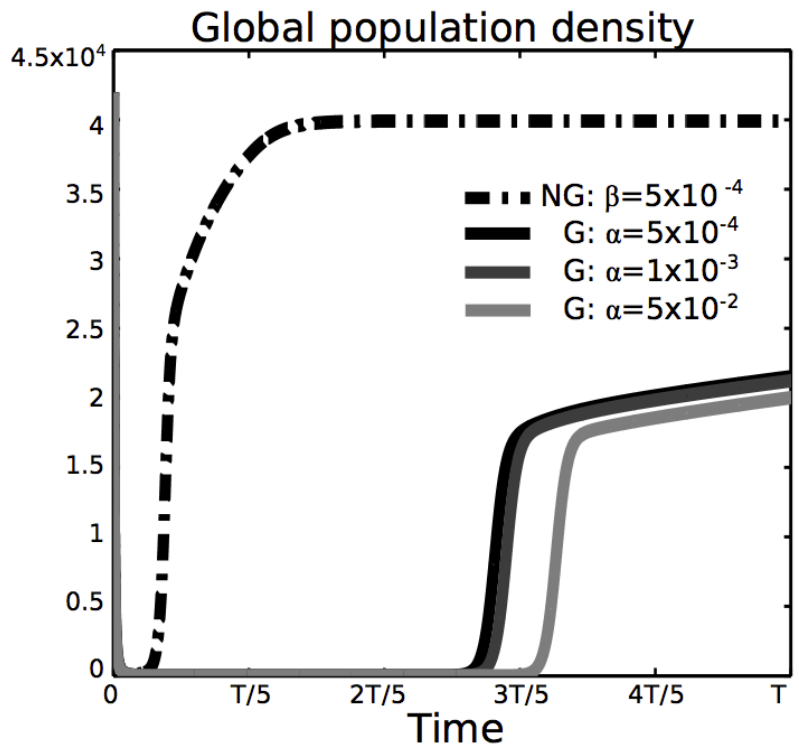
$$\int_0^1\int_0^1 M(x,y|\cdot,\cdot;\cdot)\mathrm{d}x\mathrm{d}y = 1.$$

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





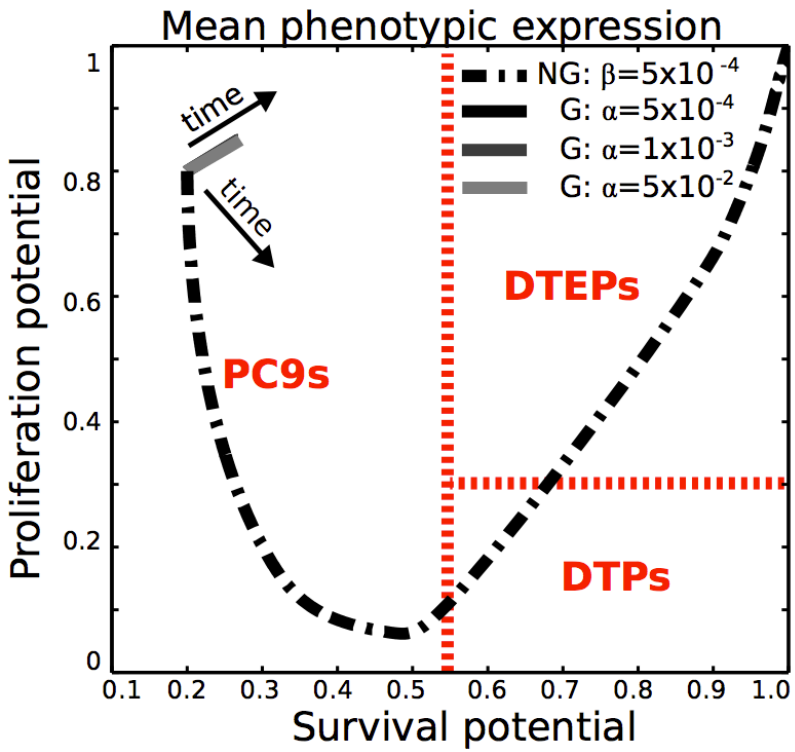
G: mutations do not let occur total recovery (NG: here, adaptation is absent $v = 0$)

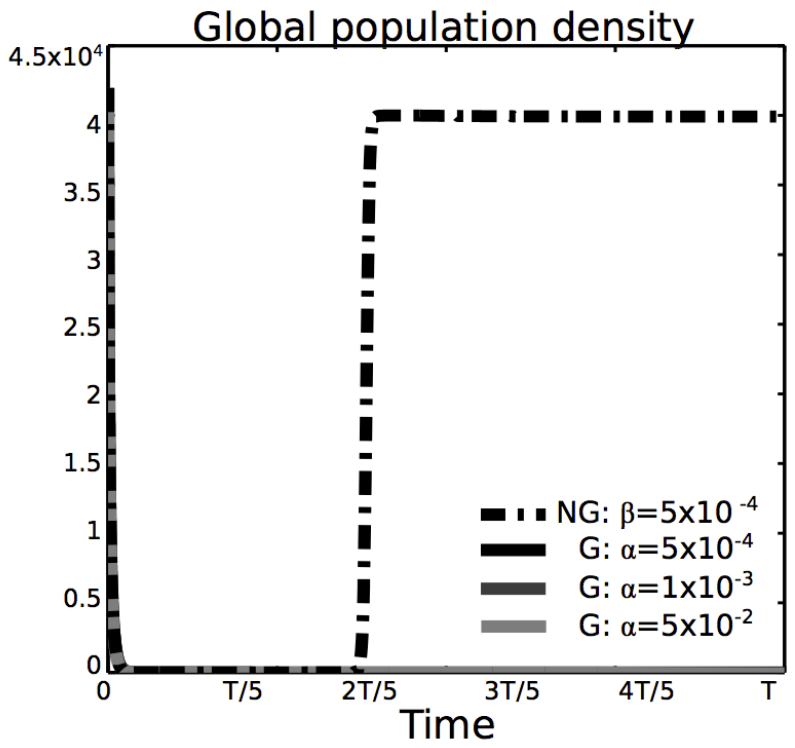
Chisholm et al., Cancer Research 2015

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

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:

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

References

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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?
- If it is so, *what is the impact of the immune system on cell differentiations?*
- Can we parallel evolution of species and evolution of their immune system?
- 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 scenarios relating 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?)

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(simplified to V=0, y=1–x, RHS=$\alpha \Delta n + n\{r(x) - \rho(t) - \mu(x) D\}$, by G. Carrère, G. Nadin)

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*
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