Drug resistance in cancer I: An evolutionary perspective on cancer, with applications to drug resistance modelling

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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]

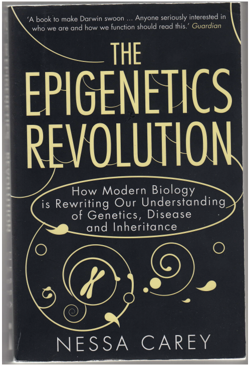
- **[Genetic] mutation**: irreversible modification of the genome (cf. Evolution)

- **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

Drug resistance: a genetic or epigenetic phenomenon?

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

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



Drug resistance: a phenomenon common to various therapeutic situations

- In therapeutic situations where an external pathogenic agent is proliferating at the expense of the resources of an organism: antibiotherapy, virology, parasitology, target populations are able to develop drug resistance mechanisms (e.g., expression of β-lactamase in bacteria submitted to amoxicillin).
- In cancer, there is no external pathogenic agent (even though one may have favoured the disease) and the target cell populations share much of their genome with the host healthy cell population, making overexpression of natural defence phenomena easy (e.g., ABC transporters in cancer cells).
- Drug resistance may account for unexpected failures in targeted therapies.

Drug resistance: how does it work?

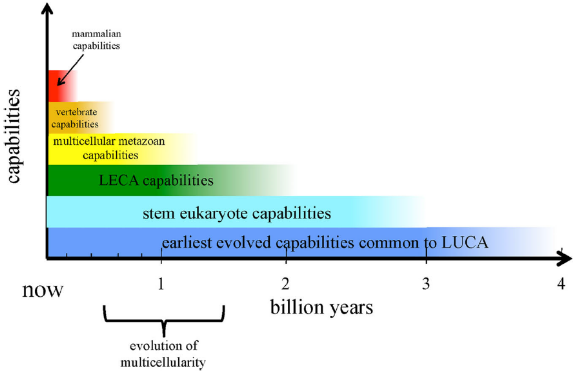
- What was formerly assumed: 0-1 expression of genes (e.g., functional or inefficient p53 due to a mutation)
- Varying expressivity of genes in a cell population, or else degree of effectiveness of mutations (e.g., mutated EGFR)
- Varying activity of ABC transporters (e.g., P-gp), main effectors of drug efflux out of cells
- Darwinian effects of drug pressure selecting subpopulations in a heterogeneously constituted (by stochastic variations: bet hedging?) cell population
- *Transient adaptation to hostile 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

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



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

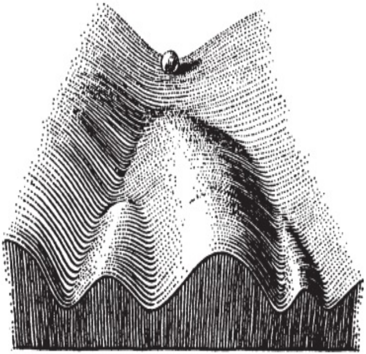
References: *Israel JTB 1996, Davies & Lineweaver Phys Biol 2011, Vincent Bioessays 2011, Lineweaver, Davies & Vincent Bioessays 2014, Chen et al. Nature Comm 2015*

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

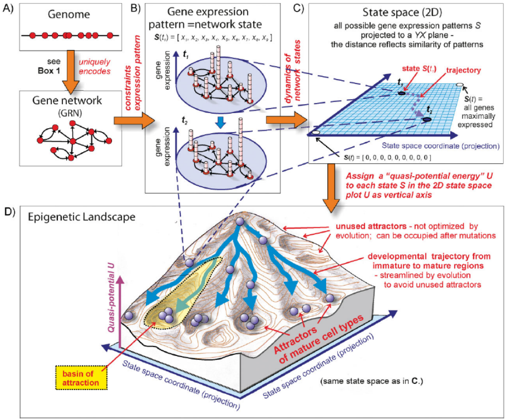
- According to the atavistic hypothesis, cancer is a ‘backward evolution’ from a sophisticated form of multicellularity (us), in which epigenetic processes control gene regulatory networks of transcription factors: differentiation factors, p53, etc., that physiologically control the basis of cellular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

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)



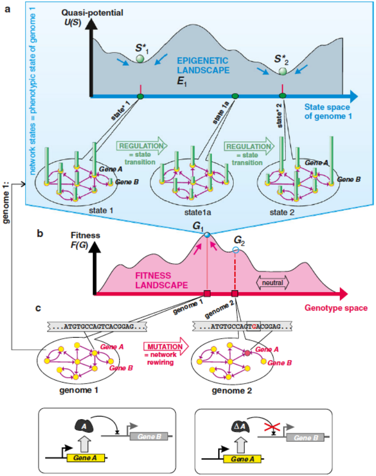
"Nothing in evolution makes sense except in the light of *systems* biology" (S. Huang, 2012)

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*)

Can resistance be assessed by biological experiments? (1)

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

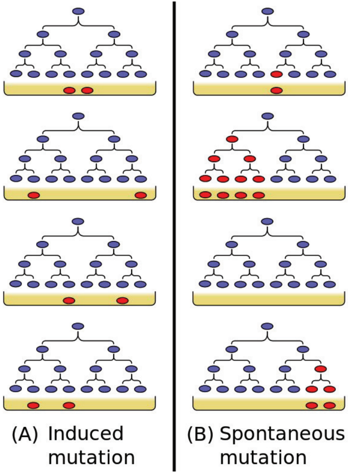
Different Petri dishes, same experimental settings

Bacterial populations firstly proliferating freely, then submitted 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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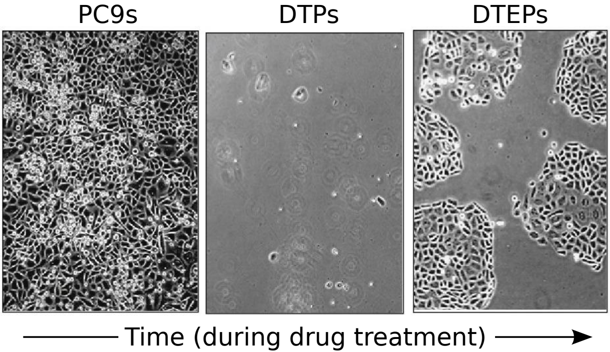
DOI 10.1016/j.cell.2010.02.027

- 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 submitted 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

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

- (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

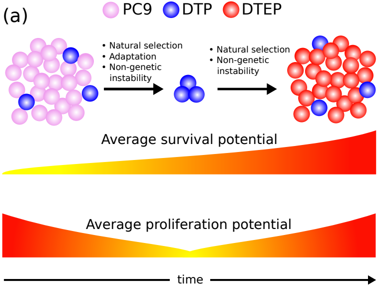
$$\frac{\partial n}{\partial t}(x,y,t) + \underbrace{\frac{\partial}{\partial y}\Big(v(x,c(t);\bar{v})n(x,y,t)\Big)}_{\text{Stress-induced adaptation of the proliferation level}} =$$

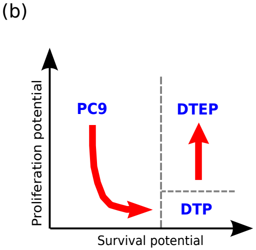
$$\underbrace{\Big[p(x,y,\varrho(t)) - d(x,c(t))\Big]n(x,y,t)}_{\text{Non local Lotka-Volterra selection}} + \underbrace{\beta\Delta n(x,y,t).}_{\text{Non-genetic phenotype instability}}$$

- The global population term $\varrho(t) = \int_0^1 \int_0^1 n(x,y,t)\, dx\, dy$ occurs in p as a logistic environment limiting term (availability of space, nutrients)
- 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

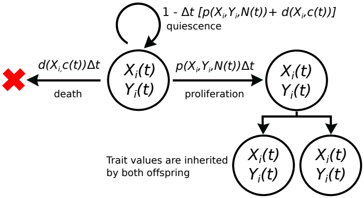
(*Chisholm et al., Cancer Research 2015*)

Agent-based model





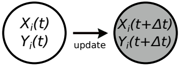
(a) Each cell i undergoes either proliferation, death or remains quiescent:



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

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

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

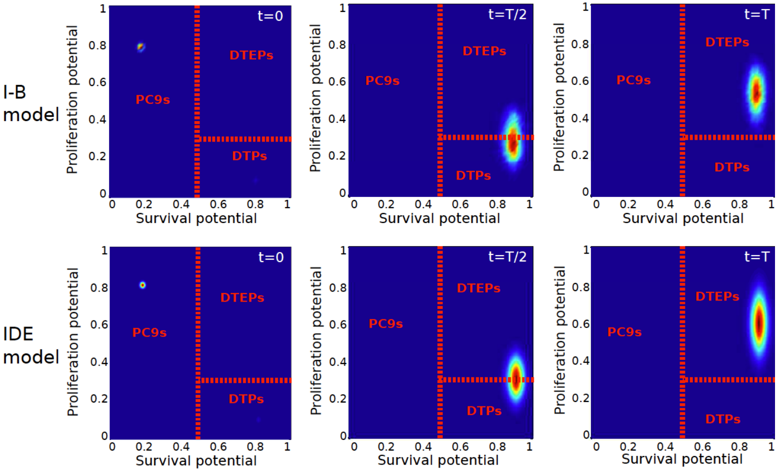


$D=\sqrt{2\beta}$: *non-genetic instability;* v: *stress-induced adaptation of proliferation level*

(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)

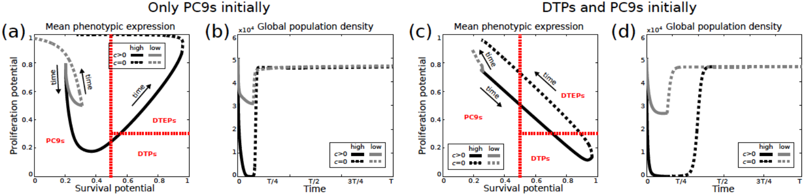


T is the simulation end-time: $0 \leq t \leq T$

(*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)

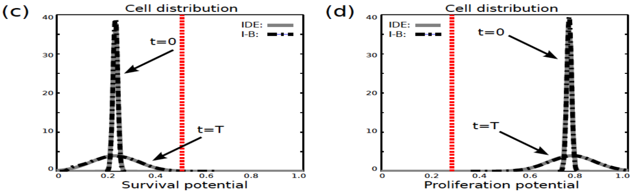


(a), (b) Only PC9s initially, adaptation on ($v \neq 0$): *'Lamarckian' scenario*, or Luria-Delbrück scenario (A)

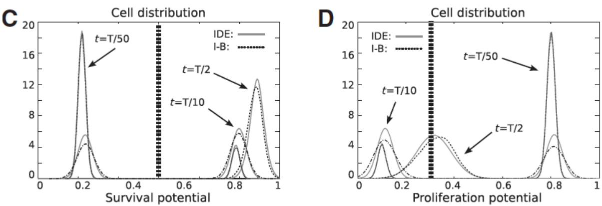
(c), (d) PC9s and DTPs initially, no adaptation ($v = 0$): *'Darwinian' scenario*, or Luria-Delbrück scenario (B)

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

Phenotype heterogeneity in the cancer cell population

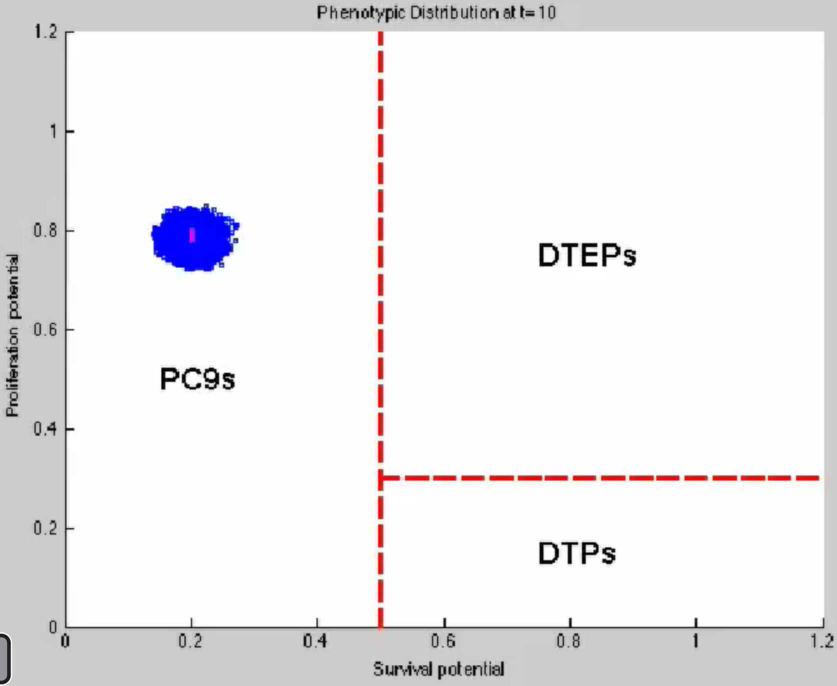


The PC9 cell population becomes more heterogeneous when it is left to evolve in the absence of drug treatment: starting from an initial concentrated phenotype (x_0, y_0), the phenotype (x, y) diffuses in the population according to a Gaussian-like curve. (c) Projection onto the x phenotype axis; (d)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

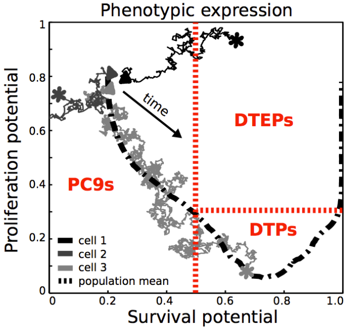


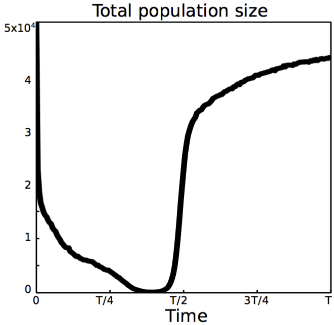
C, **D**: Under drug treatment, heterogeneity persists when phenotypes evolve (here, Darwinian scenario: DTPs are initially present)

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


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

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?

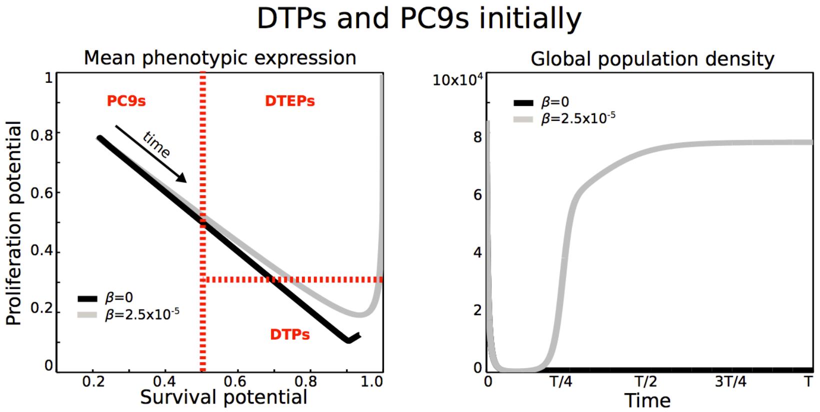
Consider $c(\cdot) = constant$ and two scenarios:

(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]

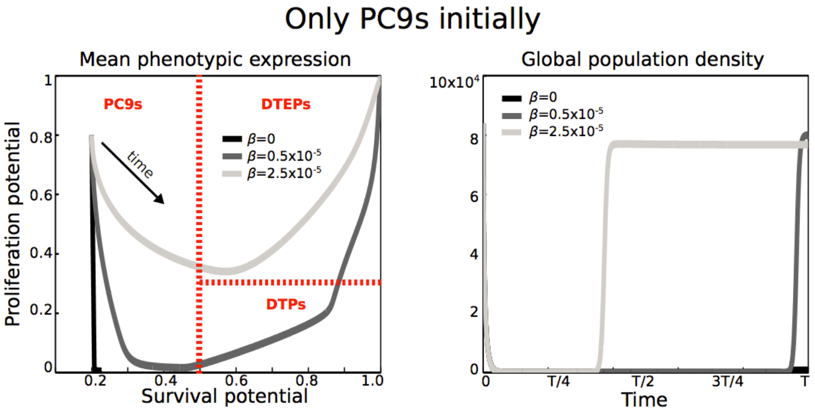


Extinction when $\beta = 0$ (here, adaptation is absent $v = 0$)

(*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?

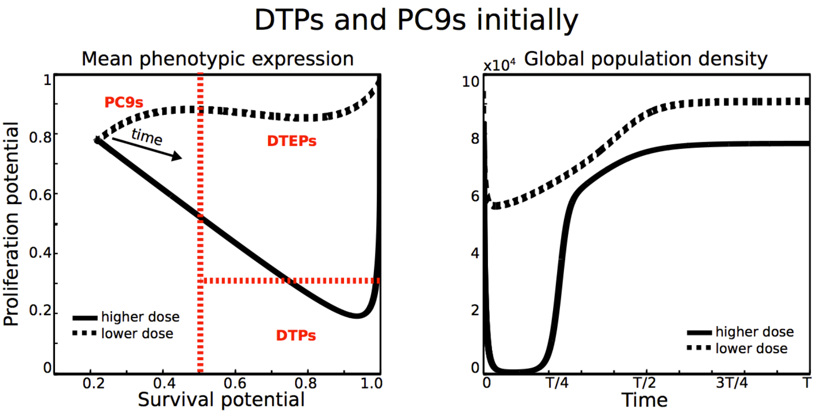
Definition (LC_γ dose)

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]

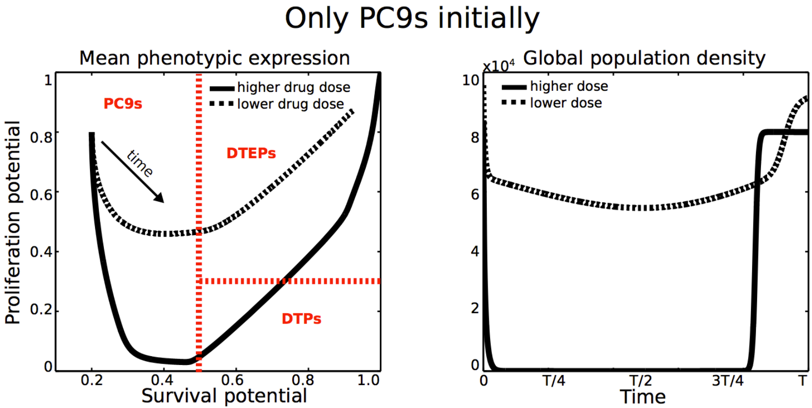


Low drug dose does not let appear DTPs (here, adaptation is absent $v = 0$)

(*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,

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

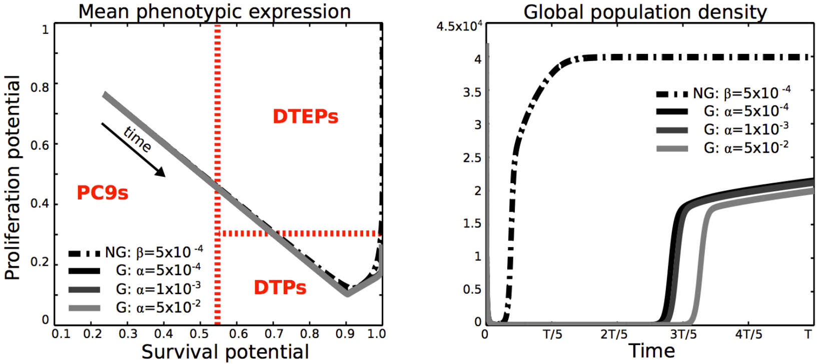
and C_M is a normalisation constant such that

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

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



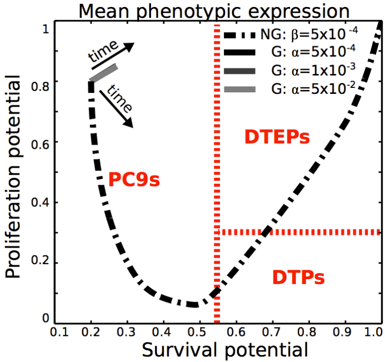
G: mutations do not let occur total recovery (NG: here, adaptation is absent $v = 0$)

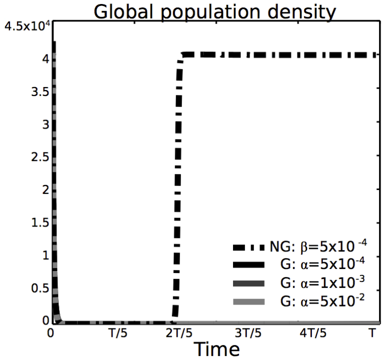
(*Chisholm et al., Cancer Research 2015*)

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

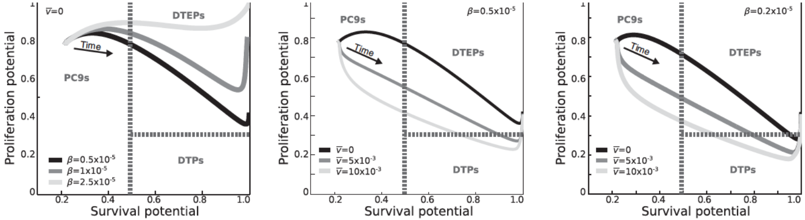
- Therapeutic consequences? Not clear yet. Epigenetic drugs? Not many of them exist (in particular no KDM5A inhibitor in the clinic). Acting on epigenetics by modifying metabolism? Combining cytotoxic (inducing drug resistance) drugs and cytostatic drugs at low doses (in principle not inducing drug resistance)? Might be assessed using this model, so far done only on a simplified model (see part II).

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)

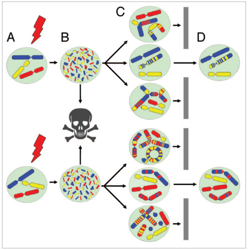


(Chisholm et al., Cancer Research 2015)

Tracks to biologically interpret adaptation to stress ($\bar{v}$)

- 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 (chromatin rearrangements, i.e., nuclear reprogramming, no gene recombinations).

(*Cartoon from Liu & Heng 2014*)



- Rather than an elusive "survival potential", could we consider that *plasticity*, i.e., the power for a cell to change its phenotype by, e.g., quick demethylation, otherwise said at the molecular level, (since methylations fix phenotypes) a high cell load in demethylases (KDM5A in the Sharma *et al.* experiment), as the main determinant structure variable, that is responsible for stress adaptation?

- Is there a succession of events from a population dynamics point of view between an epigenetic, reversible, state of drug resistance, followed by a possibly acquired, genetic, unbeatable state of resistance to a given drug? Is there a way to measure in a molecular way a *cost of resistance*, so as to design realistic model cost functions to represent the balance bertween proliferation and survival (plasticity) at the cell population level?

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- ...See also `https://www.rocq.inria.fr/bang/JC/JCarticles.html`