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Heterogeneity, drug resistance and evolution in cancer

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Mamba MODELLING AND ANALYSIS FOR MEDICAL AND BIOLOGICAL APPLICATIONS

Motivations

- Drug resistance: still a major pitfall of cancer therapeutics
- Accounting for drug resistance in cancer requires considering the level of *cancer cell populations*
- Phenotype heterogeneity in cancer cell populations is likely the main cause of drug resistance
- Heterogeneity in cancer cell populations may be due to *fast backward evolution* ('atavistic theory of cancer')
- We assess it by biological and mathematical models of evolving *heterogeneous* cell populations, structured in traits coding relevant biological variability
- Therapeutic strategies should rely on optimal control algorithms with targets in such models of heterogeneous cell populations

Summary

- Intra-tumour heterogeneity, i.e., between-cell variability within cancer cell populations, accounts for drug resistance.
- Evolutionary mechanisms that encompass the great evolution that has designed multicellular organisms, as well as smaller windows of evolution on the time scale of human disease, are in the background.
- Mathematical models used to predict drug resistance in cancer together with optimal control methods can help circumvent drug resistance in combined therapeutic strategies.
- Plasticity in cancer cells, i.e., partial reversal to a stem-like status in individual cells and resulting adaptability of cancer cell populations, may be viewed as backward evolution making cancer cell populations resistant to drug insult.
- Reversible plasticity is captured by mathematical models that incorporate between-cell heterogeneity through continuous phenotypic variables.
- Such models have the benefit of being compatible with optimal control methods for the design of optimised therapeutic protocols involving combinations of cytotoxic and cytostatic treatments with epigenetic drugs and immunotherapies.
- Gathering knowledge from cancer and evolutionary biology with physiologically based mathematical models of cell population dynamics should help oncologists to design optimised therapeutic strategies to circumvent drug resistance.

Definitions: evolution or adaptation in cell populations

[Naive and utilitary definitions]

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

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

Mutations and epimutations in cell populations

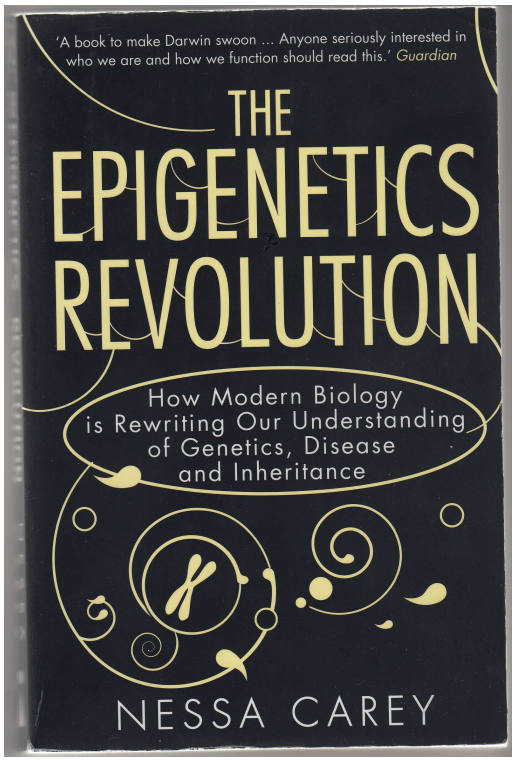
[Again, naive and utilitary definitions]

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

Initial motivation: Drug resistance, genetic or epigenetic phenomenon?

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

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



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 cellular resistance to stress, coded in 'cold', strongly preserved in evolution, rather than in 'hot', mutation-prone, genes (Wu et al. PNAS 2015).

Drug resistance: how does it work?

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

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

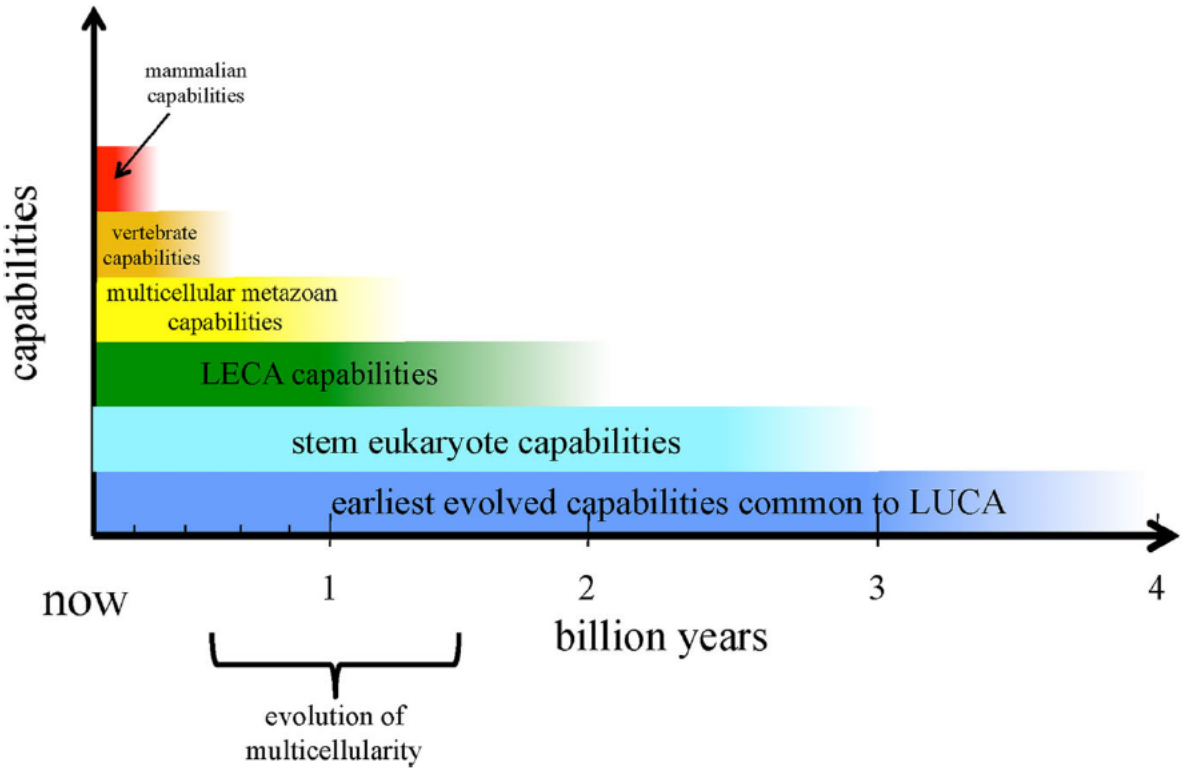
- Overexpression of ABC transporters, of drug processing enzymes, decrease of drug cellular influx, etc. are relevant to describe resistance mechanisms at the single cell level.

- At the cell population level, representing drug resistance by a continuous variable x standing for a resistance phenotype (in evolutionary game theory: a strategy) is adapted to describe evolution from sensitivity ($x = 0$) towards resistance ($x = 1$).

- Is it due to sheer Darwinian selection of the fittest after cell division or, at least partially, due to phenotype adaptation in individual cells? Not clear.

A possible evolutionary framework (*diachronic view*): the atavistic hypothesis of cancer (1)

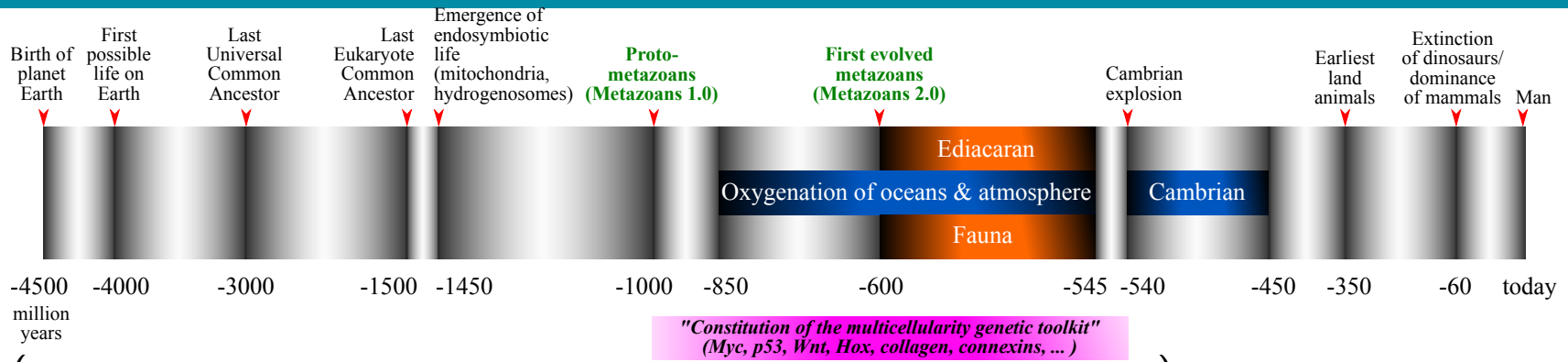
"Nothing in biology makes sense except in the light of evolution" (Th. Dobzhansky, 1973)



"Cancer: more archeoplasm than neoplasm" (Mark Vincent, 2011)

References: *Israel JTB 1996, Davies & Lineweaver Phys Biol 2011, Vincent Bioessays 2011, Lineweaver, Davies & Vincent Bioessays 2014, Chen et al. Nature Comm 2015*

A possible evolutionary framework (*diachronic 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 physiologically control the basis of cellular life, i.e., 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 local proliferations without regulation overcome; sophisticated *adaptive epigenetic mechanisms* *are present, not controlling proliferation, but serving it*

- 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

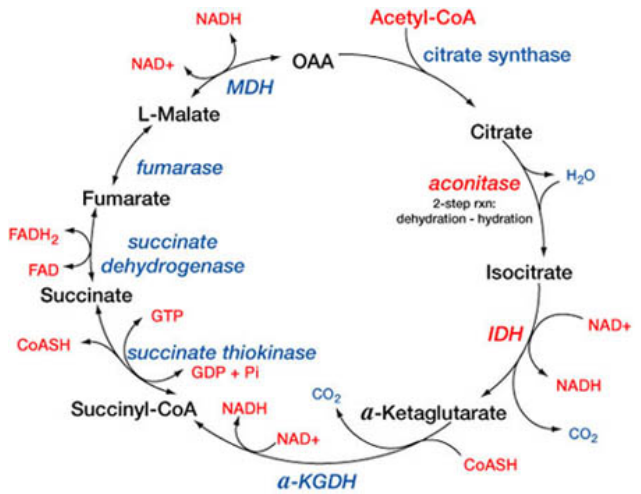
- The basic cancer cell is highly plastic and highly capable of adaptation to a hostile environment, as were its ancestors in a remote past of our planet (poor O_2, acidic environment, high UV radiations,...) *and likely presently even more*

Heterogeneity in cancer cell populations

- According to the atavistic theory of cancer, conditions of *oxygenation* and of *intercellular communications* that are quite poor in cancer cell populations send them back to very primitive forms of multicellularity
- These two conditions of multicellularity are closely related to one another, since intercellular communications, that rely in particular on gap junctions (appeared during the long oxygenation epoch of developing multicellular life and often altered in cancer), consume high quantities of energy
- High energy resources physiologically rely on the oxygen-dependent tricarboxylic acid (TCA, aka Krebs) cycle in mitochondria, that are altered in cancer: the Warburg effect describes the fact that cancer cells are hardly able to make their mitochondria work properly and depend on the poor energy-producing process of anaerobic glycolysis (aka fermentation)

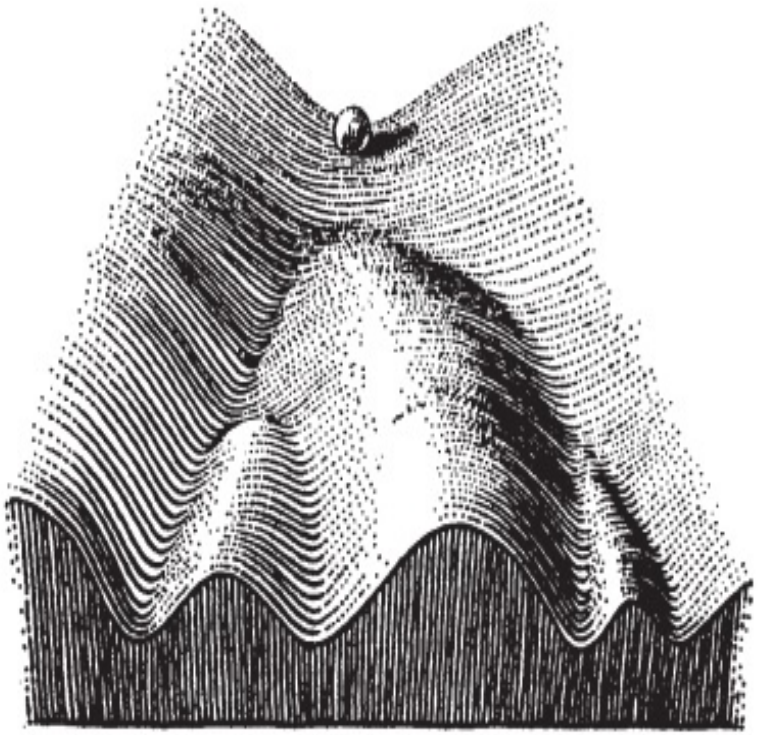
Otto Warburg has even proposed that cancer could be primarily *a disease of the mitochondria*

The mitochondrial TCA cycle →

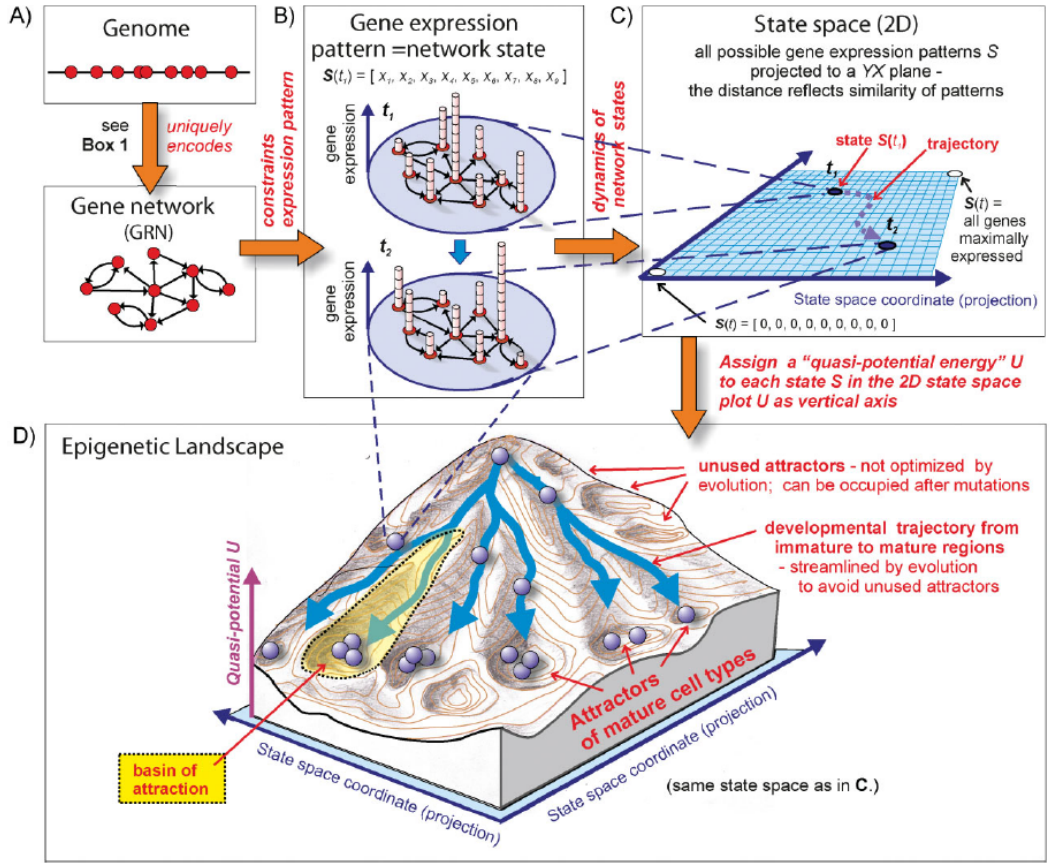


Another evolutionary framework (*synchronic view*): revisiting the Waddington epigenetic landscape

The classic Waddington landscape (1957) for cell differentiation



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



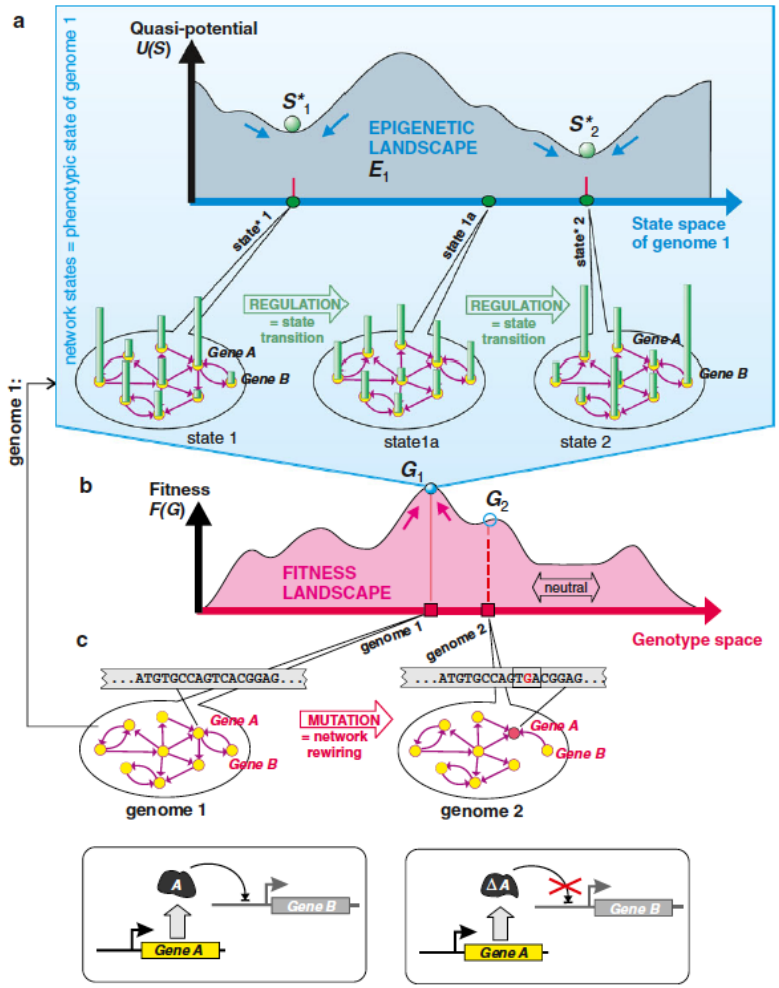
"Nothing in evolution makes sense except in the light of *systems* biology" (S. Huang, 2012)

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.

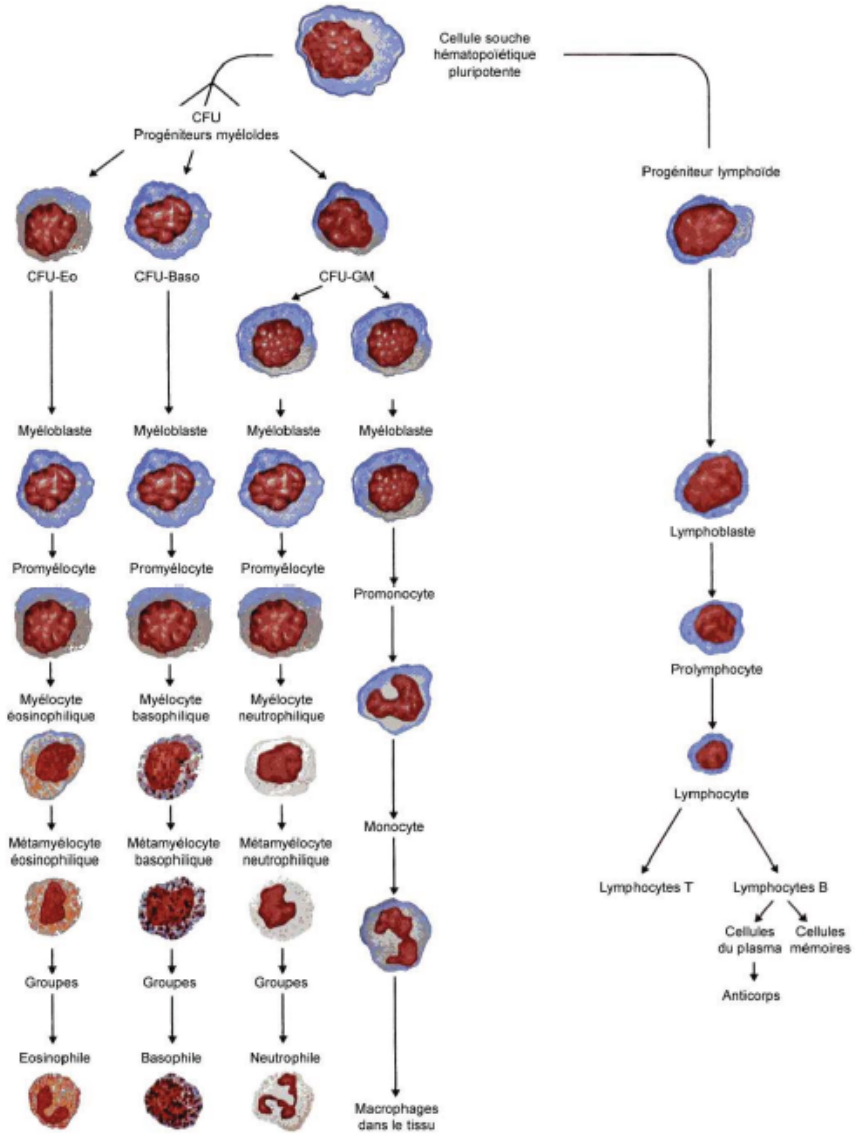
- *References: Sui Huang Sem Canc Biol 2011, Bioessays 2012, Canc Metastasis Rev 2013; Zhou Interface 2012; Pisco Br J C 2015...*

- 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.)

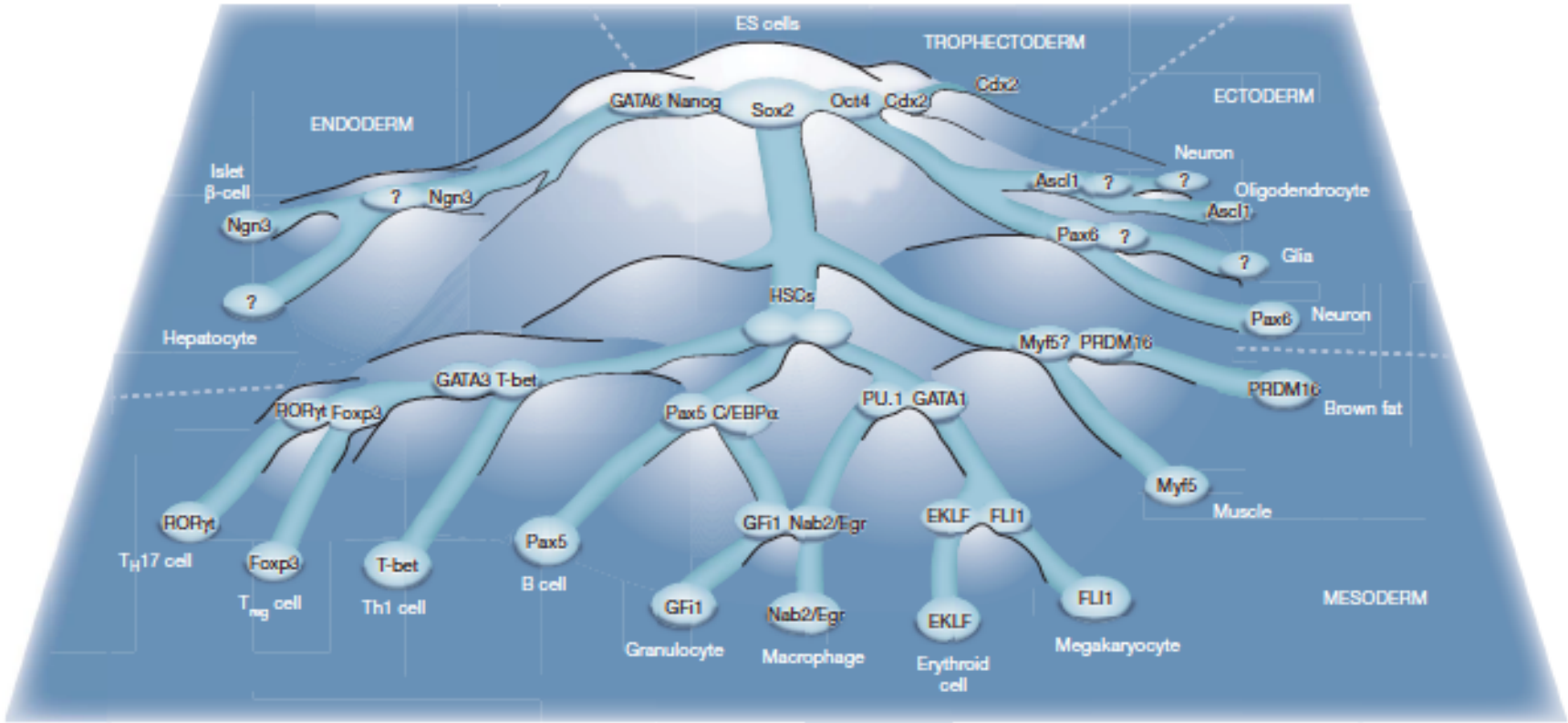


(*Sui Huang, Canc Metastasis Rev 2013*)

The best known case: haematopoiesis



Some milestones to reconstruct the global landscape



(From Tariq Enver, ASH meeting 2011)

Can resistance be assessed by biological experiments? (1)

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

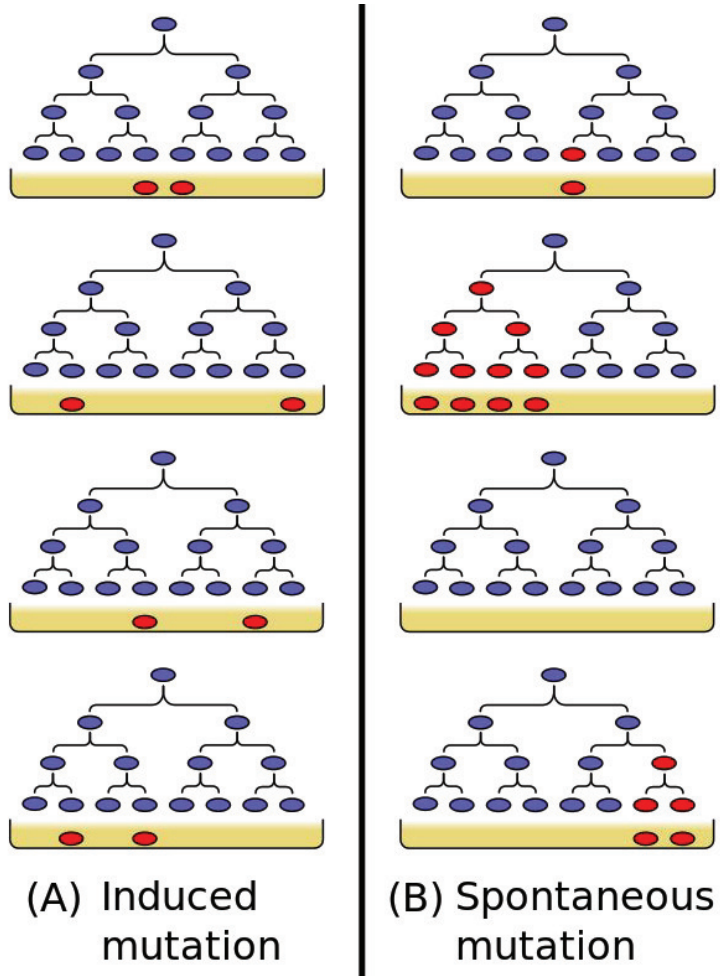
Different Petri dishes, same experimental settings

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)?

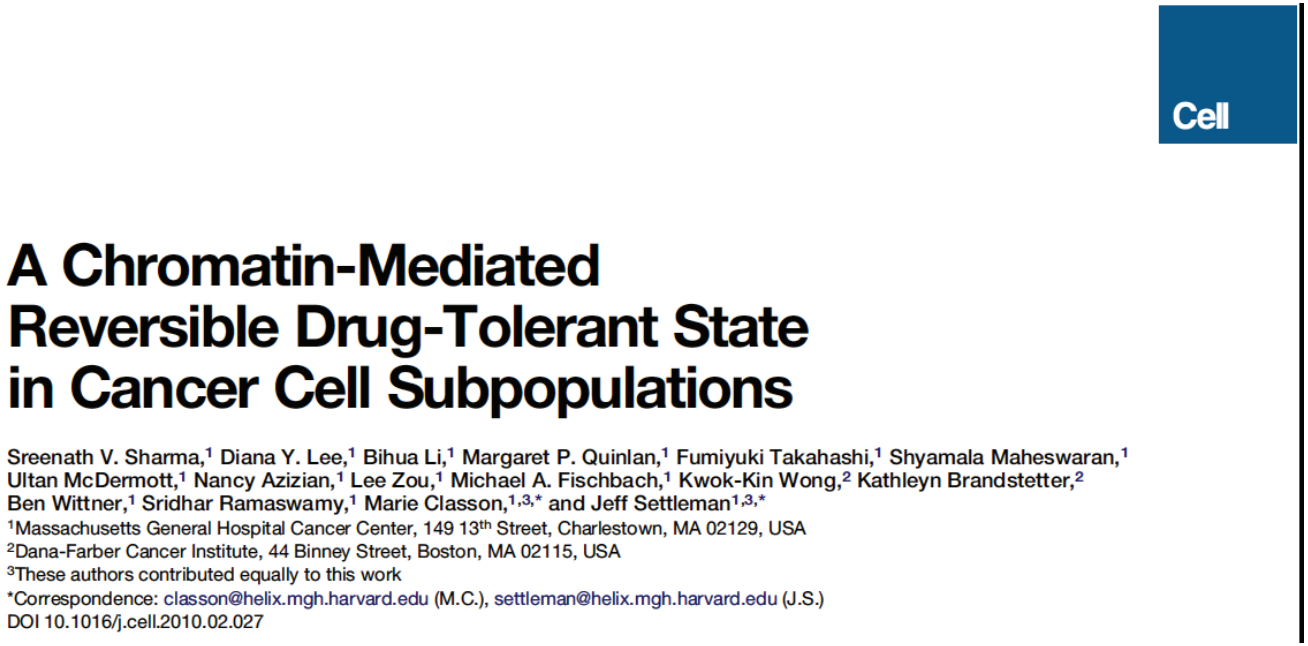
Experiment: the answer is always (B): preexistent mutations before selection

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*)

Can it be assessed by biological experiments? (2) Reversible drug resistance of cancer cells in a Petri dish



Cell

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

Sreenath V. Sharma,[1] Diana Y. Lee,[1] Bihua Li,[1] Margaret P. Quinlan,[1] Fumiyuki Takahashi,[1] Shyamala Maheswaran,[1] Ultan McDermott,[1] Nancy Azizian,[1] Lee Zou,[1] Michael A. Fischbach,[1] Kwok-Kin Wong,[2] Kathleyn Brandstetter,[2] Ben Wittner,[1] Sridhar Ramaswamy,[1] Marie Classon,[1,3,*] and Jeff Settleman[1,3,*]
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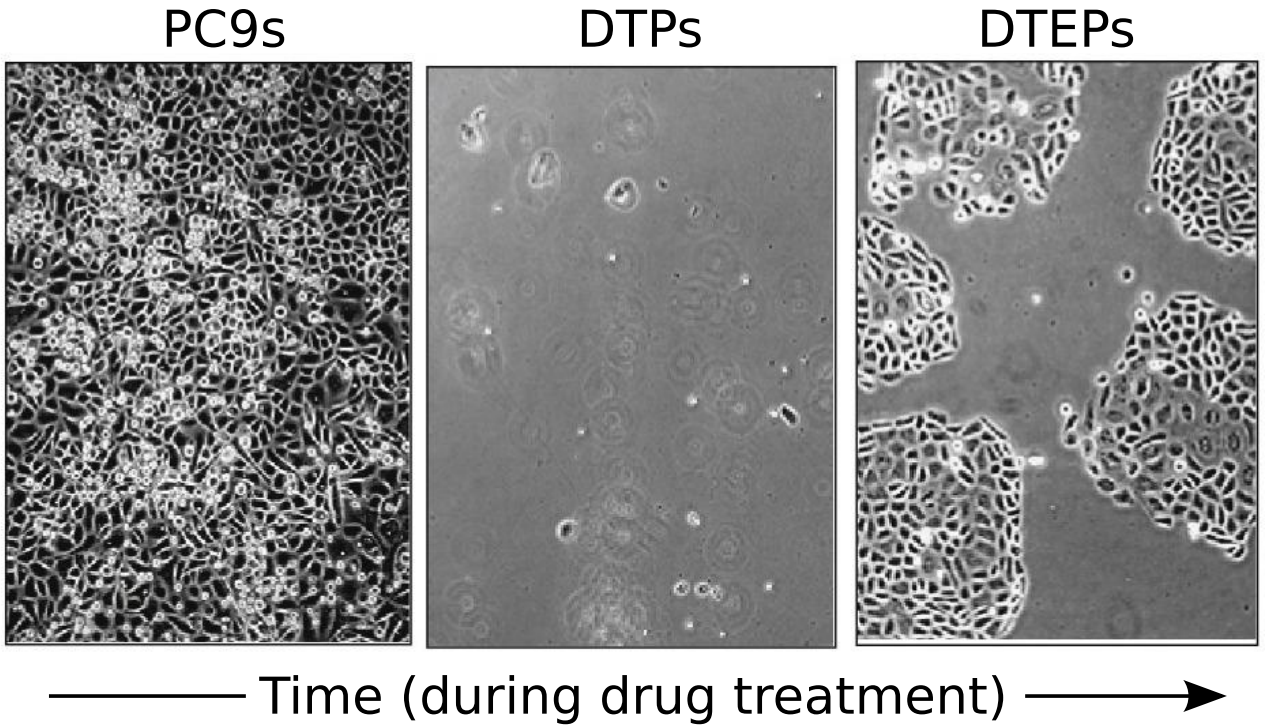
DOI 10.1016/j.cell.2010.02.027

- Motivation for math: to account for biological observations of a reversible drug-resistant phenotype in cancer cell populations, *Sharma et al., Cell 2010*
- Underlying hypothesis: epigenetic modifications affect differently survival and proliferation potentials in cancer cell populations exposed to high drug doses
- 2 proposed traits: x, stress survival potential ($\sim$ resistance to apoptosis) and y, proliferation potential ($\sim$ cell division cycle enhancement), both reversible
- A PDE model and an agent-based (AB) model show the same behaviour

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

- Population of PC9 (NSCLC) cells under high doses of drugs (e.g., gefitinib)
- 99.7% cells die, .3% survive in this maintained hostile drug environment: DTPs
- In the same hostile environment, 20% of DTPs resume proliferation: DTEPs
- Total reversibility to drug sensitivity is obtained by drug withdrawal, occurring after 9 doubling times for DTPs, and 90 doubling times for DTEPs
- Inhibition of epigenetic enzyme KDM5A blocks emergence of DTPs (*precisely: provokes rapid death of both DTPs and DTEPs, not affecting PC9s*)



(Sharma et al., Cell 2010)

Modelling framework: structured population dynamics

- Description of evolution of a population *in time t and in relevant trait x*
- 'Structure variable' x: trait chosen as bearing the biological variability at stake
- Variable : $n(x,t)$ population density of individuals bearing trait x at time t
- (1) Evolution in numbers of individuals constituting the population

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

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

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

- Cancer cell populations: (1) tumour growth; (2) asymptotic distribution of trait
- Space is not necessarily a relevant structure variable when studying drug control

2D continuous phenotype-structured PDE model

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

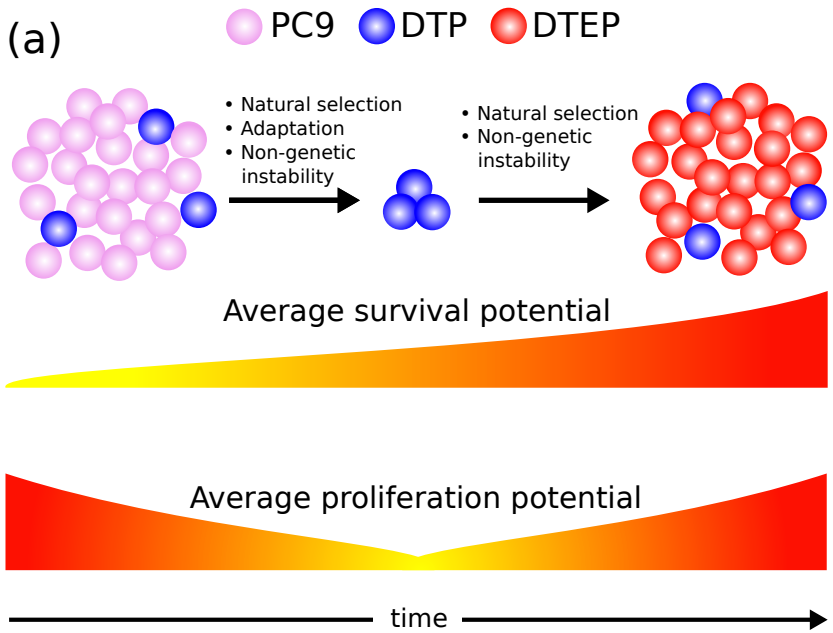
- Population density of cells $n(x, y, t)$ with phenotypic expression (x, y) at time t satisfies

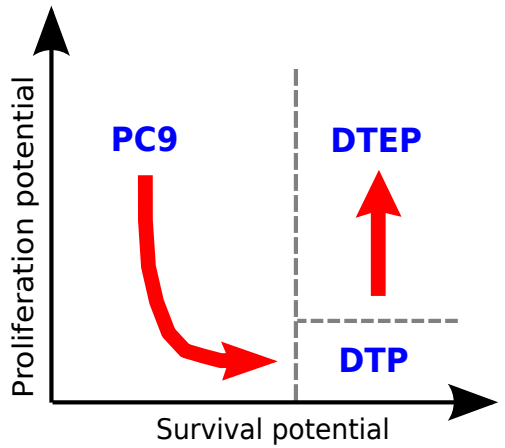
$$\frac{\partial n}{\partial t}(x,y,t) + \underbrace{\frac{\partial}{\partial y}\Big(v(x,c(t);\bar{v})n(x,y,t)\Big)}_{\text{Stress-induced adaptation of the proliferation level}} = \underbrace{\Big[p(x,y,\varrho(t)) - d(x,c(t))\Big]n(x,y,t)}_{\text{Non local Lotka-Volterra selection}} + \underbrace{\beta\Delta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

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

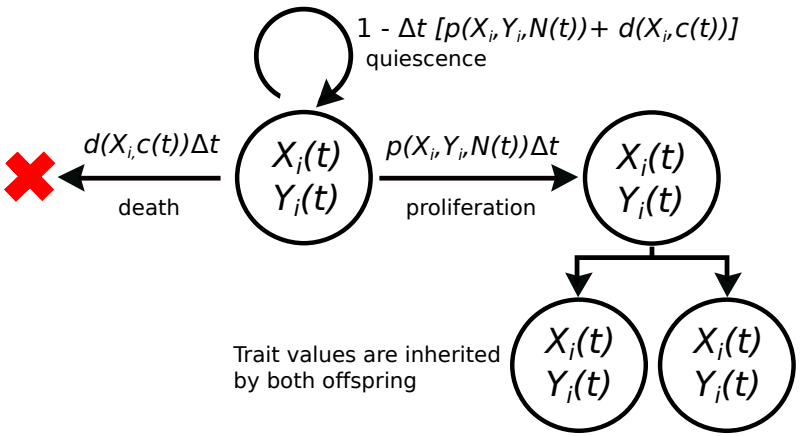
(*Chisholm et al., Cancer Research 2015*)

Agent-based model (ABM)





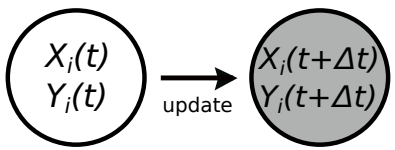
(a) Each cell i undergoes either proliferation, death or remains quiescent:



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

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

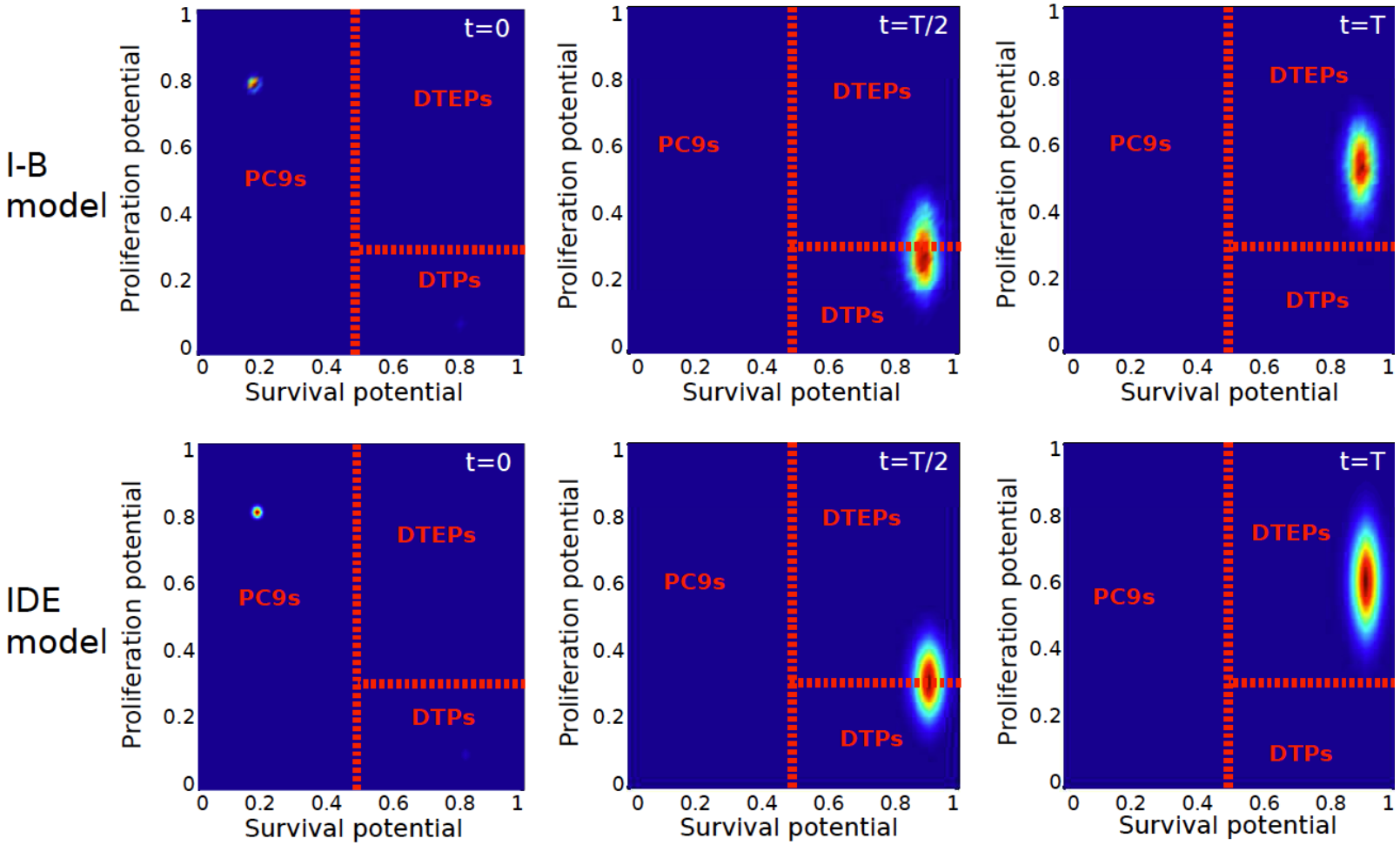
$$Y_i(t+\Delta t) = Y_i(t) + D\sqrt{\Delta t}\, W_i^2 + \Delta t\, v(X_i, c(t))$$



(*Chisholm et al., Cancer Research 2015*)

AB model and IDE model recover phenotype dynamics

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

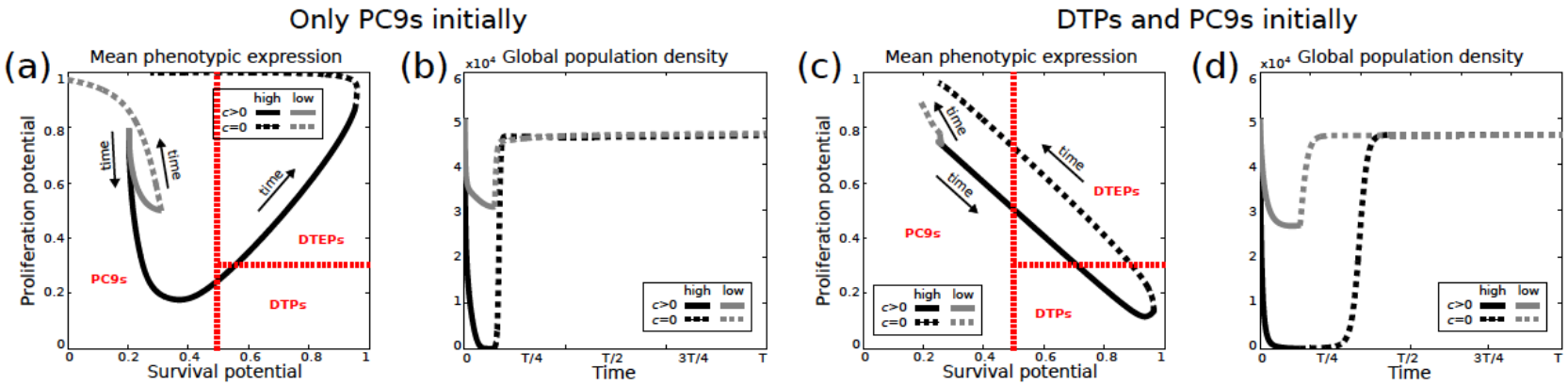


T is the simulation end-time: $0 \leq t \leq T$

(*Chisholm et al., Cancer Research 2015*)

AB model and IDE model recover phenotype dynamics

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

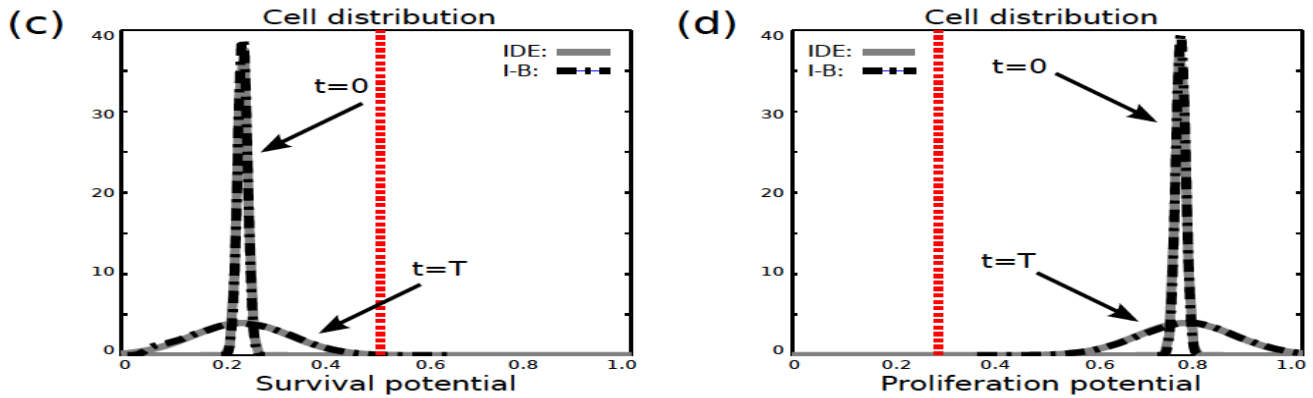


(a), (b) Only PC9s initially, adaptation on $v \neq 0$: *'Lamarckian' scenario*, or Luria-Delbrück scenario (A)

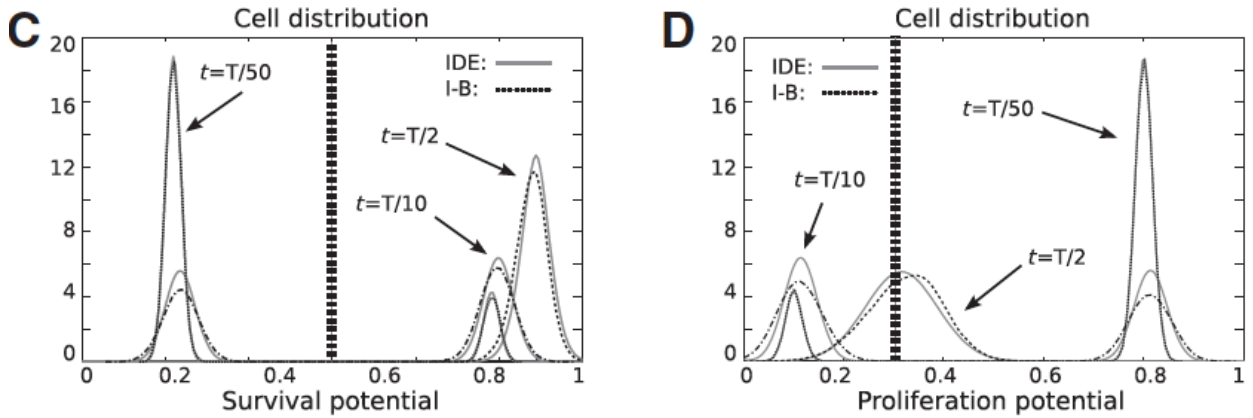
(c), (d) PC9s and DTPs initially, no adaptation $v = 0$: *'Darwinian' scenario*, or Luria-Delbrück scenario (B)

(*Chisholm et al., Cancer Research 2015*)

Phenotype heterogeneity in the cancer cell population

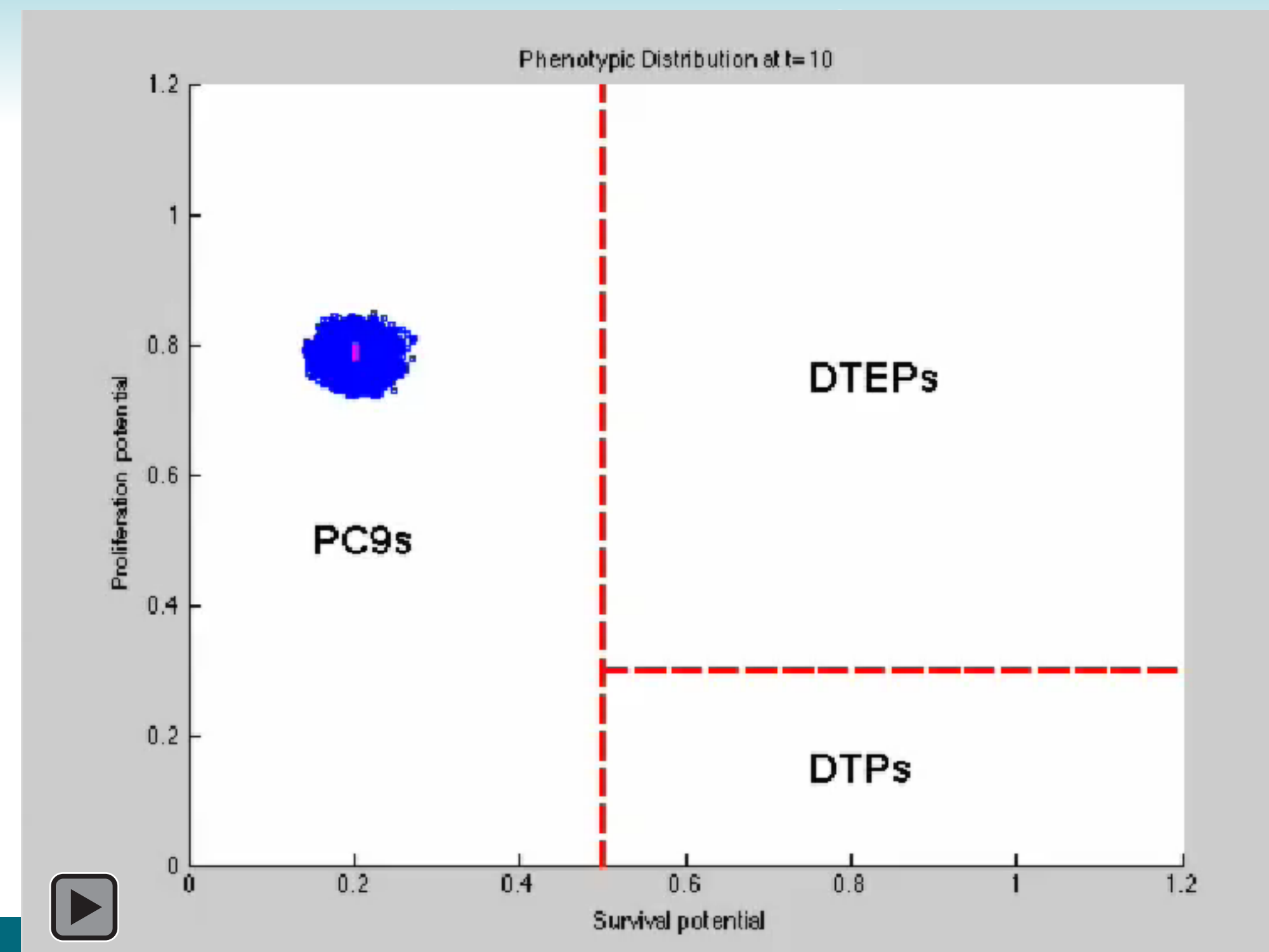


The PC9 cell population becomes more heterogeneous when it is left to evolve in the absence of drug treatment: starting from an initial concentrated phenotype (x_0, y_0), the phenotype (x, y) diffuses in the population according to a Gaussian-like curve. (c) Projection onto the x phenotype axis; (d) Projection onto the y phenotype axis.

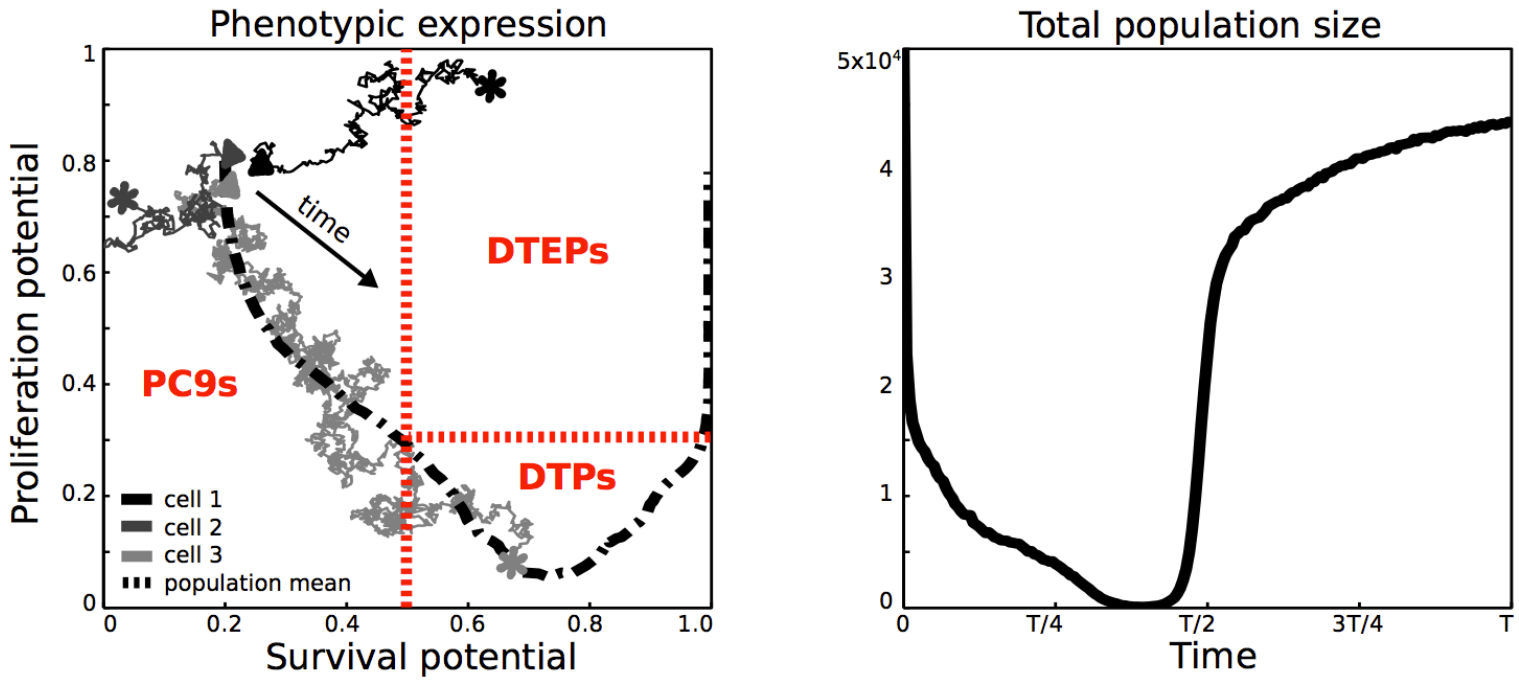


C, **D**: Under drug treatment, heterogeneity persists when phenotypes evolve (here, Darwinian scenario: DTPs are initially present)

(*Chisholm et al., Cancer Research 2015*)


Phenotypic Distribution at t= 10
Proliferation potential
Survival potential
0
0.2
0.4
0.6
0.8
1
1.2
PC9s
DTEPs
DTPs

Individual cell behaviour 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*)

Use IDE 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?

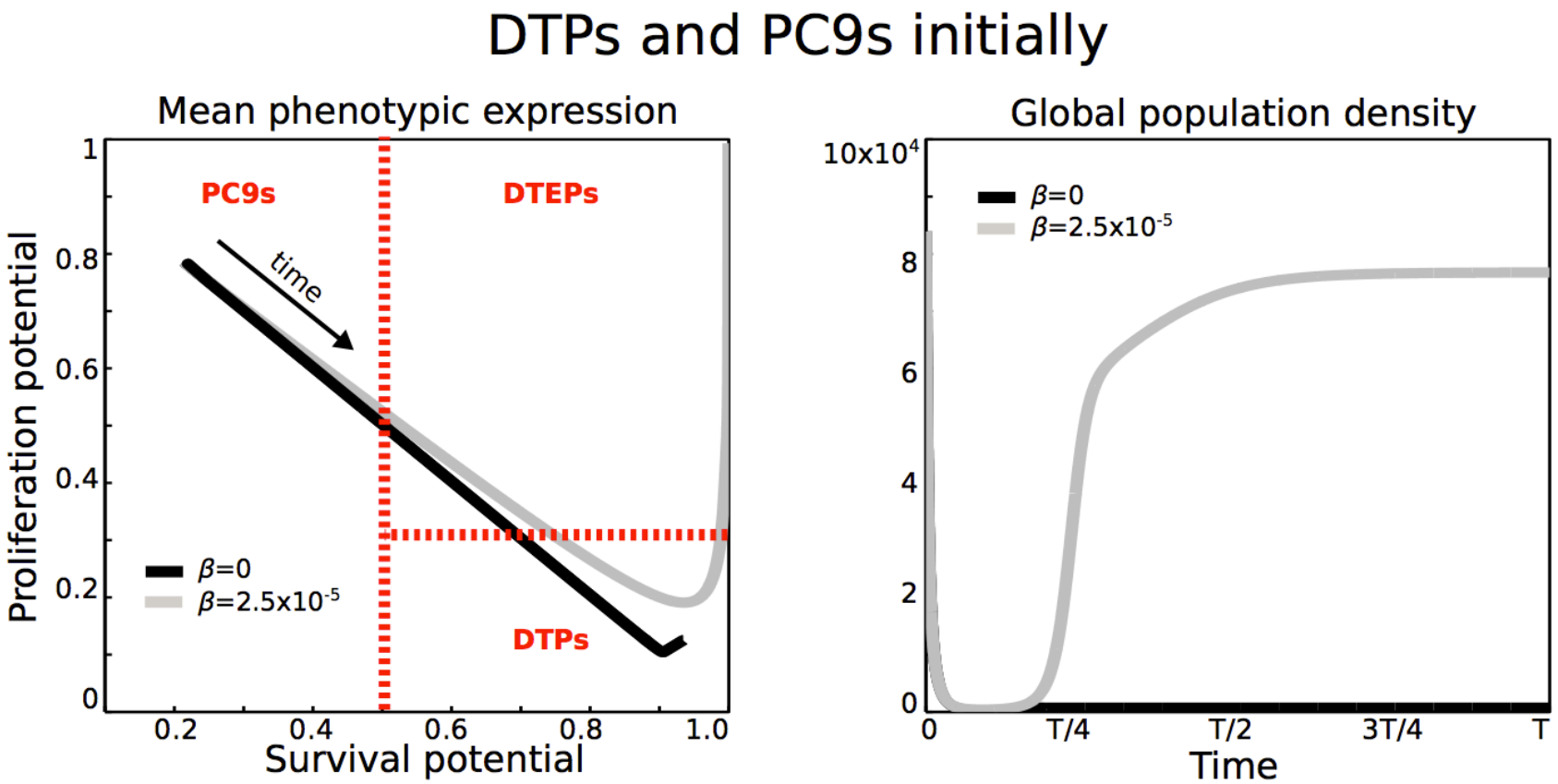
Consider $c(\cdot) = \textit{constant}$ and two scenarios:

(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 ($v \neq 0$)

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

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

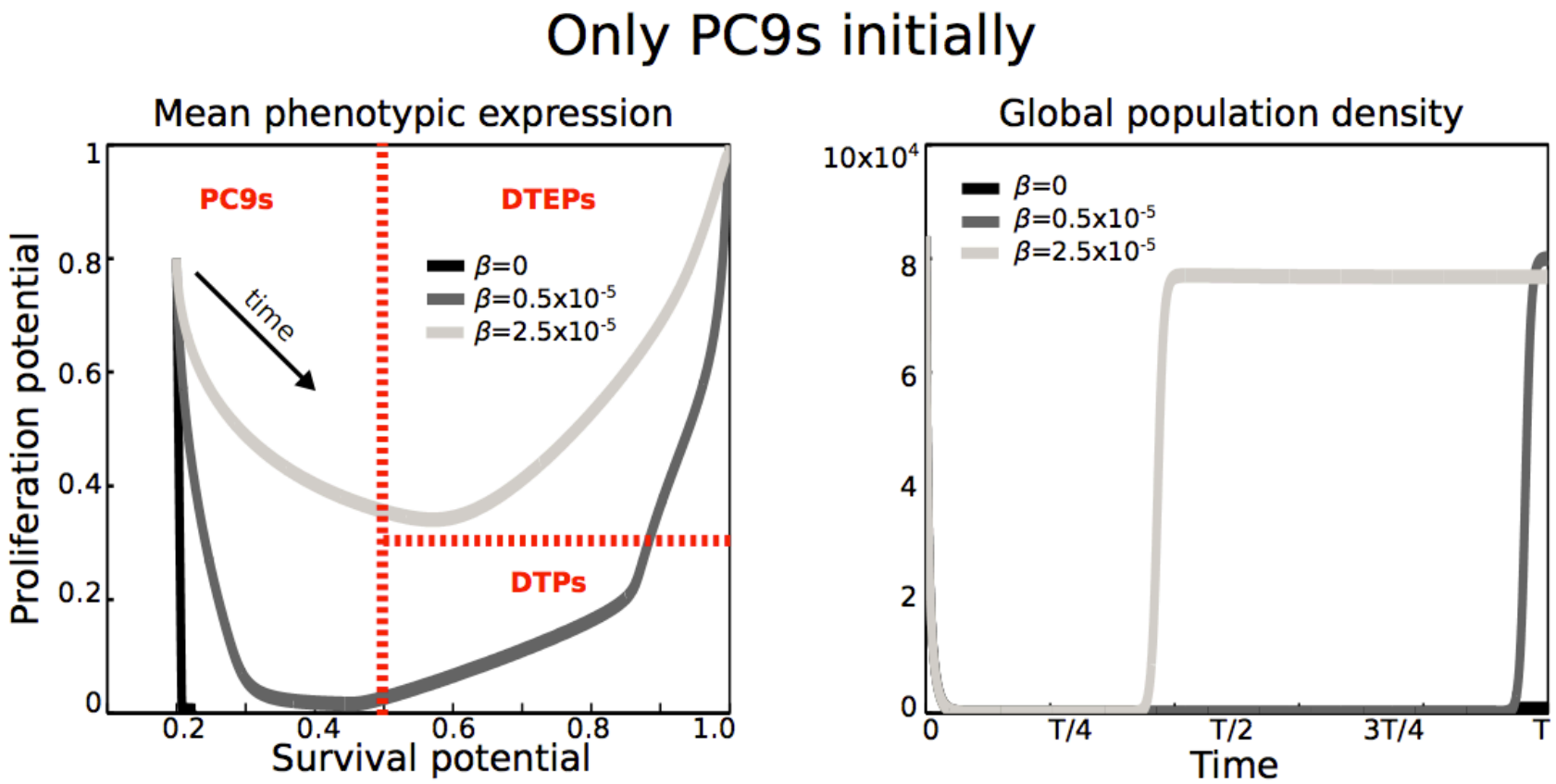


Extinction when $\beta = 0$ (here, adaptation is absent $v = 0$)

(*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?

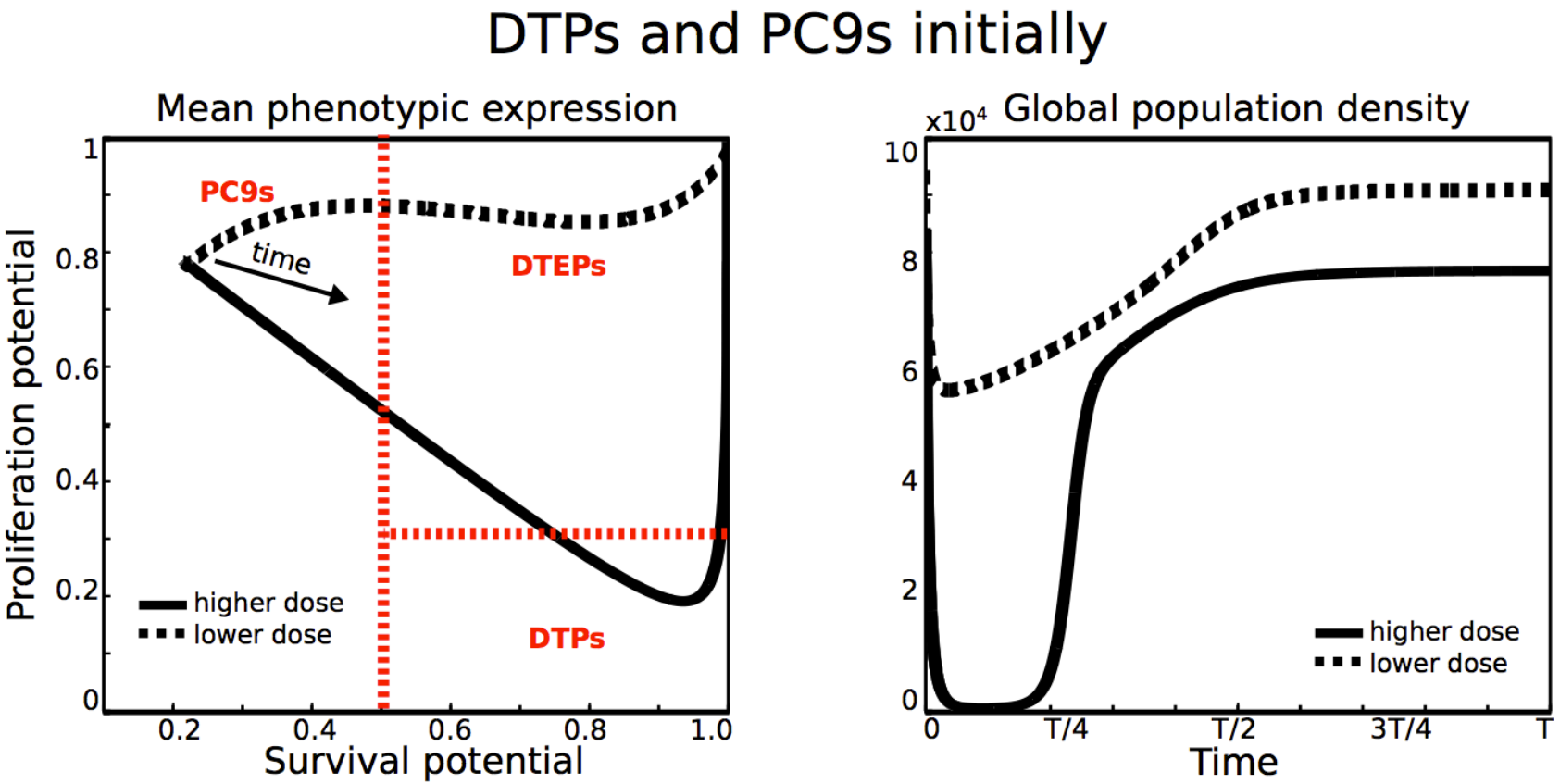
Definition (LC_γ dose)

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]

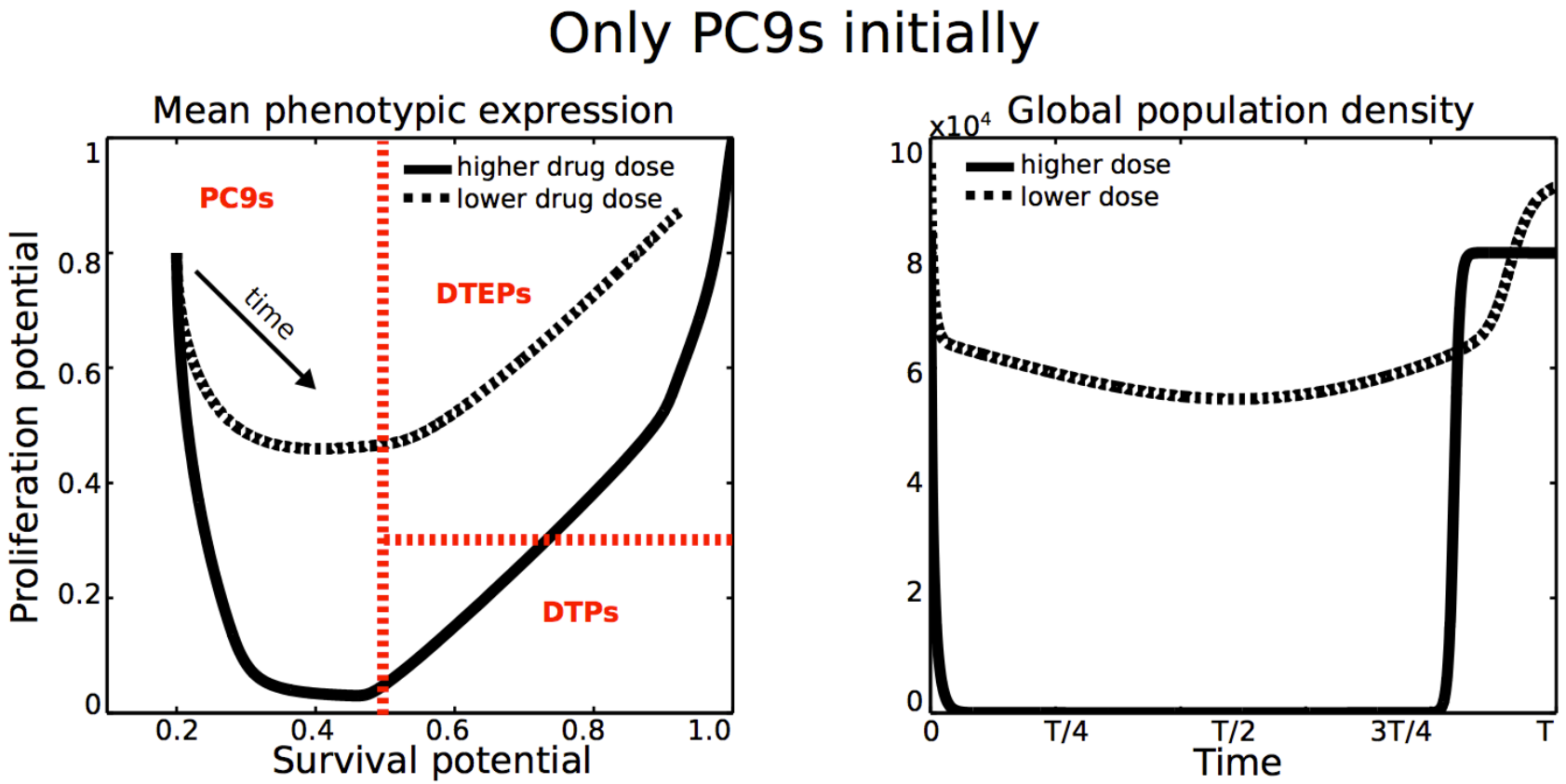


Low drug dose does not let appear DTPs (here, adaptation is absent $v = 0$)

(*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,

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

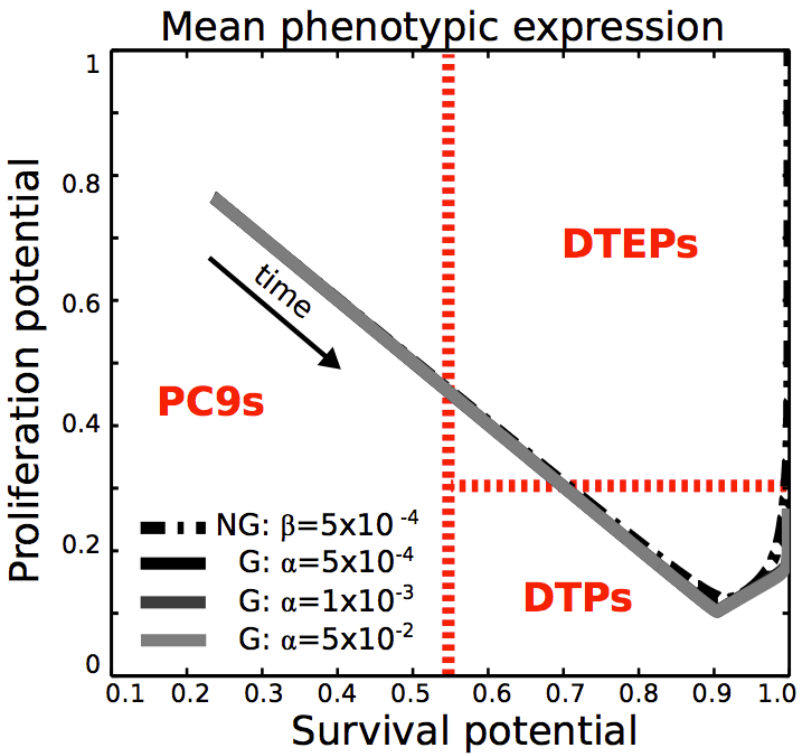
and C_M is a normalisation constant such that

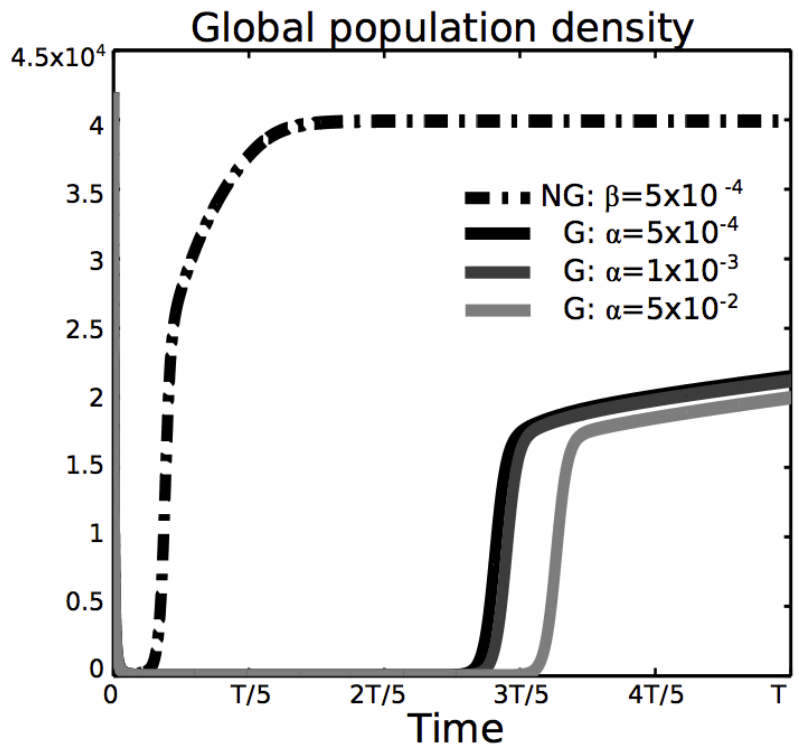
$$\int_0^1 \int_0^1 M(x,y|\cdot,\cdot;\cdot)\mathrm{d}x\mathrm{d}y = 1.$$

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





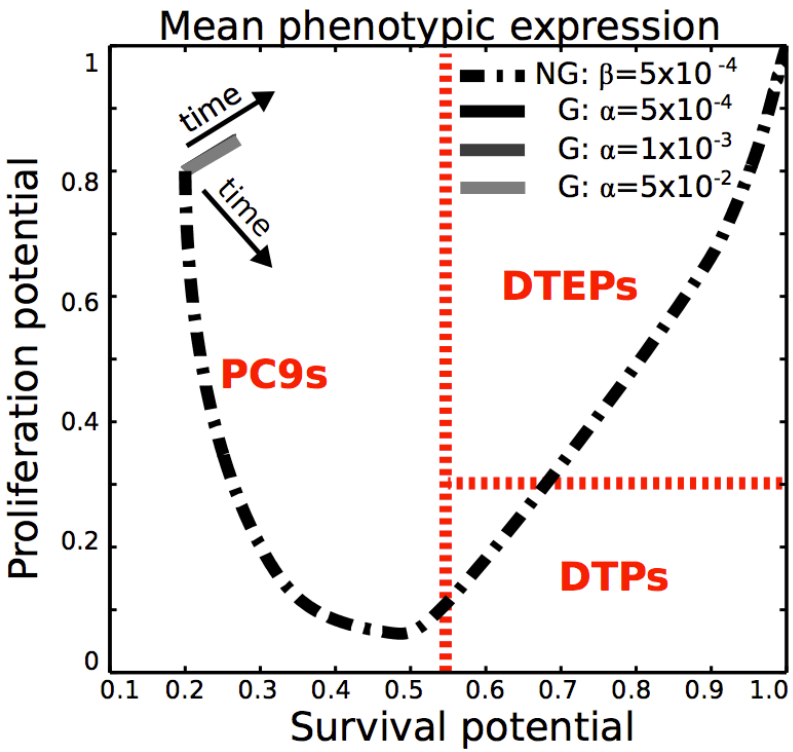
G: mutations do not let occur total recovery (NG: here, adaptation is absent $v = 0$)

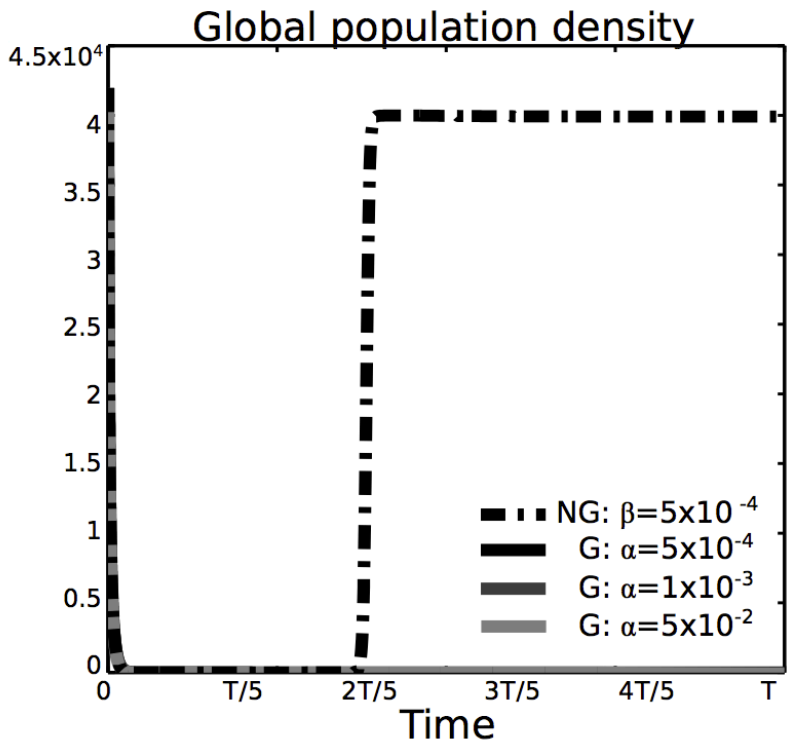
(*Chisholm et al., Cancer Research 2015*)

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

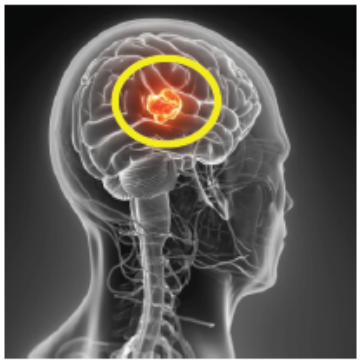
- To see the transient appearance of the DTPs during high-dose drug therapy:

  - 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 (in principle not inducing drug resistance)? Might be assessed using this model, not done yet.

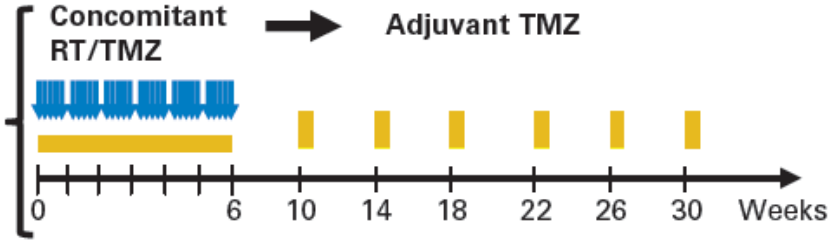
Temozolomide (TMZ) in glioblastoma (GBM)

Treatment



Surgical resection

+

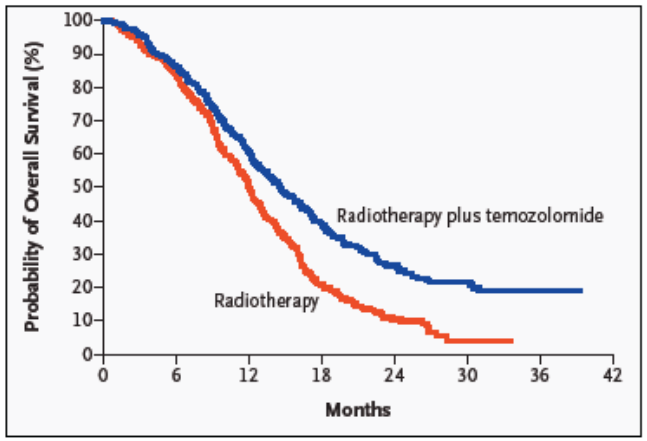


Focal RT: daily, 30 × 200 cGy
Total dose 60 Gy

Temozolomide: 75 mg/m² po qd for 6 weeks,
then 150–200 mg/m² po qd days 1–5 every 28 days for 6 months

Survival

- Median: 14,6 months
- 5 years: 3%



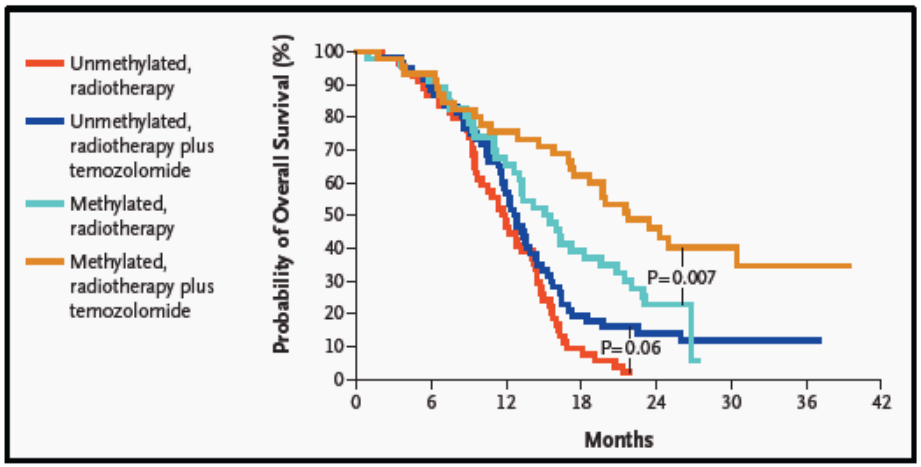
Grossman *et al*, 2009; Stupp *et al 2005;* Preusser, M. *et al*, 2011

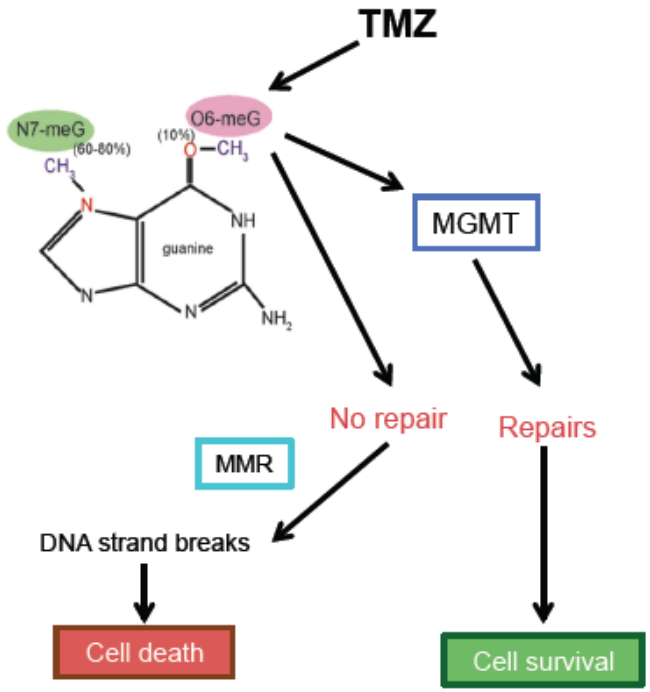
Resistance of GBM cell populations to TMZ

TMZ resistance

Main marqueur of TMZ resistance

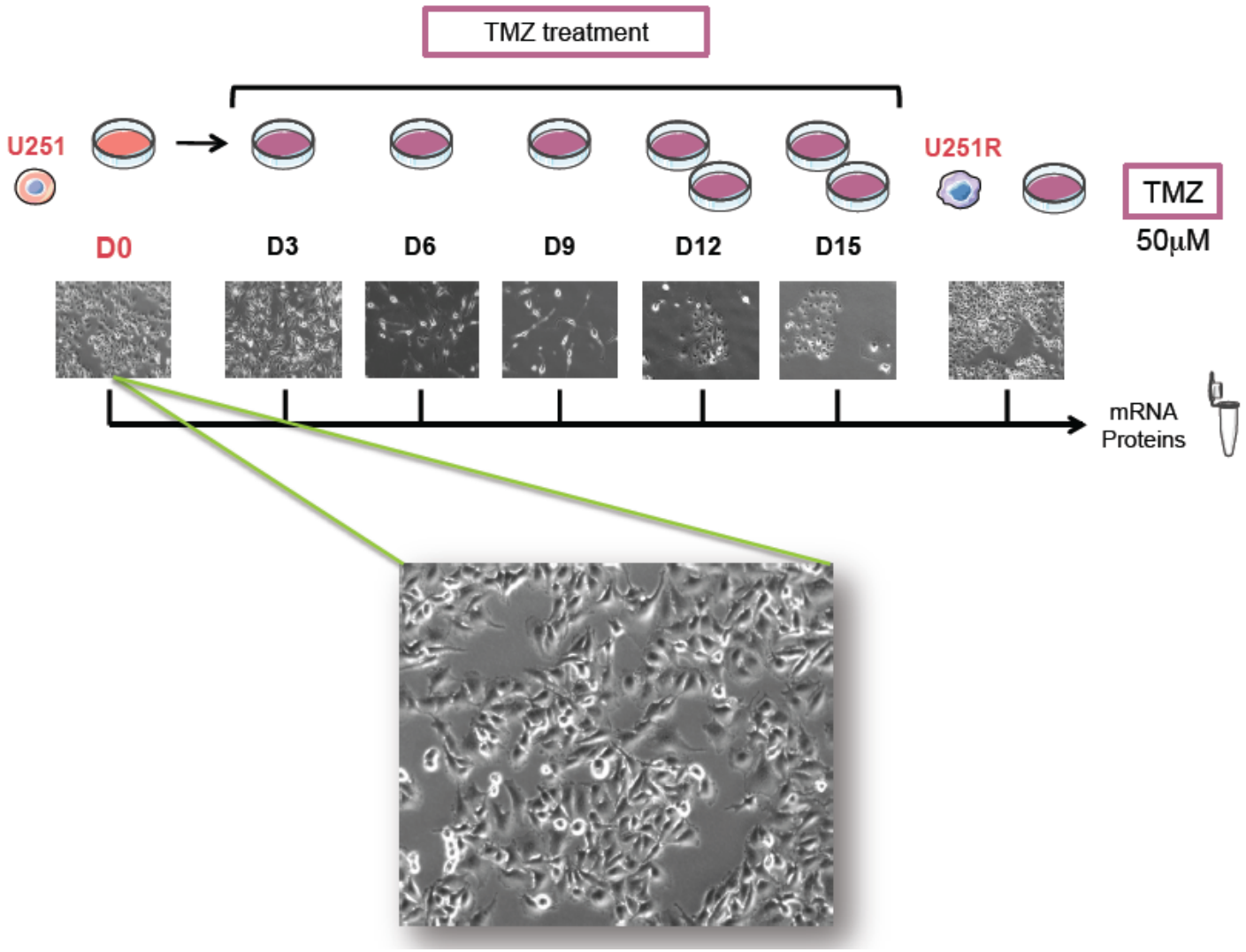
Methylation status of MGMT promoter





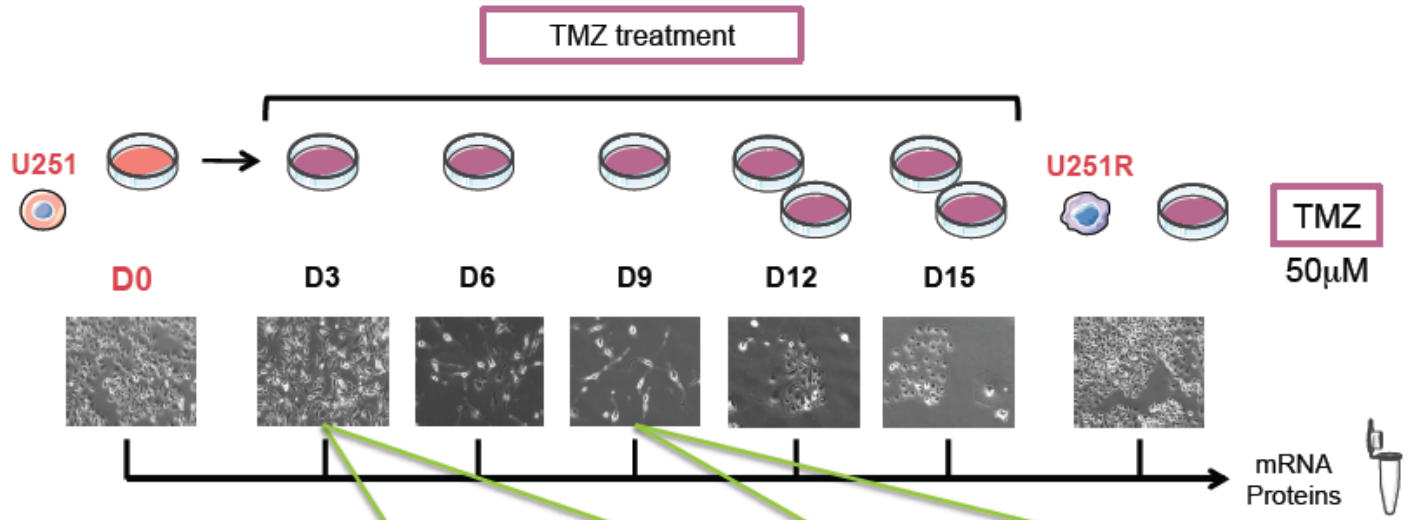
Hegi *et al*, 2005

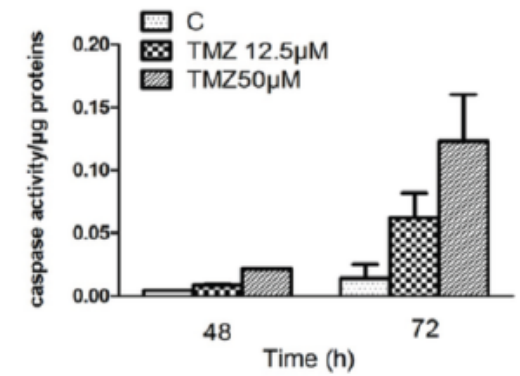
Same observations as in *Sharma et al. Cell 2010*



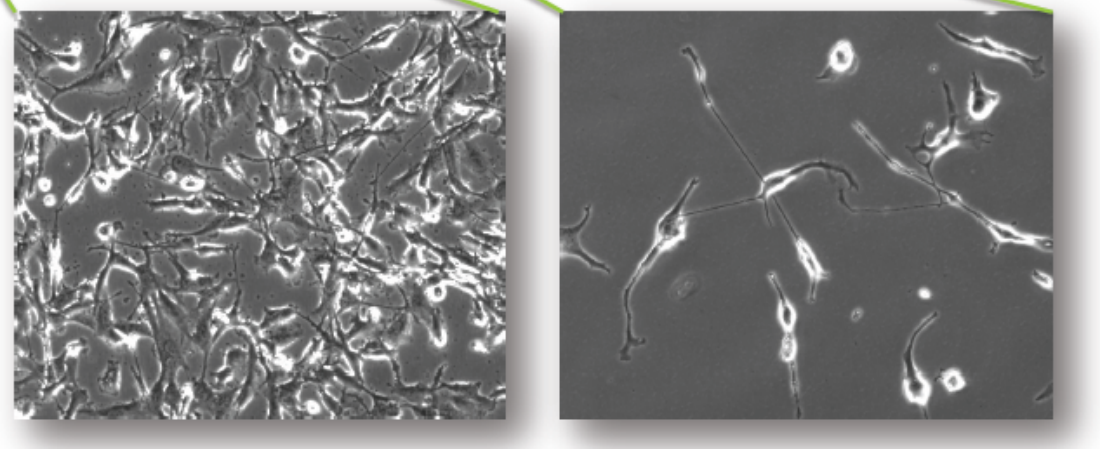
from F. Vallette's INSERM team in Nantes

Same observations as in *Sharma et al. Cell 2010*

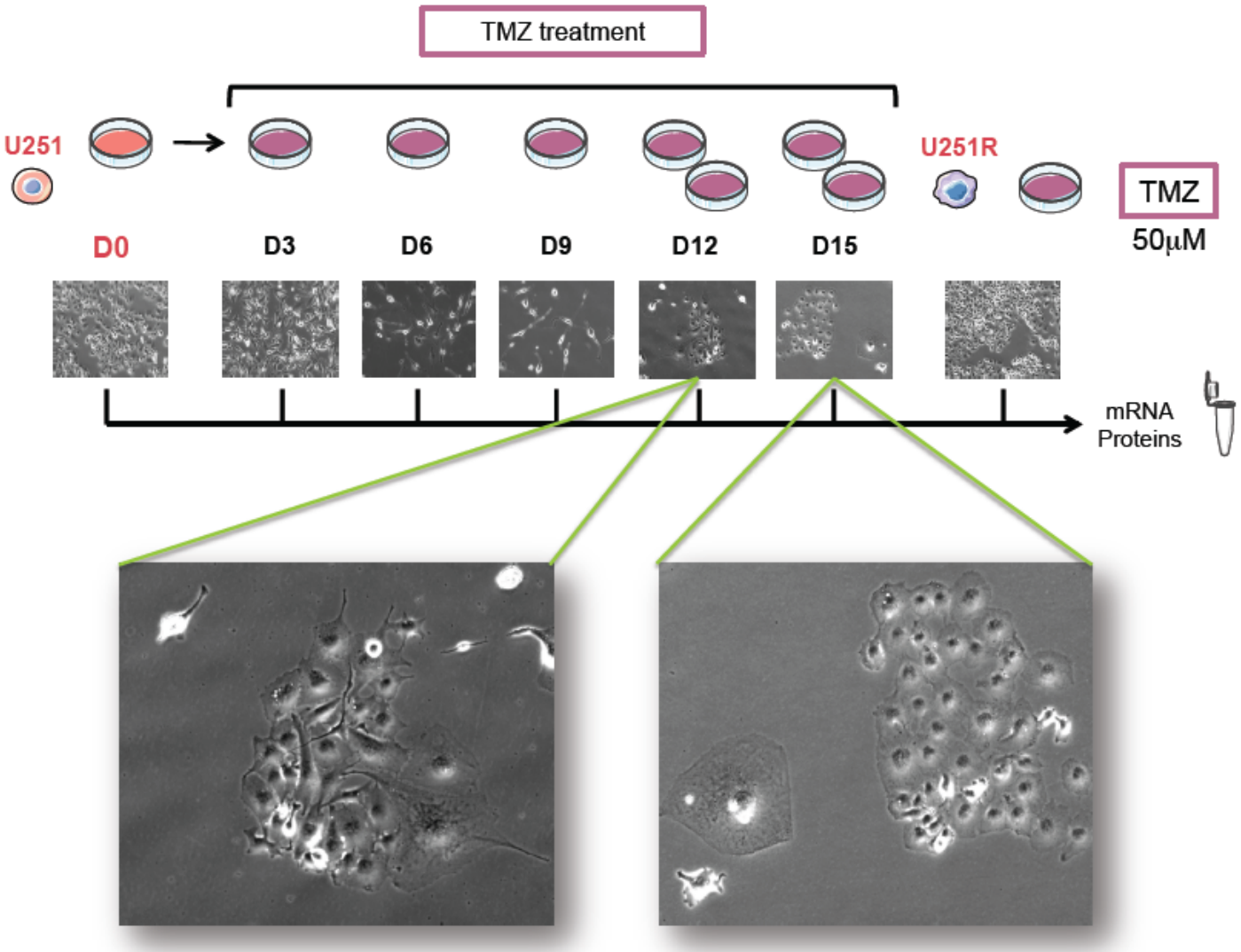




Gratas *et al*, 2014, Oncotarget



Same observations as in *Sharma et al. Cell 2010*



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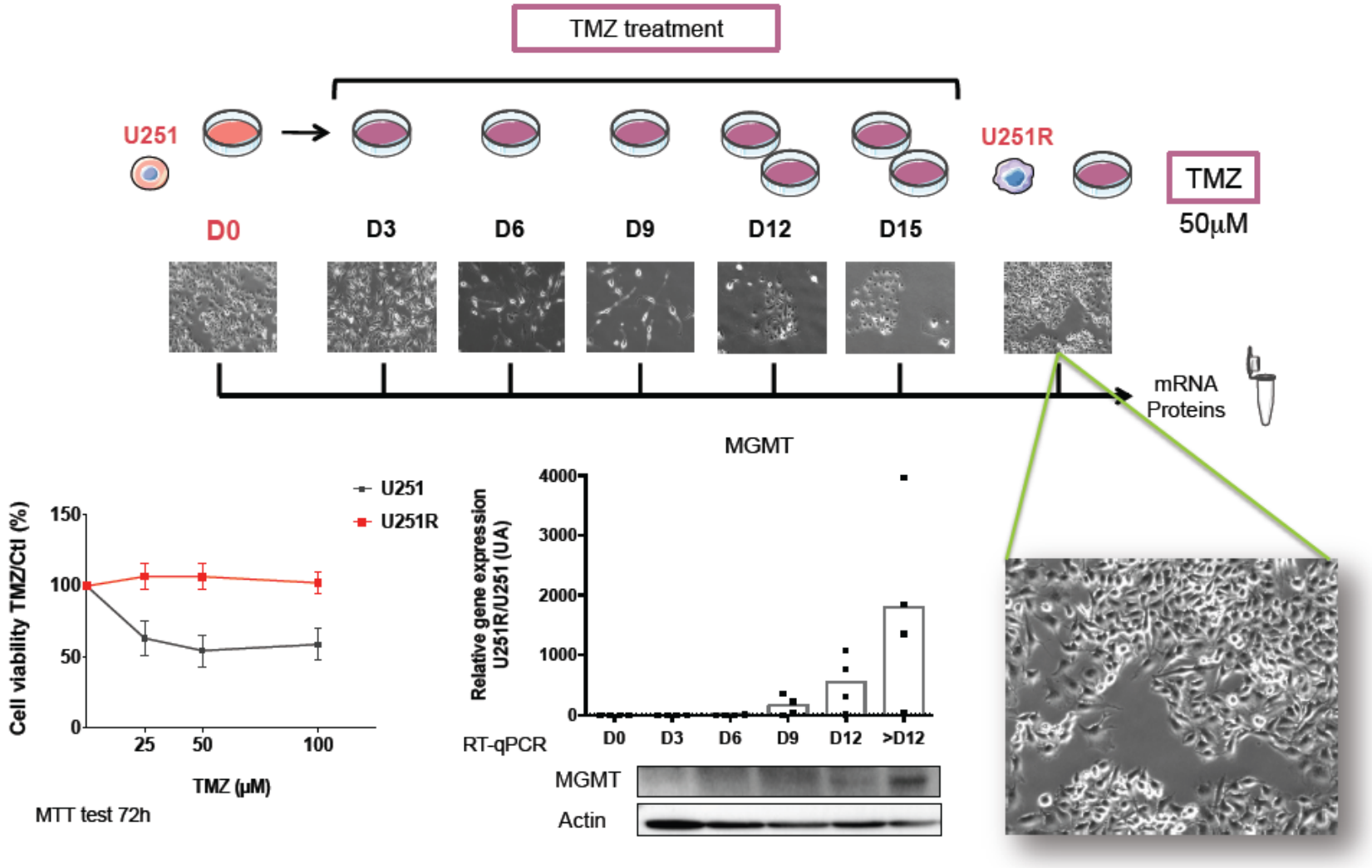
Same observations as in *Sharma et al. Cell 2010*



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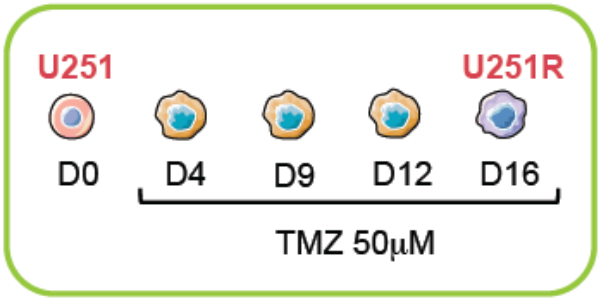
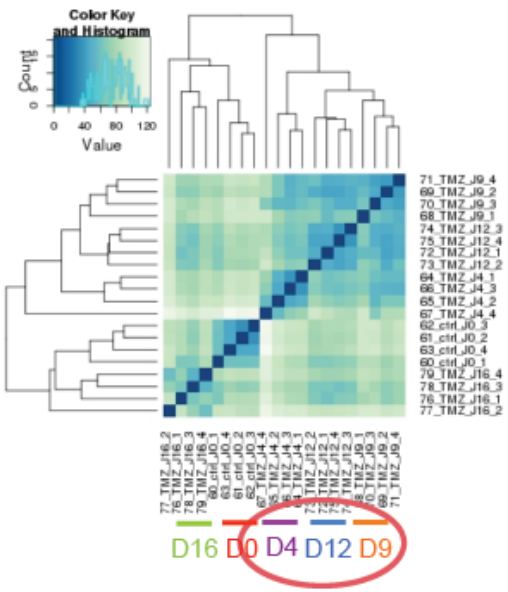
Gene expression followed from D0 to D16

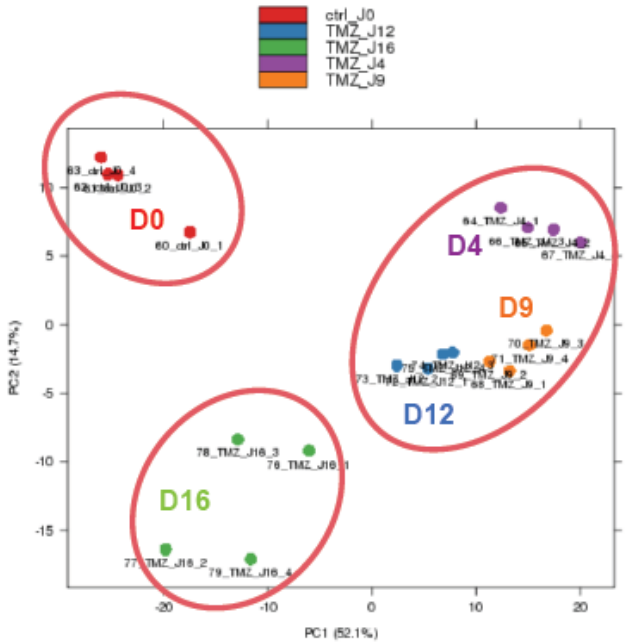
Results: Transcriptomic sequencing

Whole transcriptome sequencing

RNA-Seq







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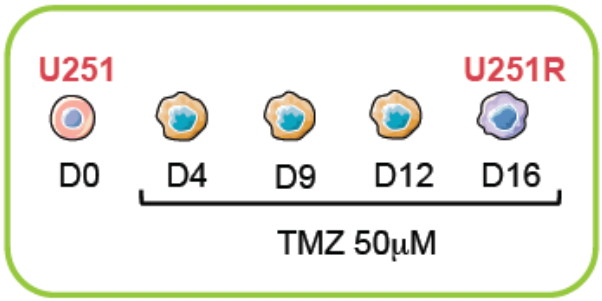
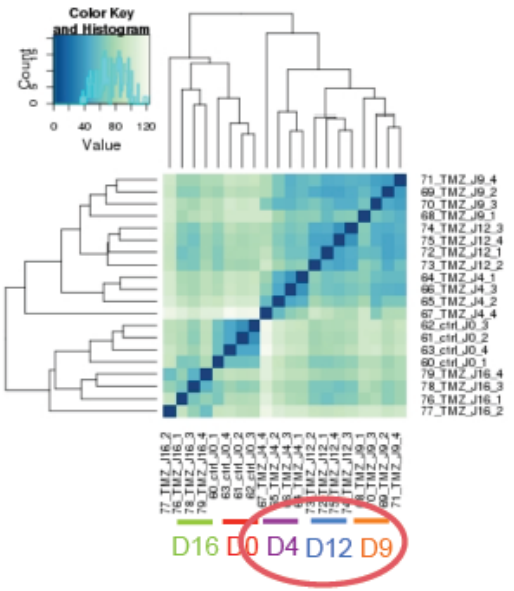
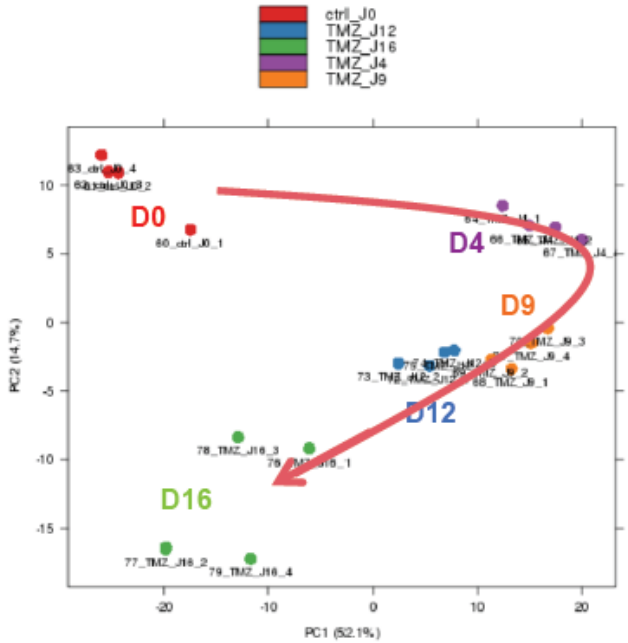
Gene expression followed from D0 to D16

Whole transcriptome sequencing

RNA-Seq







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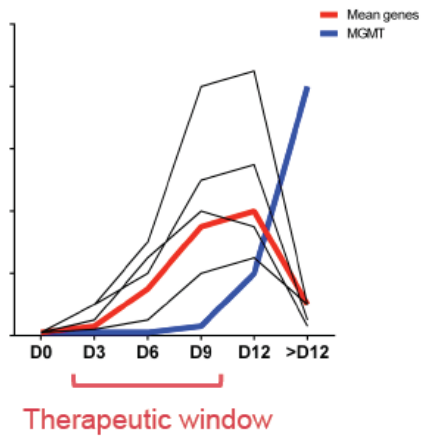
Therapeutic consequences??

Clonal selection or acquired gene expression?

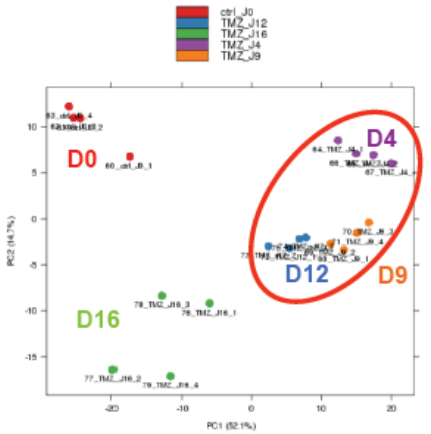
CHI3L1
FAT2
KLK5
HBEGF

➔ Transitory phase

Drug tolerant population



- Stable inhibition of target genes
- Viral barcode library



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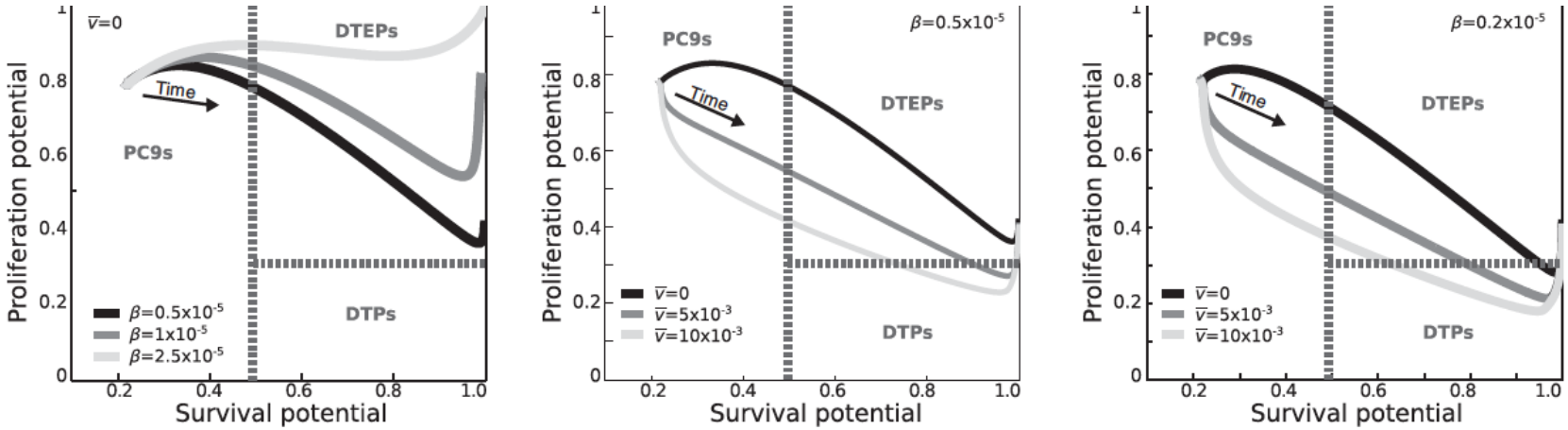
An experiment to decide between scenarios A and B

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 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: left panel, all lines; central and right panels, black line only)



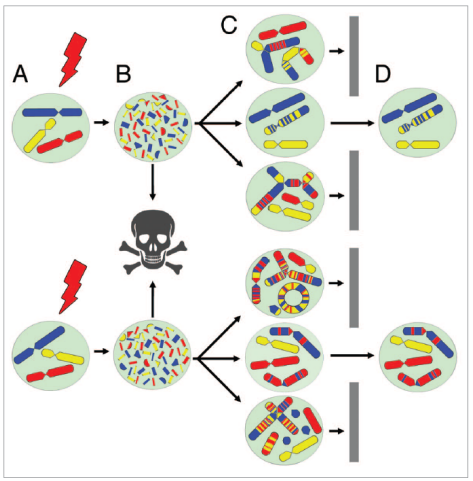
(Chisholm et al., Cancer Research 2015)

Questions and tracks to enrich and interpret this model

- Is there a succession of events from a population dynamics point of view between an epigenetic, reversible, state of drug resistance, followed by a possibly acquired, genetic, unbeatable state of resistance to a given drug?

- Hint: '[epi]genome chaos' (*Henry Heng*) triggered by stress signals, followed by epigenetic (in splicing?) rearrangements (*the drift*), and Darwinian selection?... "What does not kill me strengthens me" (*Sui Huang*, 2012, quoting Nietzsche) Note, however, that we are looking for a *reversible* and epigenetic version of chaos (massive chromatin rearrangements?)

(*Cartoon from Henry Heng 2014*)



- Is there a way to measure in a molecular way a *cost of resistance*, so as to design realistic cost functions for resistance at the cell population level?

- Can we connect stochastic events such as transcription and splicing at the single cell level - ruled by genetic regulatory networks and possibly influenced by the cellular environment - with the determination of cell fate (e.g., drug resistance, transient EMT phenotype) at the cell population level?

A general framework to optimise cancer therapeutics: designing mathematical methods along 3 axes

- Modelling the behaviour of growing cell populations on which anticancer drugs act (the targeted cell populations): proliferating tumour and healthy cell populations, including representing functional (not necessarily molecular) targets for pharmacological control
- (When PK-PD models are available) Modelling the external control system, i.e., fate of drugs in the organism, at the level of functional targets (proliferation, death, differentiation) in cell populations by functional, rather than molecular, pharmacokinetics-pharmacodynamics (PK-PD)
- Optimising therapeutic controls: dynamically optimised control of theoretical drug delivery flows representing time-dependent objectives and constraints, making use of known or hypothesised differences between cancer and healthy cell populations

Choosing the constraint to be represented determines the model of proliferation used to optimise drug delivery, aiming to avoid the two main pitfalls of pharmacotherapy:

- *Toxicity issues.* Limiting toxic side effects to preserve healthy cell populations leads to representing proliferating cell populations by ordinary differential equations, or by age-structured models: physiologically structured partial differential equations

- *Drug resistance issues.* Limiting emergence of drug-resistant cell subpopulations in tumour tissues leads to using (evolutionary) phenotypic trait-structured proliferation: physiologically structured evolutionary integro-differential equations

- In fact, one should consider the two issues simultaneously, i.e., two similarly structured cell populations, healthy and cancer, with different characteristics w.r.t. to drug effects and to evolution towards resistance: phenotypic stability of healthy cell populations vs. plasticity of cancer cell populations

Modelling framework: structured population dynamics

- Description of evolution of a population *in time t and in relevant trait x*
- 'Structure variable' x: trait chosen as bearing the biological variability at stake
- Variable : $n(x,t)$ population density of individuals bearing trait x at time t
- (1) Evolution in numbers of individuals constituting the population

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

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

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

- Cancer cell populations: (1) tumour growth; (2) asymptotic distribution of trait
- Space is not necessarily a relevant structure variable when studying drug control

Introduction to IDEs: typical 1D IDE logistic model

Prototype model, where $n(t,x)$ stands for the density of cells of 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 $n^0 \in L^1([0,1])$

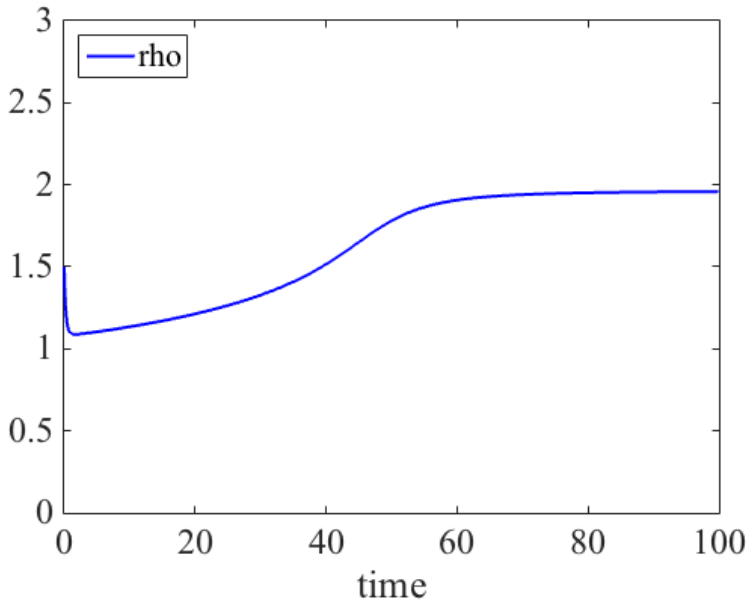
[More general settings for the growth rate $R(x,\rho(t))$, here $\Big(r(x) - d(x)\rho(t)\Big)$, have been studied in Benoît Perthame's book Transport equations in biology *(2007)]*

Questions: what is the asymptotic behaviour of

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

Introduction to IDEs: convergence and concentration (1D)

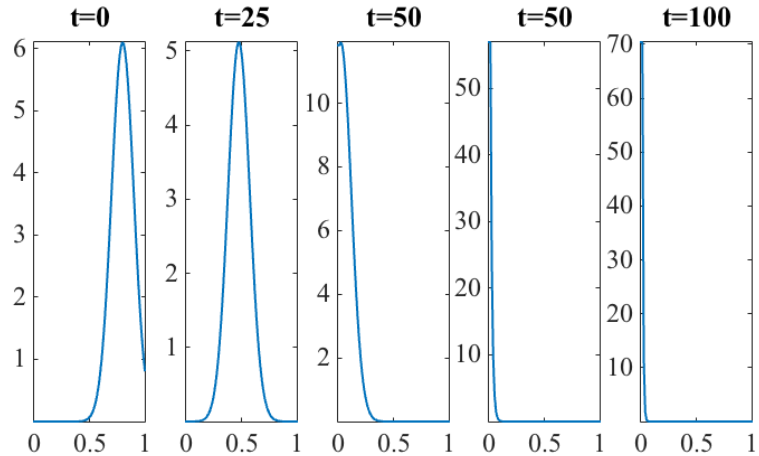
Convergence: Plot of $t \mapsto \rho(t)$



Firstly, it can be shown that: ρ converges to ρ^∞, the smallest value such that $r(x) - d(x)\rho^\infty \leq 0$ on $[0,1]$. *(Idea of proof: show that* $\int_0^{+\infty} \left|\frac{d\rho}{dt}\right|_- dt < +\infty$ *and – with additional hypotheses – that* ρ *is bounded; then convergence follows.)*

Introduction to IDEs: convergence and concentration (1D)

Concentration: 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 $\left\{x \in [0,1],\ r(x) - d(x)\rho^\infty = 0\right\}$.*
- *Furthermore, if this set is reduced to a singleton x^∞, then*

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

[Proof: 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`]

Drug effects on cell populations and their optimisation
Model with mutations, one cytotoxic drug: cancer cells

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

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

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

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

(Lorz et al., M2AN 2013)

Model with mutations, one cytotoxic drug: healthy cells

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

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

where the total population is defined as

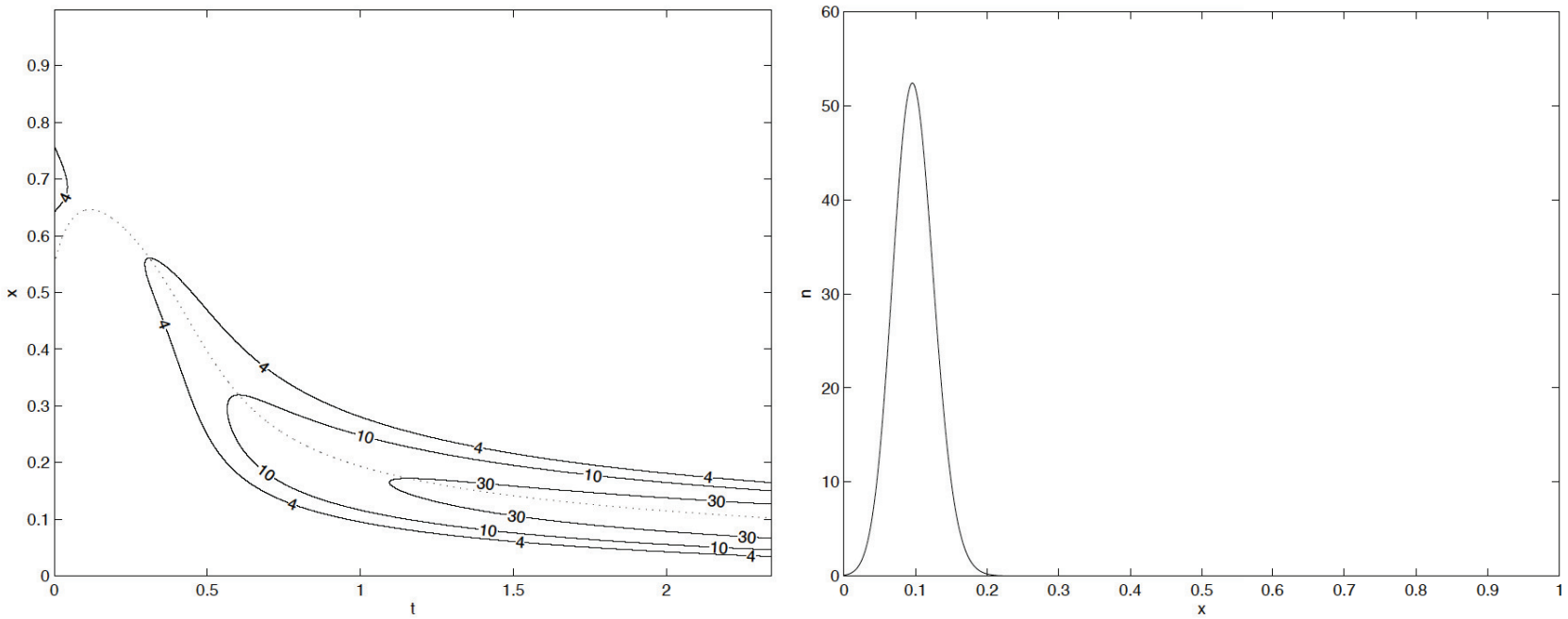
$$\rho(t) = \rho_H(t) + \rho_C(t); \rho_H(t) = \int_{x=0}^{\infty} n_H(x,t)dx; \rho_C(t) = \int_{x=0}^{\infty} n_C(x,t)dx.$$

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

(Lorz et al., M2AN 2013)

Model with mutations, one cytotoxic drug: illustrations (1)

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

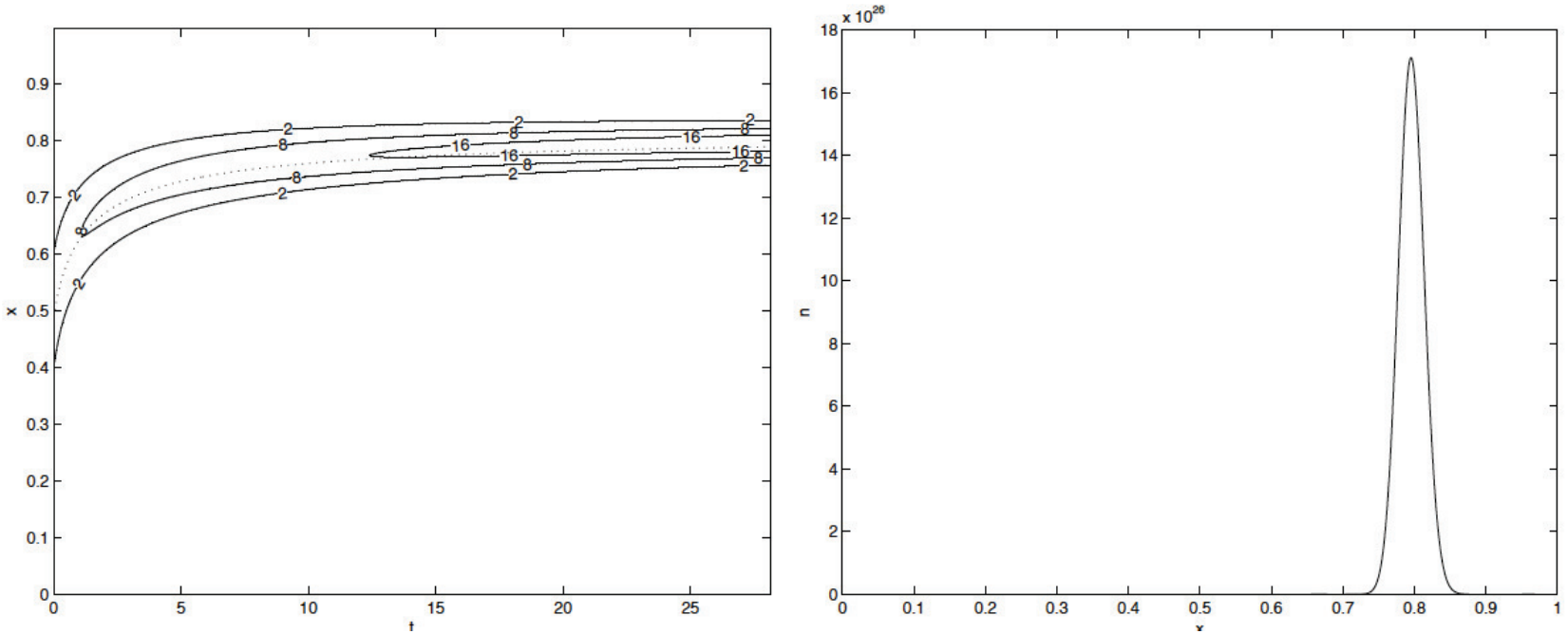


Left panel: starting from a medium phenotype $x = 0.5$, *level sets of a drug-sensitive population in the* (t, x) *plane. Right panel: asymptotic distribution of this drug-sensitive population according to the drug resistance phenotype* x.

(Lorz et al., M2AN 2013)

IModel with mutations, one cytotoxic drug: illustrations (2)

[Resistant cell population case: Gause's exclusion principle again]
Theorem: Monomorphic evolution towards drug-induced drug resistance, here with $\theta_C = 0$, $\mu_C(\cdot) > 0, r'(\cdot) < 0, \mu'_C(\cdot) < 0$ *(costly drug-induced resistance),* $u(t) = Cst$



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*.*

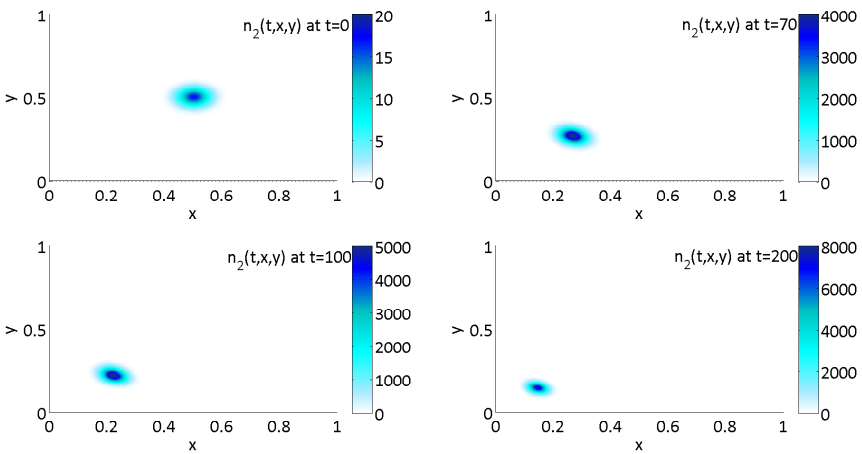
(Lorz et al., M2AN 2013)

IDE model, no mutations: phenotype-structured non-local Lotka-Volterra model with 2 drugs, cytotoxic $u_1(t)$, cytostatic $u_2(t)$, bidimensional resistance phenotype (x, y)

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

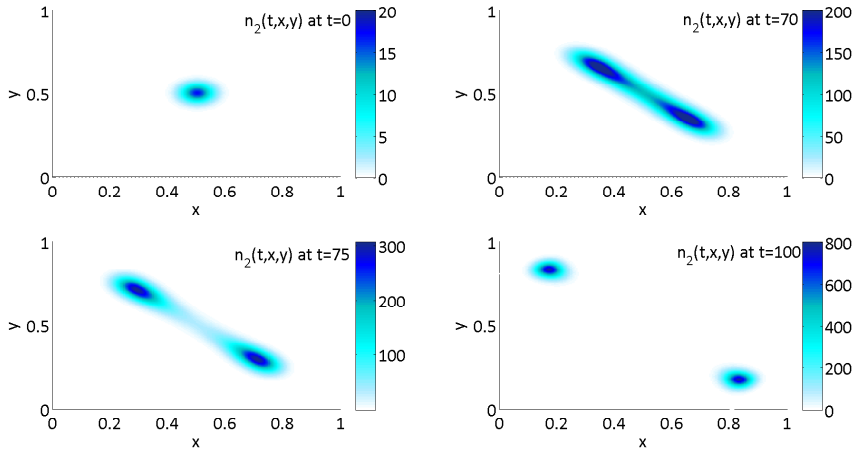
Environment: $I_C(t) = \alpha \int_0^1 \int_0^1 n_C(x,y,t)\, dx\, dy + \beta \int_0^1 \int_0^1 n_H(x,y,t)\, dx\, dy$

Sensitive cell population case:



Convergence toward total sensitivity

Resistant cell population case:



Convergence toward 2 resistant phenotypes

(Lorenzi & Lorz, unpublished)

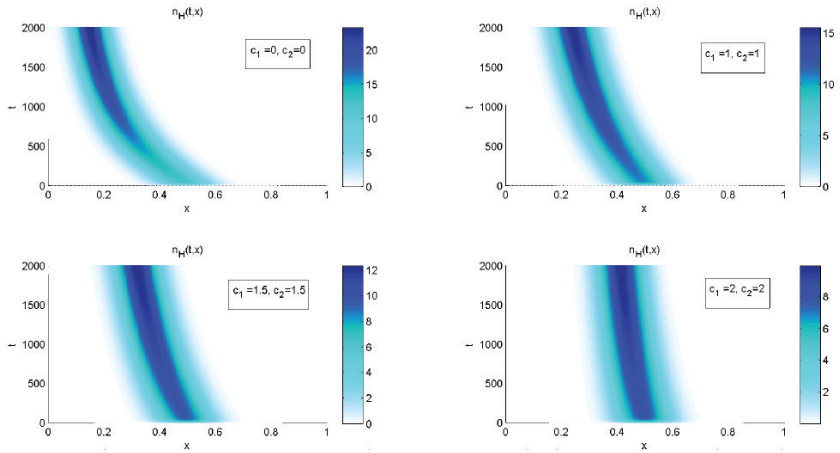
Same phenotype-structured non-local Lotka-Volterra model with 2 drugs and one (scalar) resistance phenotype x

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

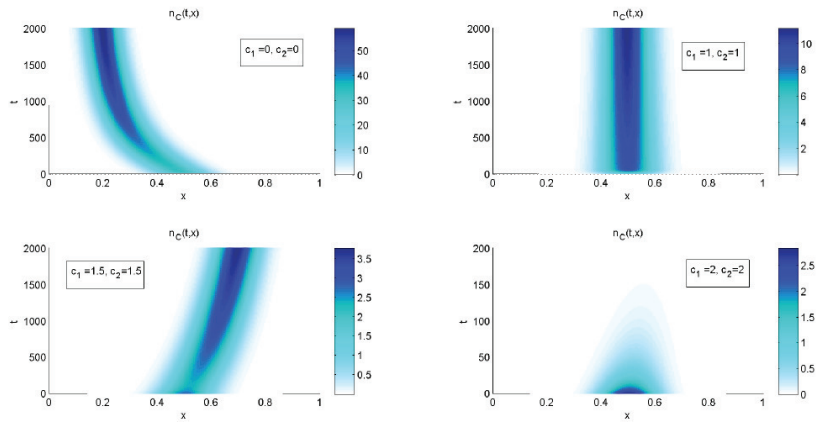
$$\frac{\partial}{\partial t} n_C(x,t) = \left[\frac{r_C(x)}{1 + k_C u_2(t)} - d_C(x) I_C(t) - u_1(t) \mu_C(x) \right] n_C(x,t)$$

Environment: $I_H(t) = a_{HH}.\rho_H(t) + a_{HC}.\rho_C(t)$, $I_C(t) = a_{CH}.\rho_H(t) + a_{CC}.\rho_C(t)$,
with $\rho_H(t) = \int_0^1 n_H(x,t)\,dx$, $\rho_C(t) = \int_0^1 n_C(x,t)\,dx$, u_1 cytotoxic, u_2 cytostatic drugs.

Simultaneous combinations of the 2 drugs, with increasing equal constant doses



Healthy cells: preserved



Cancer cells: eventually extinct

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), c_1 and c_2 in a 1D radially symmetric tumour spheroid ($r \in [0,1]$) is ruled by the following set of equations:

$$\partial_t n(t,r,x) = \left[\frac{p(x)}{1+\mu_2 c_2(t,r)} s(t,r) - d(x)\varrho(t,r) - \mu_1(x)c_1(t,r)\right] n(t,r,x), \quad (1)$$

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

$$-\sigma_c \Delta c_1(t,r) + \left[\gamma_c + \int_0^1 \mu_1(x)n(t,r,x)dx\right] c_1(t,r) = 0, \quad (3)$$

$$-\sigma_c \Delta c_2(t,r) + \left[\gamma_c + \mu_2 \int_0^1 n(t,r,x)dx\right] c_2(t,r) = 0, \quad (4)$$

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

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

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

$$c^1$$

Tumour spheroid: simulations with constant drug doses (1)

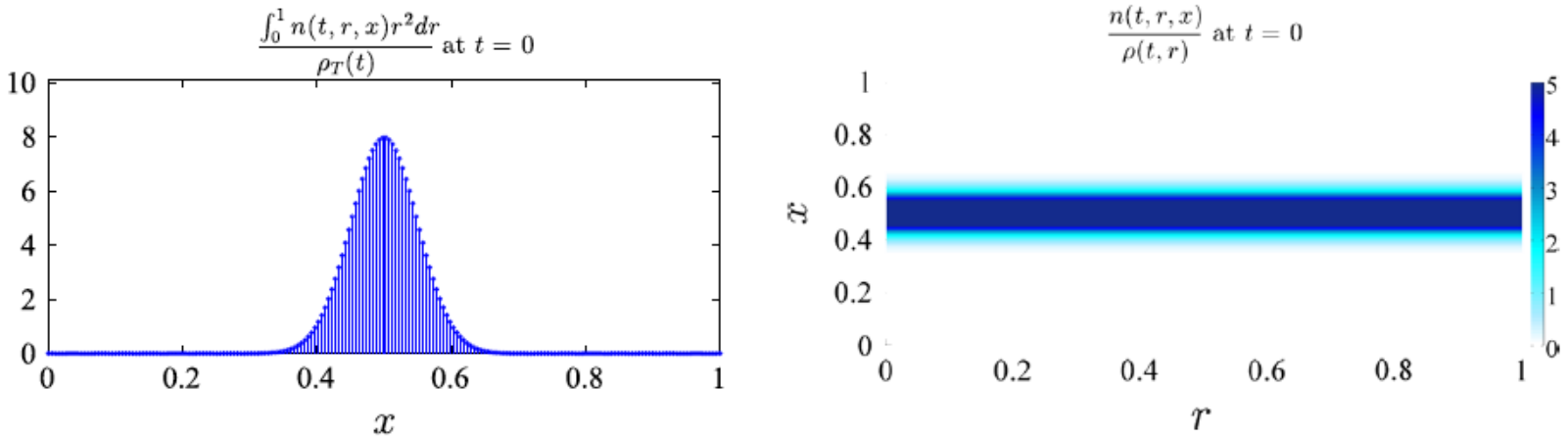
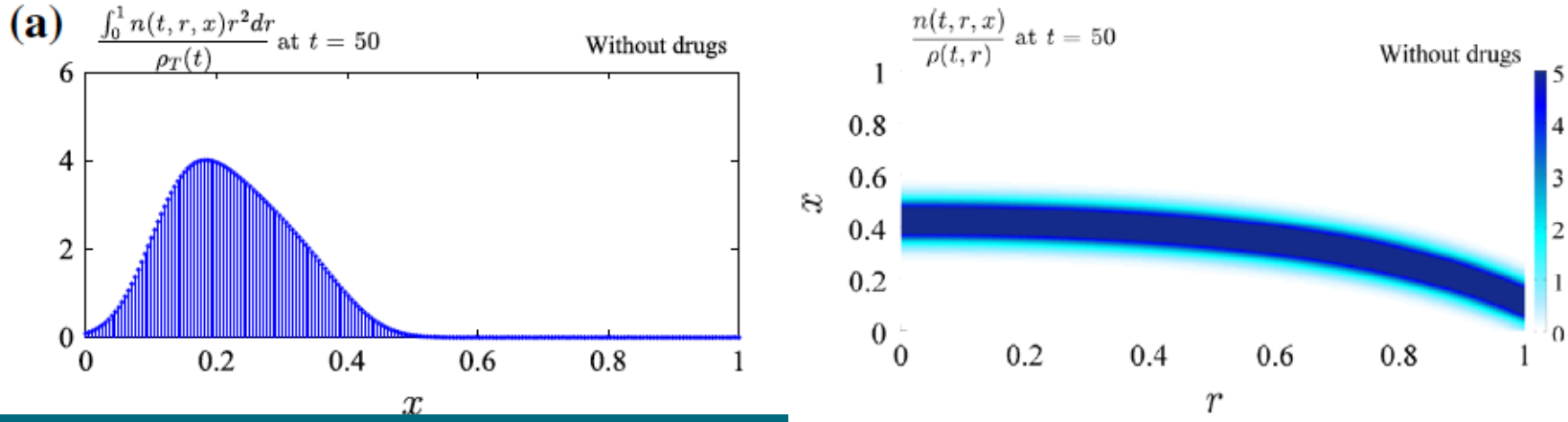
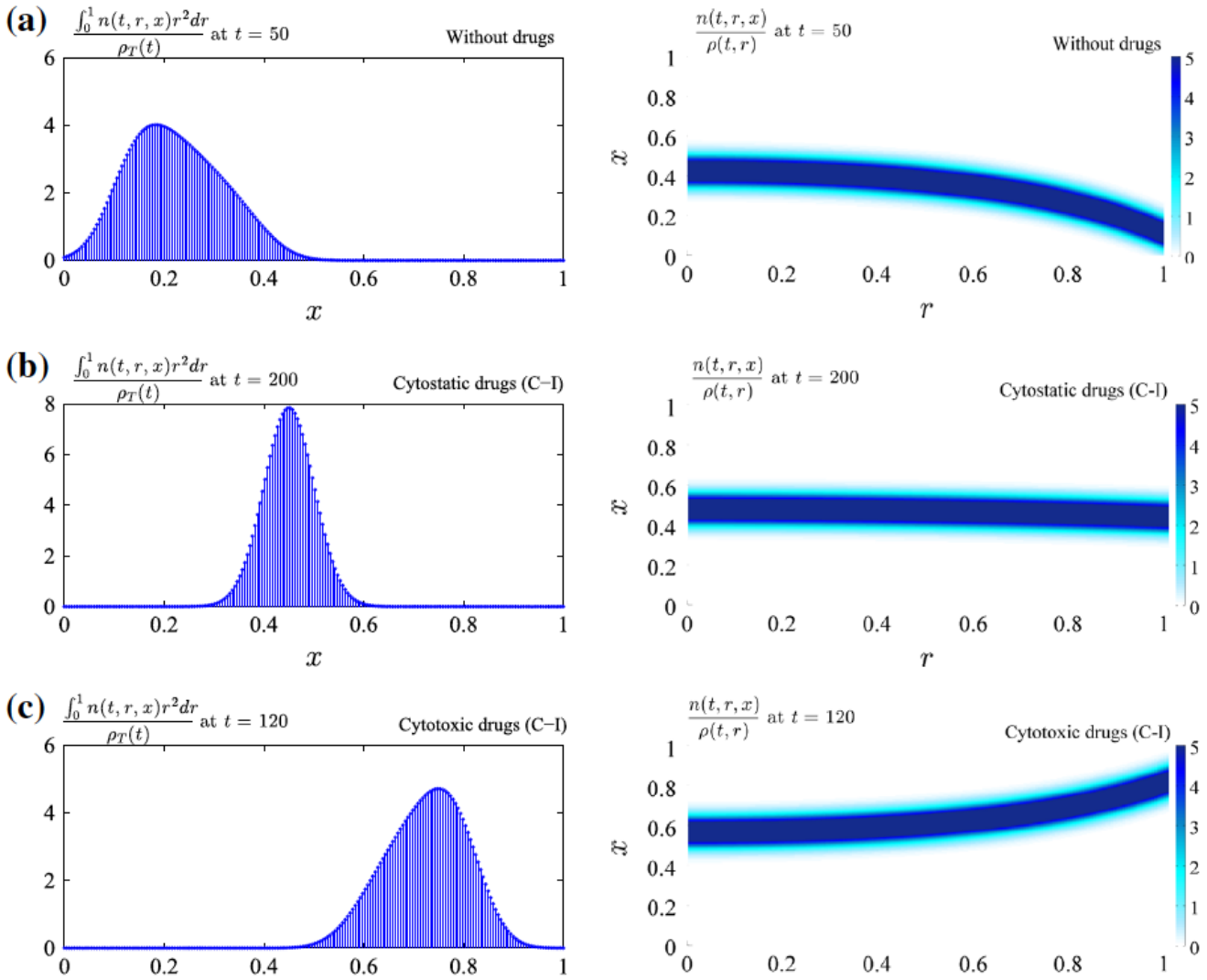


Fig. 1 Initial phenotypic distribution. Plots of $\int_0^1 n(t,r,x)r^2\mathrm{d}r/\rho_T(t)$ (*left panel*) and $n(t,r,x)/\rho(t,r)$ (*right panel*) at $t=0$. The initial cell population is almost monomorphic

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



Tumour spheroid: simulations with constant drug doses (2)



Cytotostatic c_2 has almost no effect / Cytotoxic c_1 clearly induces resistance

(Lorz et al. BMB 2015)

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

Therapeutic strategies c_1/c_2: Constant/Bang-bang vs. Bang-bang/Constant

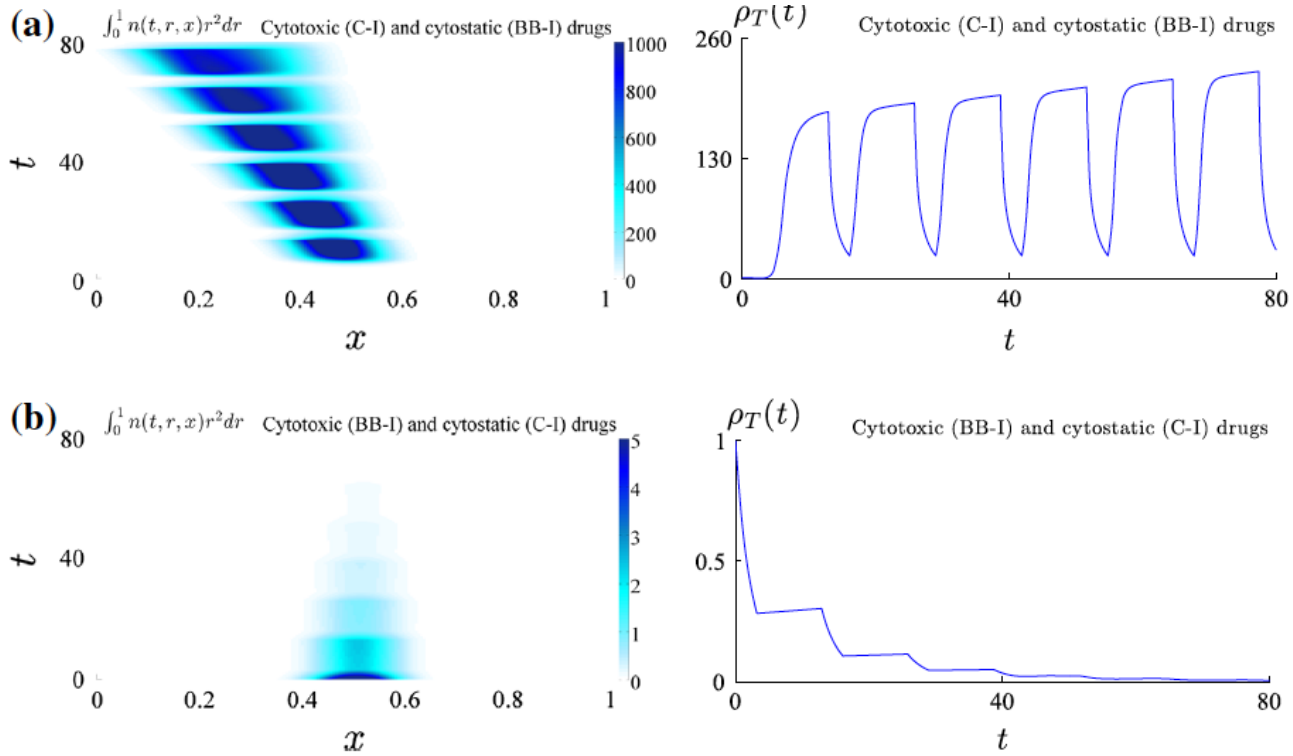


Fig. 11 **a** Cytotoxic (C-I) and cytostatic (BB-I) drugs. Plots of $\int_0^1 n(t, r, x)r^2\mathrm{d}r$ (*left panel*) and $\rho_T(t)$ (*right panel*). Bang–bang infusion of cytostatic drugs together with constant infusion of cytotoxic drugs weakly affects the dynamics of cancer cells by comparison with 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. BMB 2015)

"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,

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

  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...

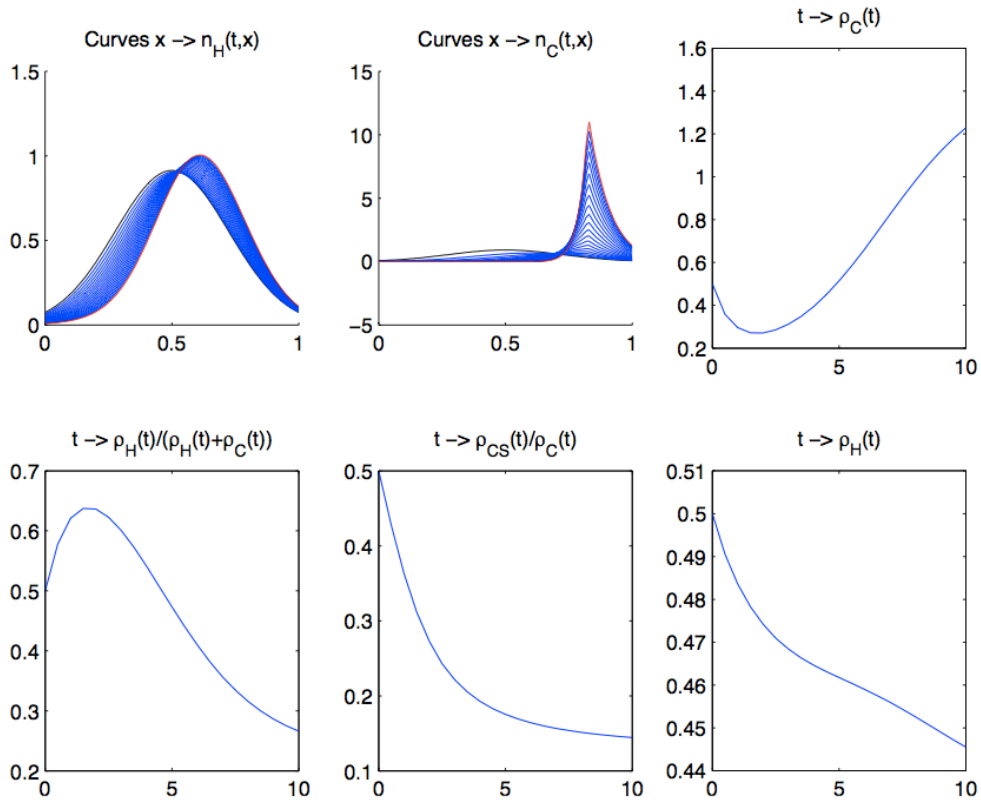
- ...whereas the cytotoxic drug u_1 *kills* the cells by increasing the death term, hence it is actually a direct life threat to the cell population, that 'defends itself' (biological bases under assessment...) by increasing its resistance phenotype x

- This resistance-inducing killing effect should be avoided as long as possible. In 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 an optimal control perspective thus far (however, see https://hal.inria.fr/hal-01302003v1).

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}.

Optimal control algorithms to improve drug delivery in cancer cell populations (with Emmanuel Trélat, LJLL)

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

$$0 \leq u_1(t) \leq u_1^{\max}, \qquad 0 \leq u_2(t) \leq u_2^{\max}, \tag{6}$$

and minimising the cost functional

$$C_T(u_1,u_2) = \int_0^1 n_C(x,T)\,dx + \gamma_1 \int_0^T u_1(t)\,dt + \gamma_2 \int_0^T u_2(t)\,dt, \tag{7}$$

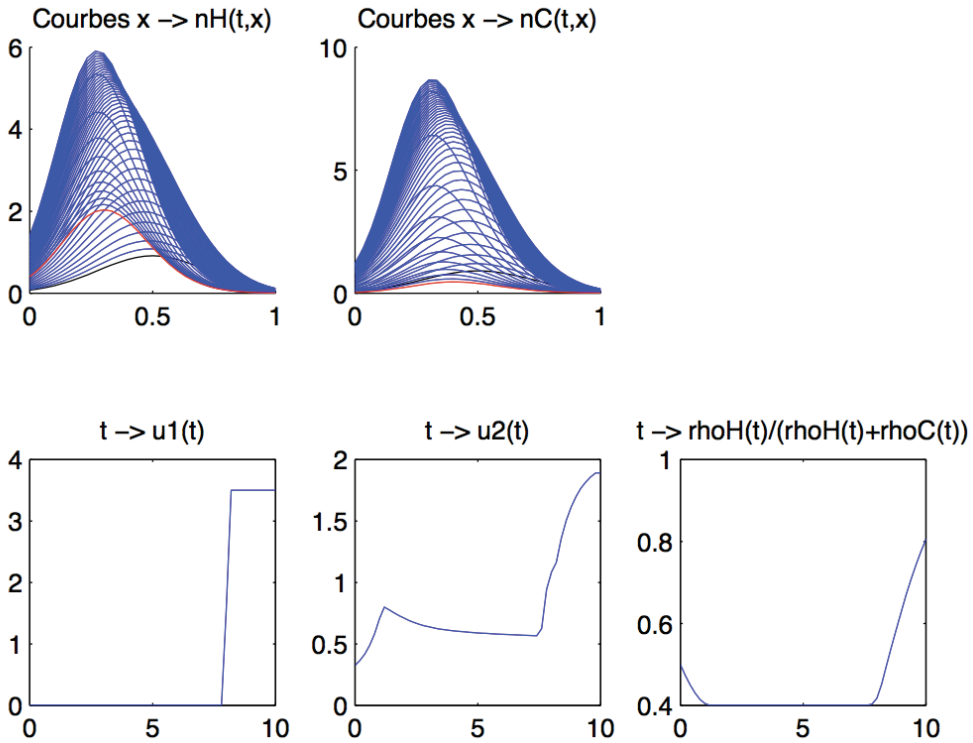
where $(n_C(\cdot,\cdot), n_H(\cdot,\cdot))$ is the unique solution of the system of PDEs corresponding to the controls u_1 and u_2, such that $n_H(0,\cdot) = n_H^0(\cdot)$ and $n_C(0,\cdot) = n_C^0(\cdot)$ and where the trajectory $t \mapsto (n_C(\cdot,t), n_H(\cdot,t))$ is subject to the dynamic state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

$$\frac{\rho_H(t)}{\rho_H(t)+\rho_C(t)} \geq \theta_{HC}. \tag{8}$$

(in simulations, e.g., $\theta_{HC} = 0.4$) We use a direct approach, discretising the whole problem and then solving the resulting constrained optimisation problem with AMPL (automatic differentiation) combined with IPOPT (expert optimisation routine)

Numerical solution to this first optimal control problem

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

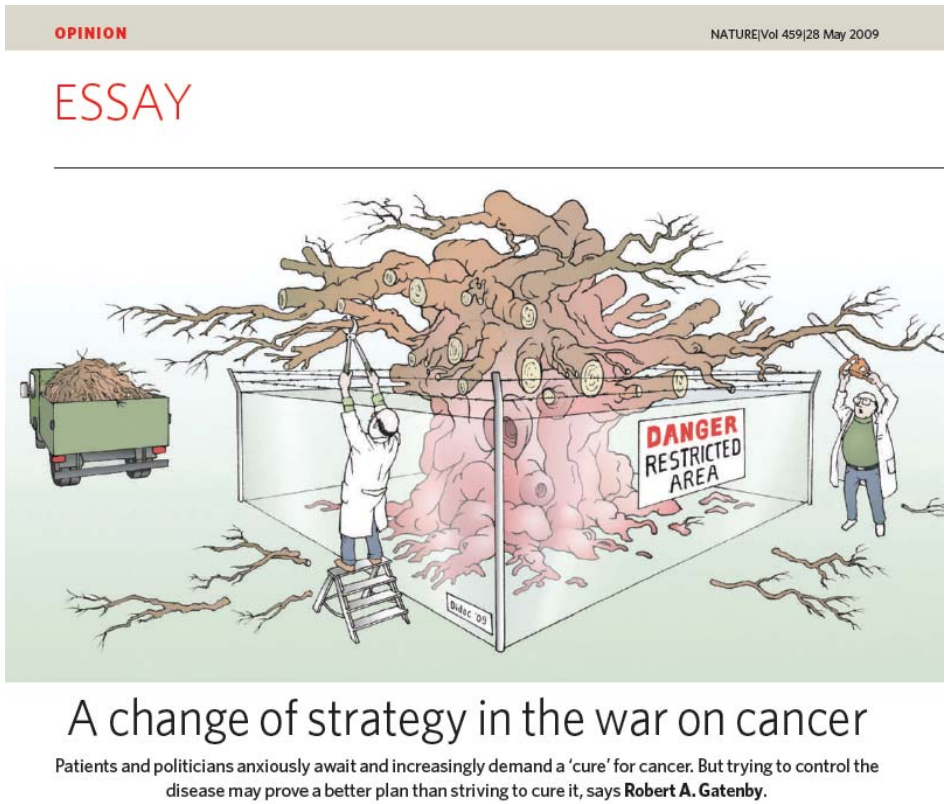


Left and centre panels: optimal drug flows for $u_1(t)$ (cytotoxic) and $u_2(t)$ (cytostatic)

Right panel: satisfaction of dynamic constraint

Introducing 'adaptive therapy', following Robert Gatenby

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



Second optimal control problem, same IDE model (1)

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

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

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

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

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

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

under the additional constraints

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

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

Second optimal control problem, same IDE model (2)

Note that we might add an "adaptive" constraint

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

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

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

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

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

However, such constraint is superfluous, as we will show - only numerically so far - that, likely due to phenotype concentration in the first phase of the optimal control, the ratio $t \mapsto \dfrac{\rho_{CS}(t)}{\rho_C(t)}$ is, *as long as* $u_1(t) = 0$, an increasing function of t without imposing this "adaptive" constraint. Nevertheless, note that when $u_1(t) > 0$, this is no longer granted, and resistance effects (evidenced on decreasing ρ_{CS}) always emerge.

Second optimal control problem: theoretical results

Theorem

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

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

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

   It leads to phenotype concentration and stationary values for cell populations

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

   for some constants ρ_H^∞ and ρ_C^∞, i.e., healthy and tumour cell populations are concentrated at some given respective phenotypes x_H^∞ and x_C^∞.
3. A transient **short-time arc**: $u_1 = u_1^{\max}$ followed by a terminal arc during which u_1 is slightly lower than $u_1^{\max}$ to ensure that the constraint $\rho_H(t) \geq \theta_H . \rho_H(0)$ is satisfied, and $u_2 = u_2^{\max}$ in the last two time intervals, during which the population of healthy and of tumour cells is very quickly decreasing.

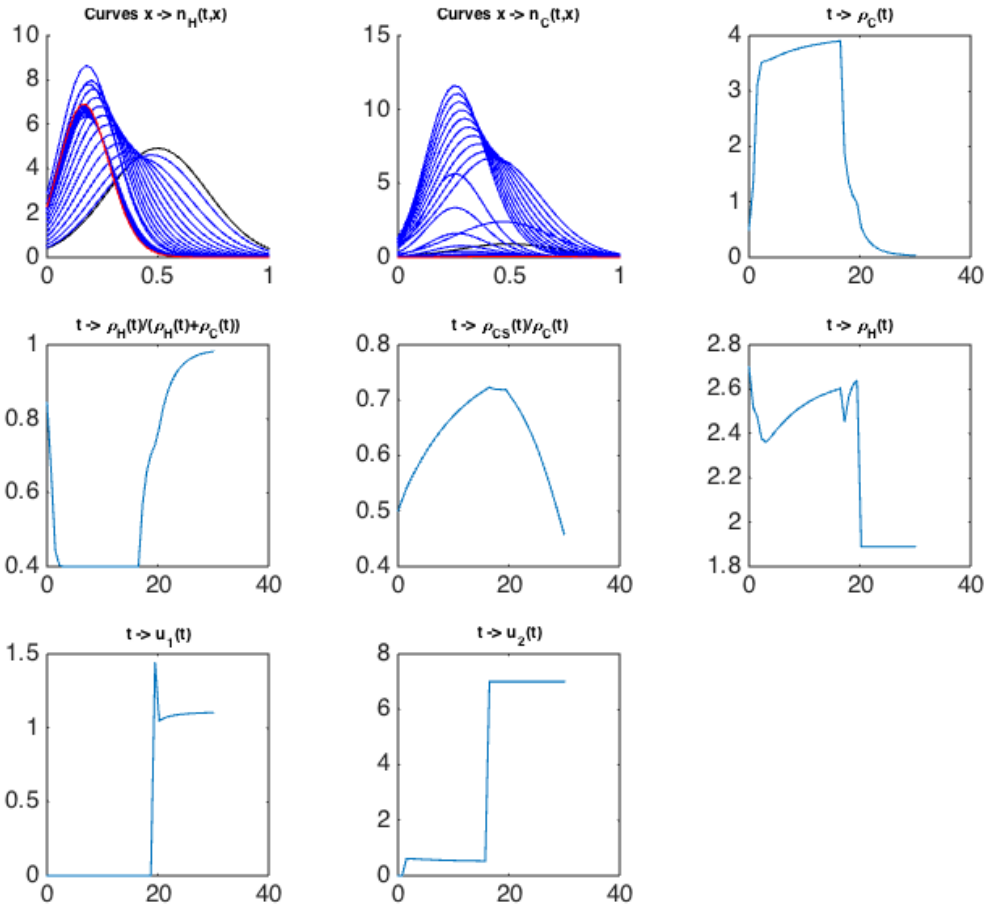
(Proof: Camille Pouchol's PhD thesis work)

Simulations illustrating this theorem

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

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

Note also that the treatment could be stopped at about $T_f = 50$ in this simulation, the last 10 days $(T - T_f)$ bringing nothing to the objective of miminising $\rho_C(T)$, except trouble, namely by triggering resistance in the few remaining resistant cancer cells, as can be seen on the curve $t \mapsto \frac{\rho_{CS}(t)}{\rho_C(t)}$.



Interpretation

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

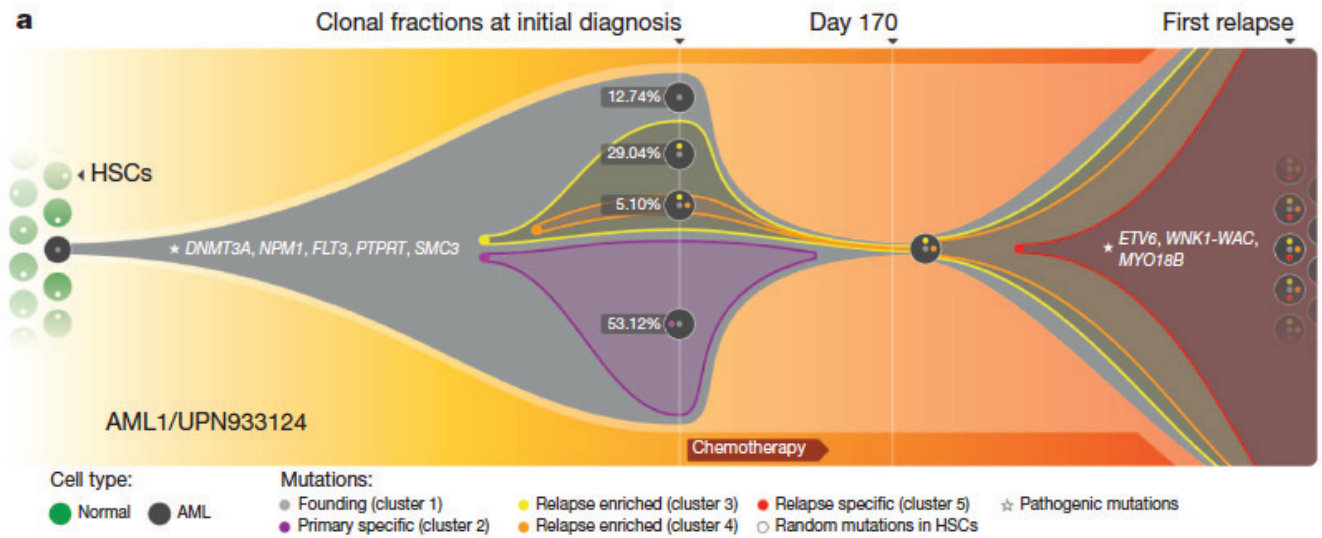
The second short-time phase is all the more efficient as the phenotypes are more concentrated (hence, as the time T is large).

We have two facts to prove: 1) convergence and concentration; 2) optimality of the concentrated state to start the final drug delivery phase. We shill prove the first fact, however the proof of the second fact is still elusive.

Looking for the proof of the theorem, beginning with the simpler case of constant controls, we investigated different tracks. The first attempt failed, but its main ingredients were used in the actual proof (with firstly constant, then piecewise constant controls), which relies on the design of a Lyapunov functional, making use of arguments taken from (*Jabin & Raoul, J Math Biol 2011*); work underway in Camille Pouchol's PhD thesis.

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

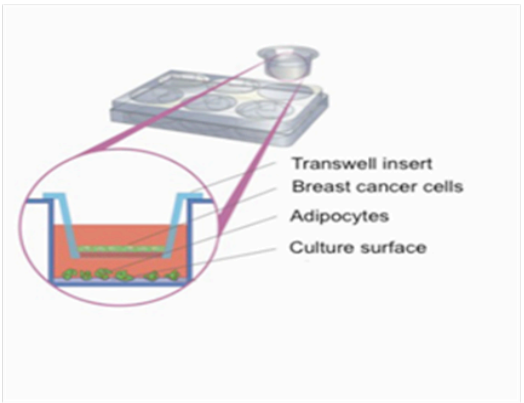
- The model assumes *one* trait of resistance corresponding to *one* cytotoxic drug.
- However, overcoming resistance using such strategy may not be successful if too many types of resistance coexist, due to high phenotype heterogeneity.
- Phenotype heterogeneity (e.g., multiclonality) within the tumour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

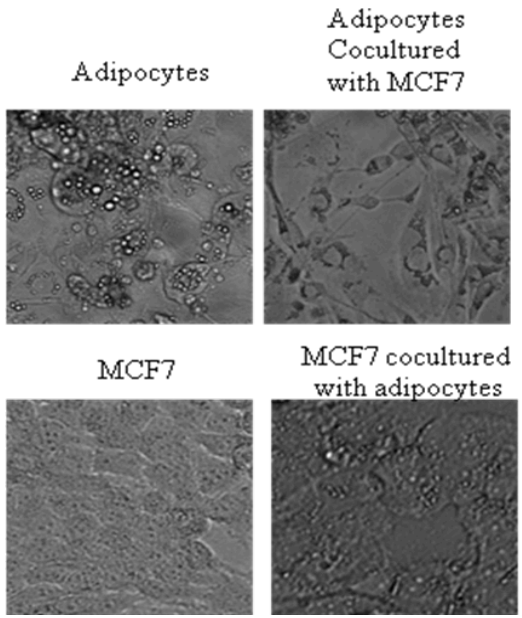


(AML relapse, cf. *Ding et al. Nature 2012*)

Extensions of the IDE model: tumour micro-environment

1) Breast cancer cell line MCF7 co-cultured with adipocytes





Control by drugs: cytostatic $v_r(t)$, cytotoxic $v_d(t)$,
plus blockade of receptors to intercellular soluble factors $\varphi_A(t), \varphi_C(t)$ by other drugs,
e.g., oestrogen receptor blockers $w_{sC}(t)$, antiinflammatory molecules $w_{sA}(t)$

$$\frac{\partial}{\partial t} n_C(u,t) = \left[\frac{r_C}{1+v_r(t)} + \varphi_A(t) \frac{s_C(u)}{1+w_{sC}(t)} - (1+v_d(t)) d_C(u) \rho_C(t) \right] n_C(u,t),$$

$$\frac{\partial}{\partial t} n_A(x,t) = \left[r_A + \varphi_C(t) \frac{s_A(x)}{1+w_{sA}(t)} - d_A \rho_A(t) \right] n_A(x,t).$$

(Camille Pouchol's PhD thesis 2015-. . .)

Extensions of the IDE model: tumour micro-environment

2) Haematopoietic stem cells interacting with support stromal cells (no drugs so far)
Model of mutualistic interactions between the two cell populations:

$$\frac{\partial h(t,u)}{\partial t} = \left\{ \alpha(u)(1 - \frac{\varrho(t)+\sigma(t)}{K}) + \gamma(u)\Sigma(t) \right\} h(t,u) \; + D\frac{\partial^2 h(t,u)}{\partial u^2},$$

$$\frac{\partial s(t,v)}{\partial t} = \left\{ \beta(v)(1 - \frac{\varrho(t)+\sigma(t)}{L}) + \delta(v)P(t) \right\} s(t,v) \; + E\frac{\partial^2 s(t,v)}{\partial v^2},$$

$h(t,u) :=$ haematopoietic stem/progenitor cells (HSPCs) of plasticity phenotype u
$s(t,v) :=$ stromal cells in the bone marrow of supporting capacity phenotype v,
$\varrho(t) = \int_0^1 h(t,u)\, du$ / $\sigma(t) = \int_0^1 s(t,v)\, dv$ total HSPCs/stromal cells.

Mutualistic terms: $P(t) = \int_0^1 \varphi(u)h(t,u)\, du$ and $\Sigma(t) = \int_0^1 \psi(v)s(t,v)\, dv$

Sensitivities $\gamma(u)$ and $\delta(v)$ quantify interaction strength in the target populations: $\gamma(u)$ for the sensitivity of haematopoietic cells to messages from stromal cells, and $\delta(v)$ for the other way round; parameters D and E quantify non-genetic instability of the cell populations w.r.t. phenotypes u and v.

... Model to be identified after dynamic recordings from genomic expression data??

Going forward, from local to global: models of cancer cell population dynamics from genome samples in single cells?

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

- A (time) dynamic deterministic structured model of cell population behaviour with phenotype variability and evolutionary (relevant trait) dynamics; we have experience of such PDE models: transport, reaction-diffusion, integro-differential

- Intracellular molecular deterministic models for the concentration of relevant mRNAs and proteins to determine cell fates, e.g., of nodal antagonist pairs X,Y such as transcription factors PU.1/GATA1 for the choice of myeloid vs. erythroid lineages in HSCs: relatively easy to design and classic by sets of ODEs

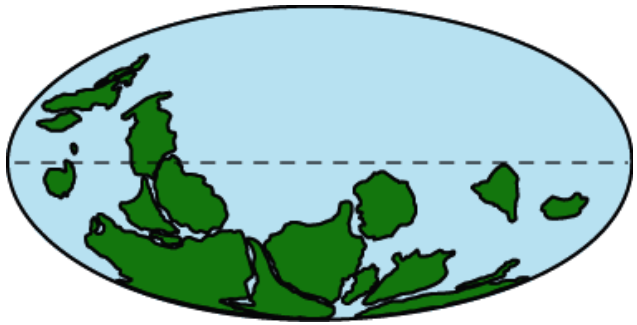
- For each antagonism, a stochastic process Z at the gene expression level, where would lie the (epigenetic?? TET2, etc.) source of phenotypic heterogeneity, randomly determining ODE parameters and whose parameters would themselves depend on tissue environment variables

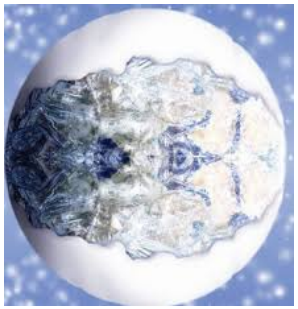
- Upscaling principles to integrate models from cell ODEs to tissue physiologically structured PDEs, making phenotype signatures from single cell genome samples

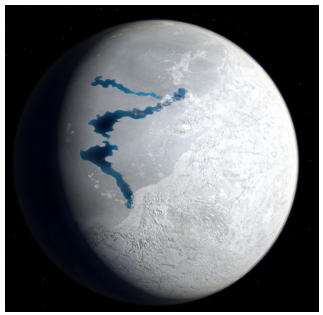
- Environment variables would result from integration, at the tissue level, of such "readouts" from single cell characteristics; their concentrations would determine phenotypes in cell populations; see e.g., Friedman et al. J Diff Eq 2009, 2012

From local to global, and back: a geophysical metaphor

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



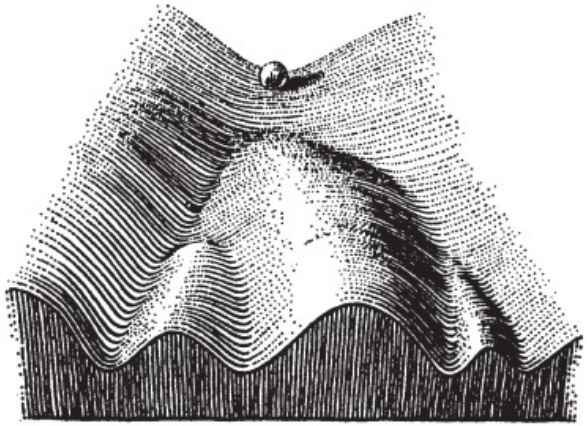




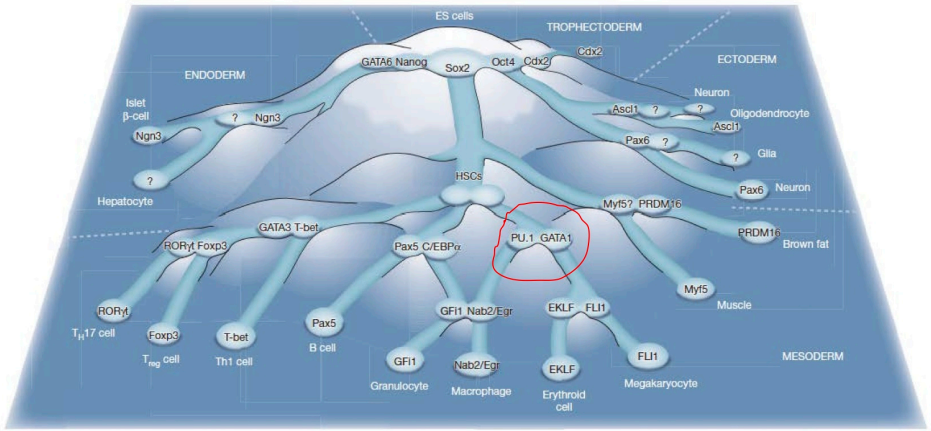
[NB: Stable equilibrium? (Budyko 1969)... yes, but only if one does not take into account volcanic activity, that can pierce the ice crust and release enormous quantities of gases (CO_2, CH_4), contributing to re-establishing the greenhouse effect, which actually happened (ice melt -635 My?) and led to the Cambrian multicellular explosion 540 million years ago, from which we were all begotten]

Global and local, from Graf & Enver, Nature 2009

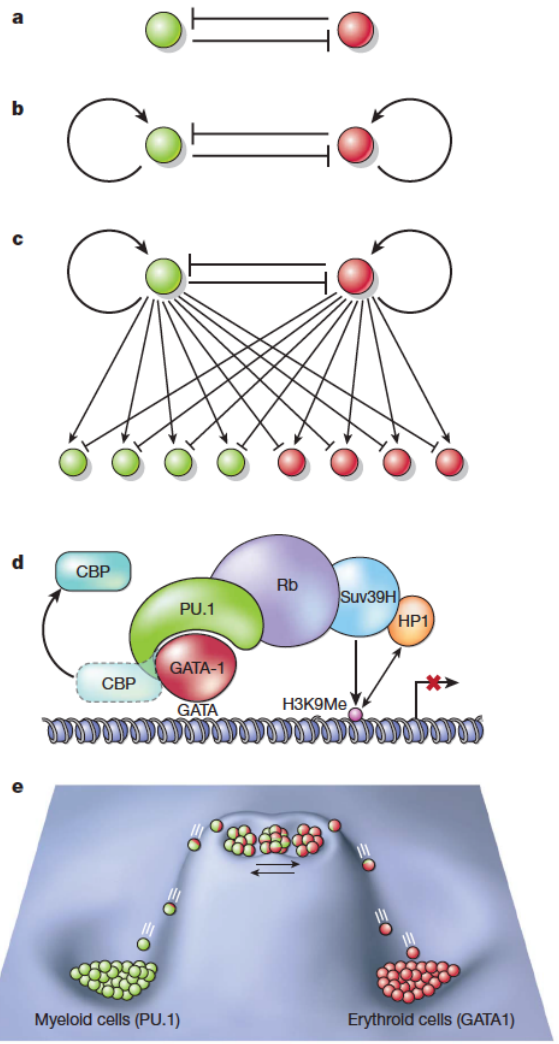
In one multicellular organism:



[Classic Waddington landscape]



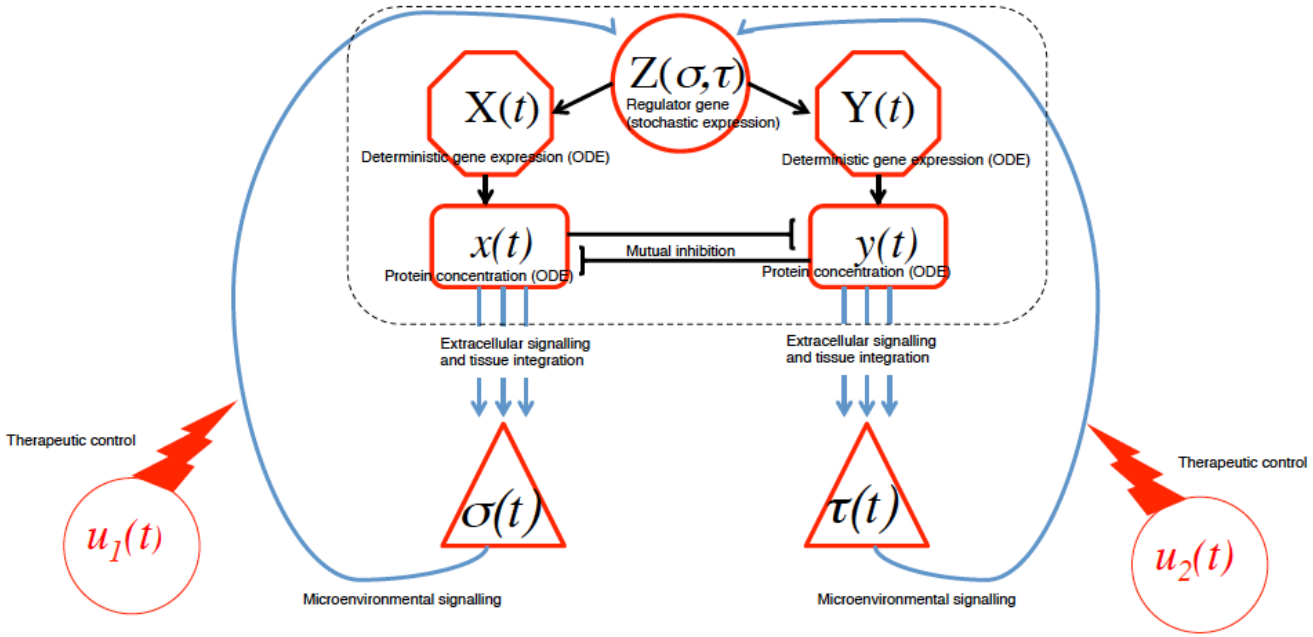
Stem cell fate: modern version by Tariq Enver (from ASH meeting 2011)



Zoom on the PU.1/GATA1 node:
Choice between red/white lineages

Sketched candidate model for 2 antagonistic genes X, Y

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



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

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

Possible candidate equations for the dynamics of the model

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

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

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

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

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

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

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

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

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

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

Other extensions: dealing with the immune response

- Remarkable recent and longlasting therapeutic results have been obtained in various cancers by using immune checkpoint inhibitors (anti-CTLA-4, anti-PD1, anti-PDL1), monoclonal antibodies that *inhibit inhibition* of immune effector cells, see, e.g., Naidoo *et al.* in Br J Cancer 2014

- However, remarkable though they are, these results remain limited, long survivors (18 months) in melanoma passing from 0 to 25-40 % in the best cases (Nivolumab in melanoma without *BRAF* mutation, *C. Robert NEJM 2015*)

- Using chemotherapies to decrease cancer cell populations, not to eradicate them, but to make them amenable to be kept in check by the immune system, raises reasonable hopes to increase these (already remarkable) results

- This calls for models of the immune response in cancer to optimise cancer treatments by combining chemo- and immunotherapies...

- ... Keeping in mind the urge by Charles Lineweaver, Paul Davies and Mark Vincent (Bioessays 2014) to *target cancer's weaknesses (not its strengths)* by triggering the adaptive immune response (William Coley revisited)

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