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Mathematical modelling of cancer growth and drug treatments: taking into account cell population heterogeneity and plasticity.
Part II: fundamentals on cancer and models

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Joint work at LJLL with Frank Ernesto Alvarez Borges, Rebecca Chisholm, Tommaso Lorenzi, Benoît Perthame, Camille Pouchol and Emmanuel Trélat

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Plan of the talk

1. Generalities on cancer and cancer studies
2. Cell plasticity in multicellular development and in cancer
3. Modelling reversible drug tolerance in cancer by structured equations
4. Theoretical therapeutics: drug resistance and unwanted side effects
5. Multicellularity and atavism in health and in cancer

Cancer puzzle: beyond intracellular signalling pathways

- Cancer is a disease of multicellular animals, characterised by loss of control on differentiations and on proliferation of cells and tissues, in solid tumours or in malignant haemopathies, resulting in tumoral incoherent tissue organisation.

- Two complementary ways of approaching cancer: at the tissue and organism level, cell population dynamics, with continuous deterministic equations, vs. at the individual cell level, investigating mutations in genes and gene regulatory networks (GRNs), in molecular biology settings, using probabilistic methods.

- Proliferation at the tissue level relies at the cell level on the cell division cycle: one cell becomes two, a process determined by gene expression and resource availability, approached both by individual cell and cell population studies.

- Differentiation of cells at the tissue level of an animal, into the different mature cell types arising from the original egg by successive changes of phenotypes, is physiologically controlled at the gene level by epigenetic enzymes that regulate gene expression, and that may themselves be altered in cancer. They may be represented in cell population dynamics by phenotype-structured equations.

- Cancer studies are thus concerned both by *irreversible mutations of genes* themselves (on A,T,G,C beads) and by fast, frequent, *reversible epigenetic modifications* (aka *epimutations*) of the expression of genes, that only change phenotypes of the adaptable, plastic, cancer cells, in which differentiations are out of control by the organism.

A few definitions for cell populations

- **[Genetic] mutations**: irreversible modifications of the genome at the cell level, including nucleotide (A,T,G,C) substitutions, deletions, translocations..., i.e., irreversible modifications of the genome 'written in the ATGC sequence code'.

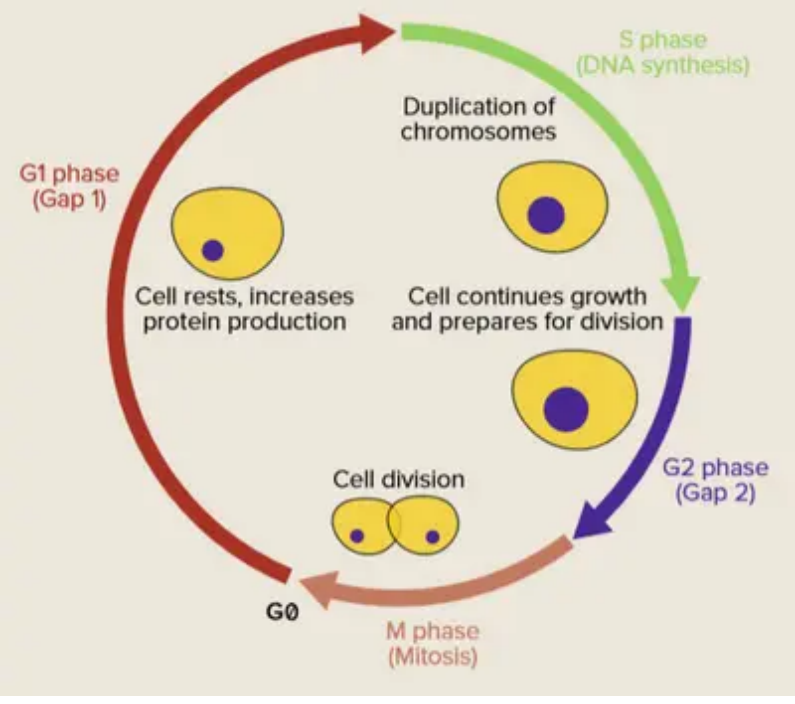
- **Evolution in the Darwinian sense**: constitution of a new cell population by irreversible *genetic mutations*, resulting in new phenotypes in the cell population.

- **Adaptation of cells**: modification by *differentiations* of an isogenic cell population into another one, resulting in a new phenotype in the cell population, but reversible, i.e., amenable to complete restitution of the initial phenotype, with preservation of the genome (= of the ATGC code); here adaptation is taken in a sense different from *adaptation of species* in Darwinian evolution of species.

- **Epigenetic modifications** = 'epimutations': modifications at the cell level of the phenotype due to mechanisms (activation of *epigenetic enzymes*) that do not affect the genetic sequence code. They are changes in the chemical structure of DNA that do not change the DNA sequence, but silence (or enhance) the expression of genes by DNA methylation and histone methylation or acetylation.

- **Cell differentiations** are changes of phenotypes in cells, resulting from epimutations, by silencing or enhancing gene expression without mutations. *Stem cells* are those cells that initiate in a *stem cell niche* the sequence of differentiations of a lineage, in a tissue of the organism, e.g., in haematopoiesis (blood is a tissue).

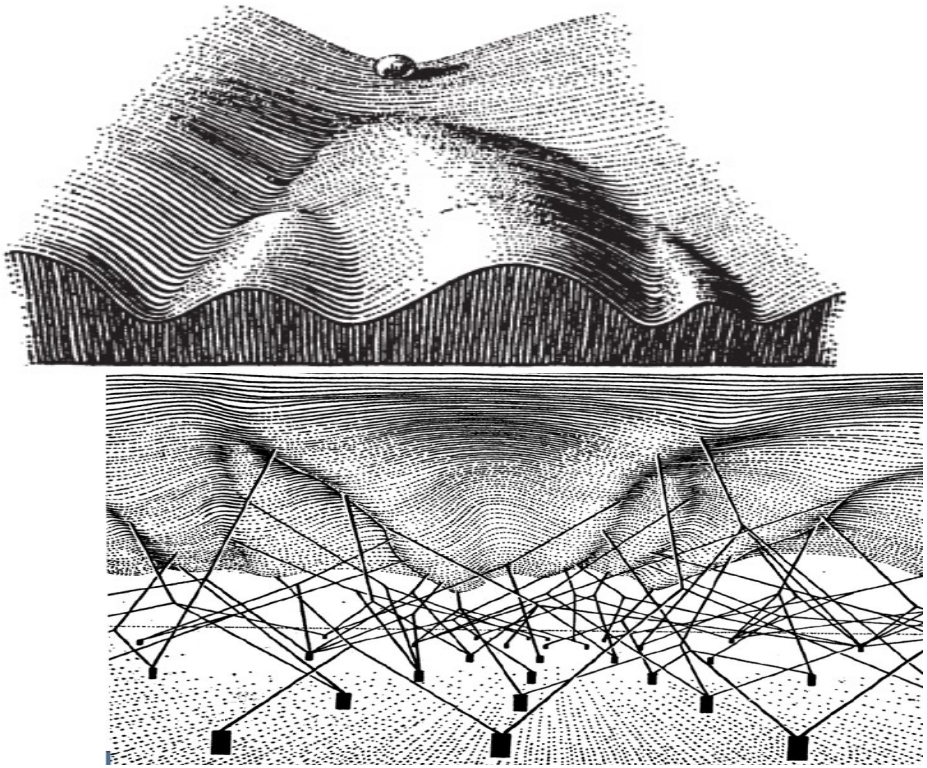
Proliferation relies at the cell level on the cell division cycle



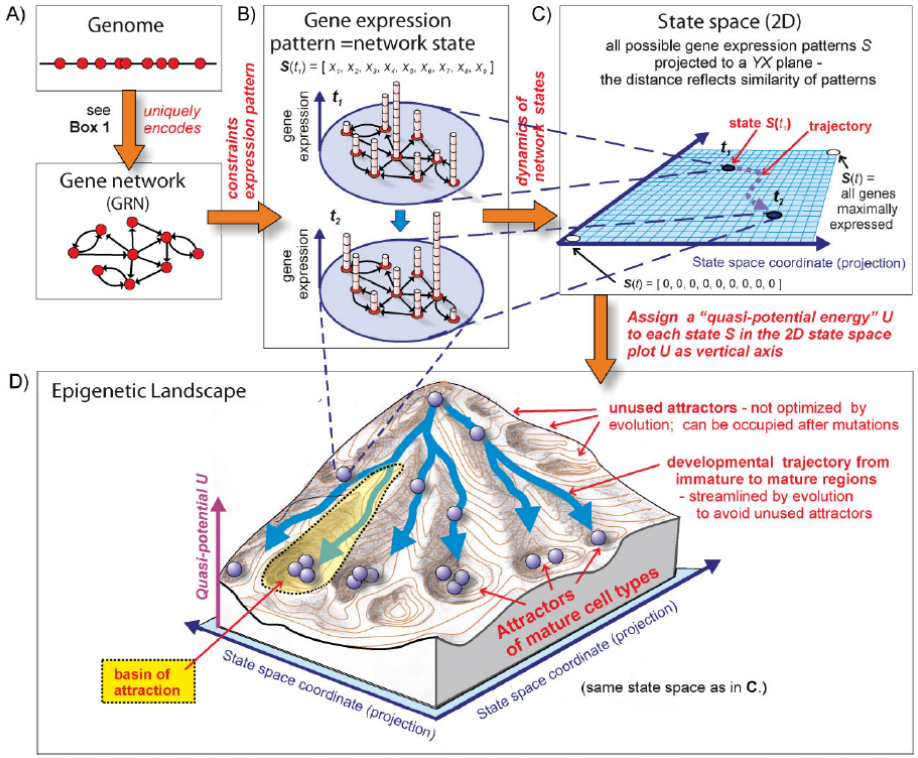
- Proliferation in cell populations results at cell level from the cell division cycle
- Classically divided into G1, S (DNA synthesis), G2 and M (mitosis) phases
- Multiplication of cells results from individual cell divisions: one cell becomes two at mitosis
- The cell division cycle is physiologically strictly controlled, but deregulated in cancer

Differentiation in the Waddington epigenetic landscape

The classic Waddington landscape ("The strategy of genes", 1957): cell differentiation *in an isogenic organism*



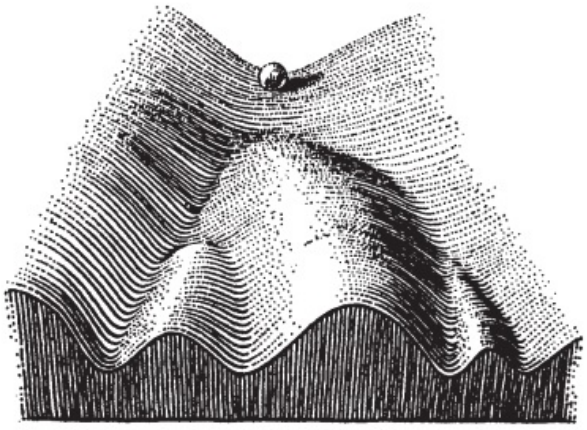
Waddington landscape revisited (S. Huang 2011, .., 2013)



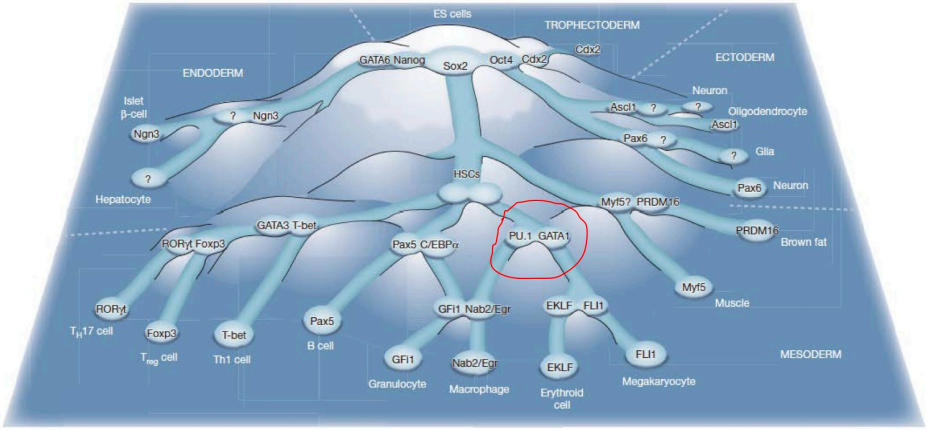
"Nothing in biology makes sense except in the light of evolution" (Th. Dobzhansky 1973), revisited as:

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

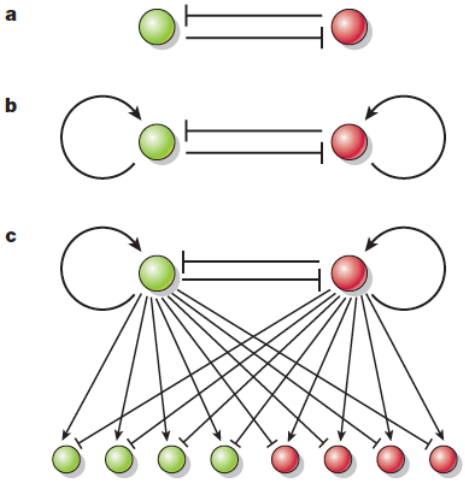
Milestones to reconstruct the global differentiation landscape

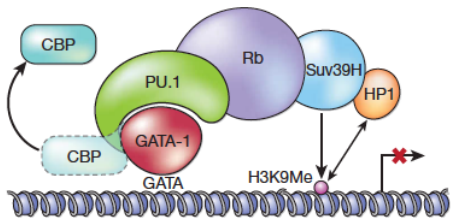


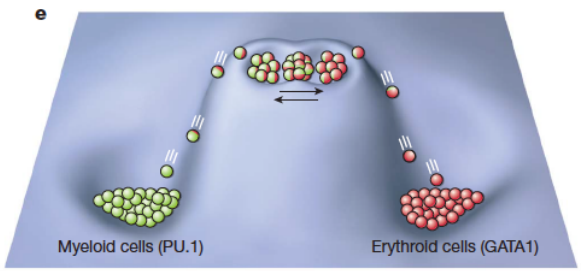
[Classic Waddington landscape]



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

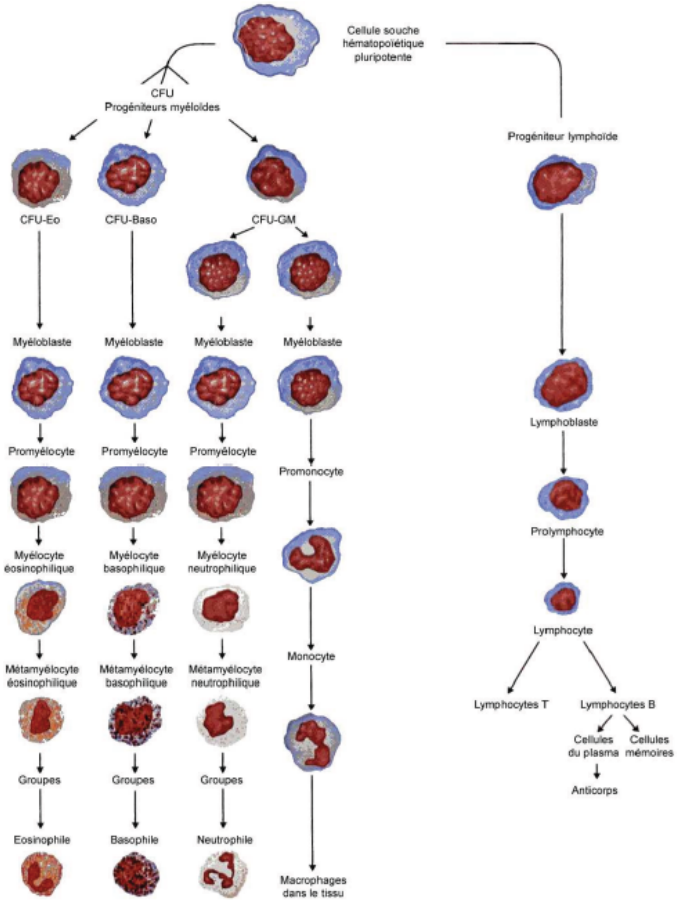






Zoom on the PU.1/GATA1 node (for equations and bifurcations, see Huang, Guo, May & Enver *Devel Biol 2007*)

Focus on Acute Myelogenous Leukaemia (AML)



- AML is a blood cancer, that alters the myelocytic lineages (left lineages on the figure) of haematopoiesis, i.e., making of blood cells in the bone marrow from pluripotent haematopoietic stem cells to terminally differentiated blood cells.
- In this classic representation of physiological haematopoiesis, are suggested in the different lineages of the left part of the figure the different possible versions of AML, different possible blockades of differentiations (from one cell a to a more mature one, closer to the terminal type) at different stages, and subsequent uncontrolled proliferation of the resulting immature cells in AML.
- AML will be the frame of different presentations in this tutorial session.

The stem cell niche

- Stem cells require close interactions with their micro-environment to preserve their functionality
- The micro-environments supporting stem cell function are referred to as stem cell niches. The stem cell niche plays a major role in regulating stem cell proliferation, self-renewal, quiescence and differentiation
- The cellular composition of the hematopoietic stem cell niche(s) remains incompletely defined. Endosteal, perivascular and endothelial cells play a role (e.g., Boulais & Frenette, Blood 2015; Calvi & Link, Blood 2015; Morrison & Scadden, Nature 2014)
- Acute myeloid leukemia perturbs the physiological function of the stem cell niches

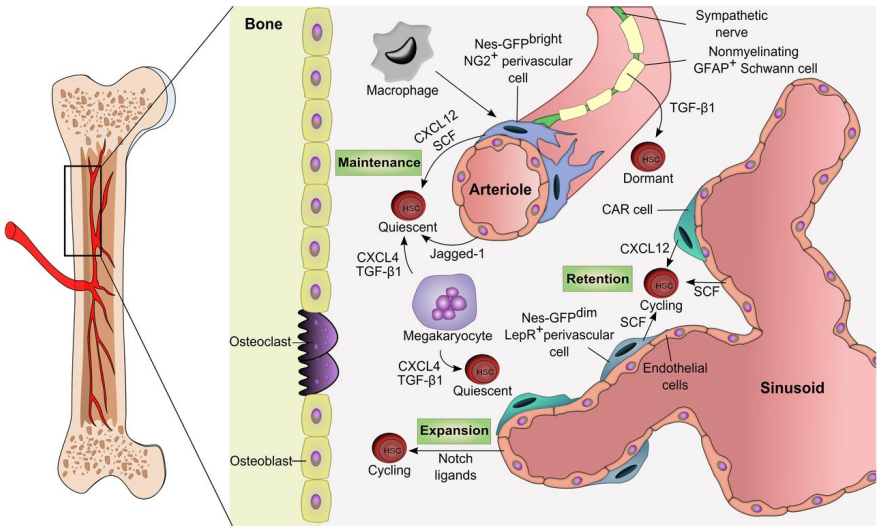


Image from *Blood (2015) 125 (17): 2621-2629*

Gene mutations in AML and Myeloproliferative Neoplasms

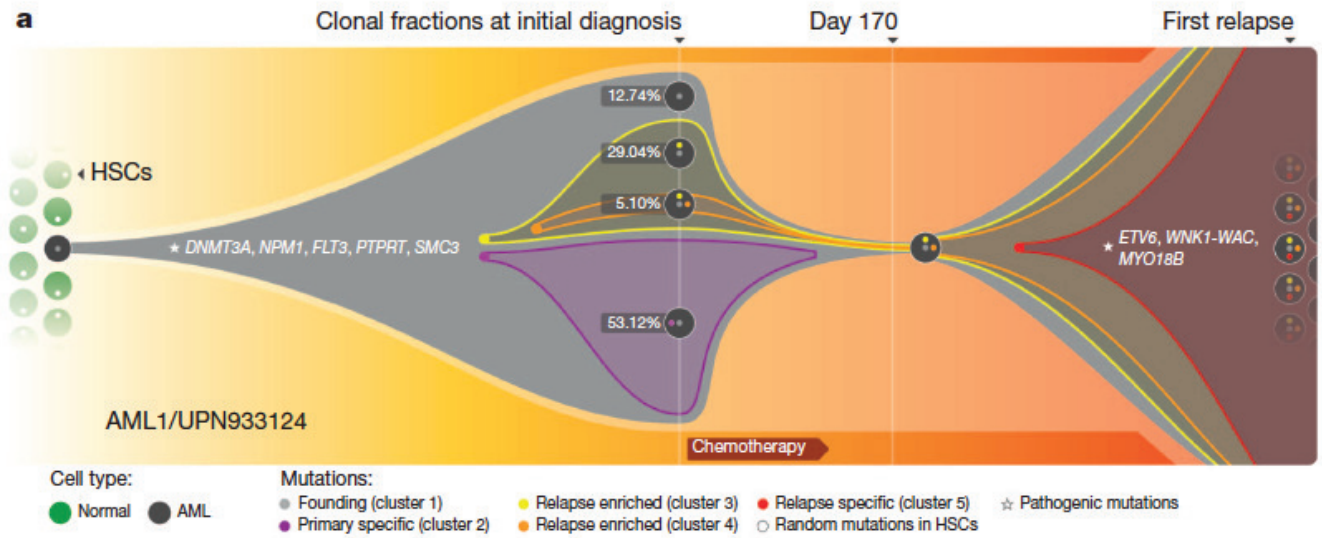


Figure: Mutations in AML1, from Ding *et al.* Nature Lett. 2012

- Myeloproliferative Neoplasms (MPNs) are clonal disorders of the haematopoietic stem cells.
- MPNs are mainly due to the acquisition of a driver mutation to the gene JAK2 (JAK2V617F), CALR (CALRm) or MPL.
- MPNs are characterised by an overproduction of some blood cells, but without a blast phase.
- MPNs can evolve towards AML, mainly with accumulation of additional mutations.

Tumour-immune interactions

The three possible outcomes for tumour cells confronted to immune cells: elimination, equilibrium, escape

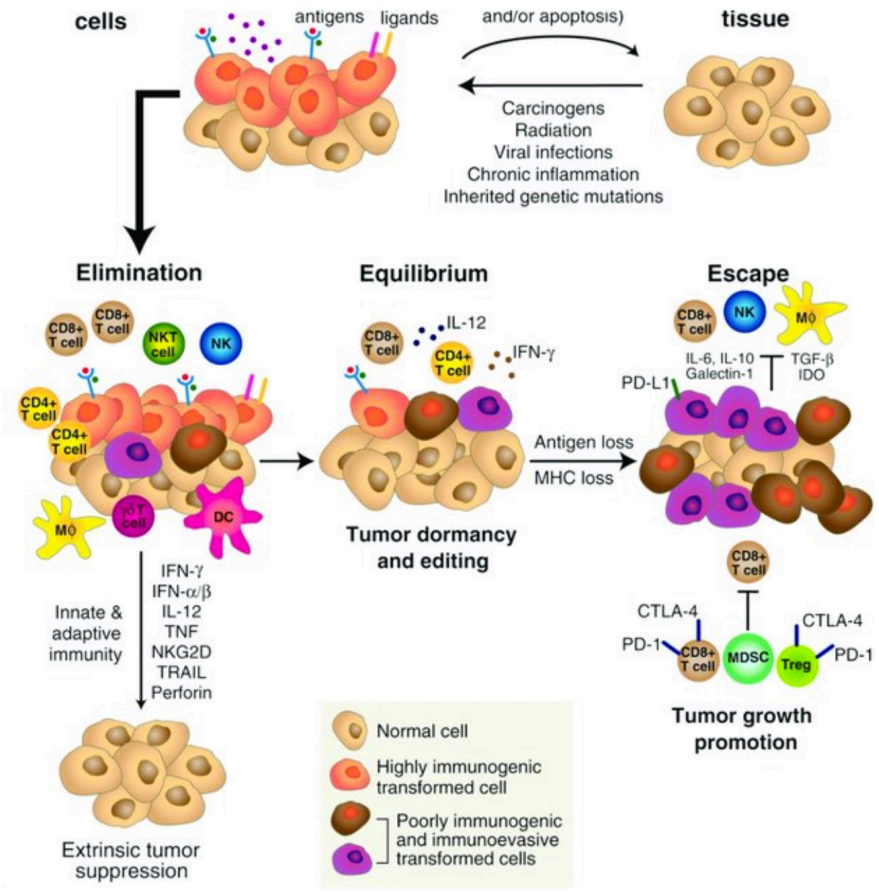


Figure: The three Es of immunoediting, after Schreiber, Science 2011

Continuous cell population dynamics models can represent these interactions.

Debates on the origin of cancer

- The present mainstream admitted cause of cancer, among oncologists and cancer biologists, follows SMT (somatic mutation theory), that states that one somatic cell one day became mutated in some critical genes, giving rise to the development of cancer in a stochastic way, by succession of mutations. Such mutations may affect firstly the genes coding for epigenetic control.

- However, the tissue organisational theory (TOFT), proposed by Ana Soto and Carlos Sonnenschein, emphasises the importance of the tissue ecosystem, denying that one single cell might be at the origin of the disease.

- The atavistic theory of cancer, proposed in 2011 by physicists Paul Davies, Charles Lineweaver and oncologist Mark Vincent, is a new way of understanding cancer, according to which tumours are the result of a *reversal of evolution* in the physiological development of the host tissues from the initial cell to finally differentiated cell types, leading to plastic, poorly organised populations of cells, *Metazoa 1.0*, incoherently organised tissues escaping organism control, but able to develop cooperation between subpopulations of cells, and drug resistance, due to some preservation of their genome of a multicellular species.

- Nevertheless, the atavistic theory tells us nothing about the origin of the disease. It is however possible that mutations or tissue microenvironment changes affecting epigenetic enzymes may account for the primary loss of control on differentiations, which *is* plasticity in cancer cell populations.

Heterogeneity in cell populations

- Heterogeneity is just biological between-cell variability in cell populations, w.r.t. chosen relevant traits. Numerous continuous models of structured cell populations exist, using cell size, cell cycle age, time since last neuronal fire, expression of drug resistance...
- Heterogeneity is meant here as structuring populations w.r.t. continuous cell functional phenotypes governing cell population fate; it may be understood as between-individual variability identified in *phenotypic* (not Cartesian) space.
- Chosen traits in a structured cell population model, *aka cell phenotypes*, are assumed to be *continuous* and to characterise cells in the population in a way that is relevant to a given biological question.
- They may be identified as linked to biological gene expressions and/or protein concentrations, but more fundamentally (and abstractly in these mathematical models) such traits are *functional*, supposed to govern cell population fate.
- They are *adaptive*, continuously changing according to changes in the environment of the cell population; following their probability distribution in phenotype space (e.g., yielding concentration on asymptotic phenotype values) allows to identify such adaptation in the long run.

Mathematics of heterogeneity and plasticity in cancer

- The privileged point of view here: PDEs for phenotype-structured cell population dynamics (nonlocal logistic or Lotka-Volterra type): Variable $n(t,x)$ for cell population density at time t and phenotype $x \in \mathbb{R}^n_+$ with $\rho(t) = \int_{\mathbb{R}^n_+} n(t,x)dx$ as nonlocal logistic term limiting growth.

- Many other dynamic models may be used: agent-based models (ABMs), compartmental ODEs with discrete traits, age-structured differential models involving the cell division cycle at the cell population level, delay-differential models, stochastic differential models... possible translations between them.

- Single population models of one cell population or models of competition or mutualism between cell populations are used, according to the question at stake.

- All of them are amenable to asymptotic analyses and optimal control methods applied to external means of action (e.g., drugs) acting on built-in targets.

Sketch of the first stages of multicellularity in evolution

- Emergence of multicellularity appeared several times in evolution, with possible species extinction, and there are cases in which it may be considered as optional, e.g., in Volvocine green algae (*Volvox carteri*), which have evolved from colonies of unicellular *Chlamydomonas rheinhardti*, with many known intermediate states.

- Primitive multicellular organisms, such as the sponges *Porifera* (closest existing descendants of the *"Urmetazoa"*, *Müller et al. 2001*), have been extensively studied. Although endowed with about only 20 cell types (200 to 400 in Humans), they share with us fundamental characteristics at the molecular stage: cell-cell and cell-matrix adhesion molecules, morphogens, transcription factors, tight-junction proteins, making them able to separate the cohesive multicellular individual they constitute from the surrounding environment.

- Furthermore, although they have no proper organs, sponge cells have the ability to differentiate, they are endowed with an apoptotic machinery, and, most of all, sponges have an immune system, an essential capability to distinguish friend and foe, and thus to define a coherent individual.

- The existence of tumours has not been documented thus far in *Porifera*, but it has been evidenced in *Hydra* (*Ćetković 2018*), a rather primitive non-bilaterian Metazoan, which may hint to the expected fact that all multicellular organisms may be prone to develop cancer. On the contrary, it makes little sense to try and characterise cancer in unicellular organisms.

What makes an individual in the animal kingdom

- Although sexual reproduction may be bypassed in a variety of animals under particular circumstances, it is the rule in Metazoa, and the zygote (fecundated egg by the union of male and female gamete, *Wolpert & Szathmáry 2002*) is the primitive cell containing coded in its genome the *Bauplan* (or body plan), i.e., the design program mandatory to build a coherent isogenic multicellular organism (*Davidson et al. 1995, Müller et al. 2004*). It has no other existence than the one of a program of instructions written in genetic code.

- The production of cellular matter by successive divisions from the zygote, conserving in each new cell the *Bauplan*, transmitting and updating *positional information* (*Wolpert 2011*) to the descendants, and on the other hand obeying physical laws of fluid dynamics (*Collinet & Lecuit 2020*), is the basis of embryological development, which by successive differentiations yields in evolved animals the different terminal cell types, basis of the anatomic organs that support the great physiological functions.

- To ensure compatibility between tissues and cooperativity between organs and functions, which is arguably from a teleological viewpoint what multicellularity is made for, i.e., division of work (as it is at higher levels of evolution, in anthropology and in sociology), molecular mechanisms of cohesion between tissues and of coherence between signals, excluding uncontrolled plasticity, must exist all along the process of development and organism maintenance, i.e., such molecular mechanisms must also be coded by instructions in the *Bauplan*.

Physiological plasticity in development and tissue repair

- In development (in particular in embryogenesis), physiological cell populations are initially functionally indeterminate and very plastic; they depend for their differentiation on the genetic programme they bear in their genome and on close interactions (contact, delta/notch, connexons?) between neighbouring cells.

- Such plasticity is epigenetically determined (differentiation is of epigenetic nature, it is not due to mutations), transient during development, but can be reactivated under physiological circumstances such as tissue repair. In particular some vertebrate species (e.g., axolotl) are able to regenerate a missing limb.

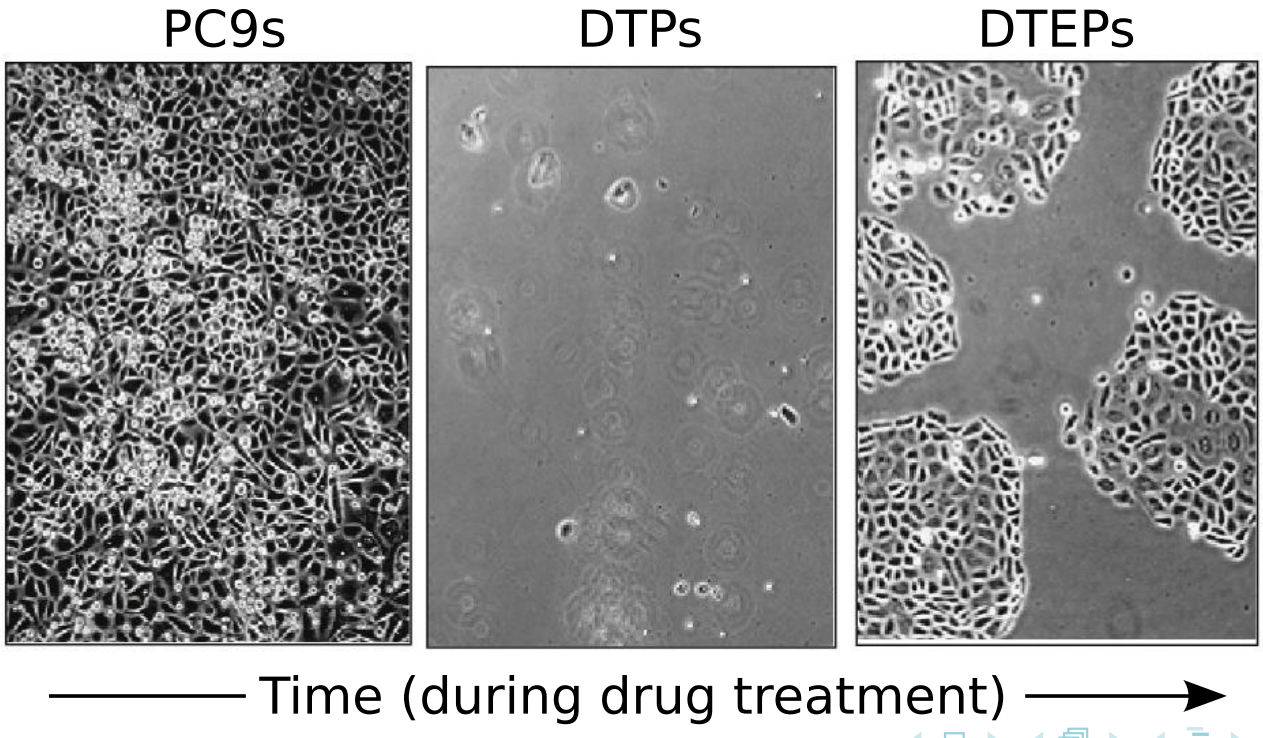
- The possibility of de-differentiation, although normally repressed, is thus naturally present in the genome of all cells in multicellular organisms, and can easily be exploited by plastic cancer cells - *and not by healthy cells* - to adapt their phenotypes to a hostile environment (e.g., drug insult), or to the cancer invasion process through EMT, recovering normally lost motility in epithelia.

Plasticity in cancer *is* loss of control on differentiations

- Blockade of differentiation of the myeloid lineage leads to immature (myeloblast) cell proliferation in acute myeloid leukaemia (AML).
- Uncontrolled differentiation leads to immature cell proliferation in unclear histological zones: Barrett's oesophagus, ductal carcinoma in situ (DCIS).
- Transdifferentiation from interfollicular epidermis (IFE) cells to bulge cells, favours basal cell carcinoma (BCC) upon activation of the Hedgehog oncogenic pathway, and vice versa from BCC to an IFE/isthmus mixed cell state upon inhibition of Hedgehog (reviewed in S. Shen & *JC*, *F1000 Research* 2020).
- In castration-resistant prostate cancer, transdifferentiation of epithelial cells may lead to a neuroendocrine cellular type.
- Epithelial to mesenchymal transition (EMT) and its reverse (MET): normally differentiated epithelial cells are unable to move, a capacity mainly left to immune or mesenchymal cells (fibroblasts). De-differentiation of transformed epithelial cells into a mesenchymal state endows them with the mandatory motility to invade remote tissues where they re-acquire an epithelial state and proliferate, making metastases.

Evidence of cell plasticity in cancer: non-genetic mechanisms

- Population of PC9 (NSCLC) cells under high doses of drugs (e.g., gefitinib)
- 99.7% cells die, .3% survive in this maintained hostile drug environment:
  Drug Tolerant Persisters, DTPs
- In the same hostile environment, 20% of DTPs resume proliferation:
  Drug Tolerant Expanded Persisters, DTEPs.
- Total reversibility to drug sensitivity is obtained by drug withdrawal, occurring after 9 doubling times for DTPs, and 90 doubling times for DTEPs.
- Inhibition of epigenetic enzyme KDM5A blocks emergence of DTPs



from Sharma et al. Cell 2010

2D phenotype-structured model for drug tolerance

- Initial (PC9) cancer cell population structured by a 2D phenotype (x, y):
  $x \in [0,1]$: expression level of survival potential phenotype (*viability*), and
  $y \in [0,1]$: expression level of proliferation potential phenotype (*fecundity*)
  (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

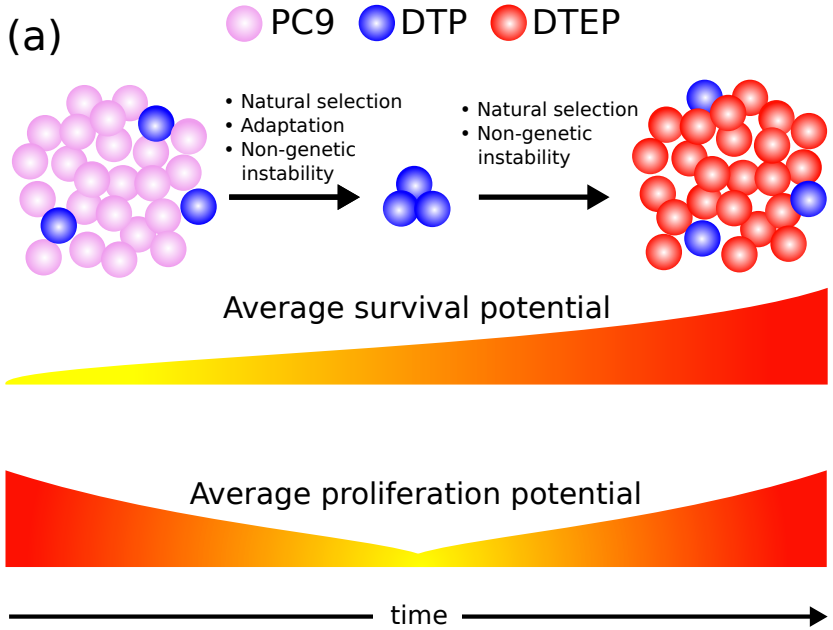
$$\frac{\partial n}{\partial t}(x,y,t) + \underbrace{\frac{\partial}{\partial y}\Big(v(x, c(t); \bar{v}) n(x,y,t)\Big)}_{\text{Stress-induced adaptation of the proliferation level}} =$$

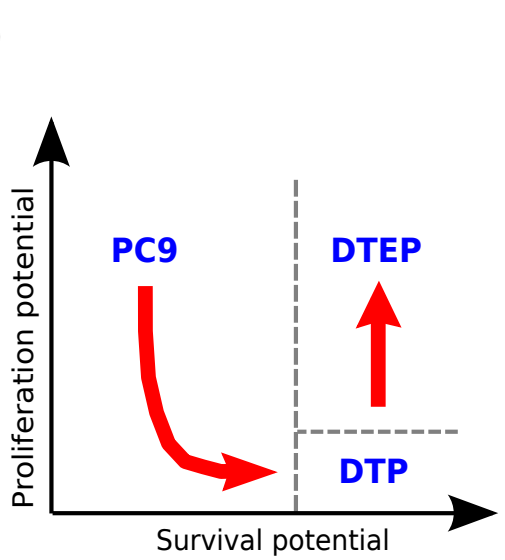
$$\underbrace{\Big[p(x,y,\varrho(t)) - d(x,c(t))\Big] n(x,y,t)}_{\text{Non local Lotka-Volterra selection}} + \underbrace{\beta \Delta n(x,y,t).}_{\text{Non-genetic phenotype instability}}$$

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

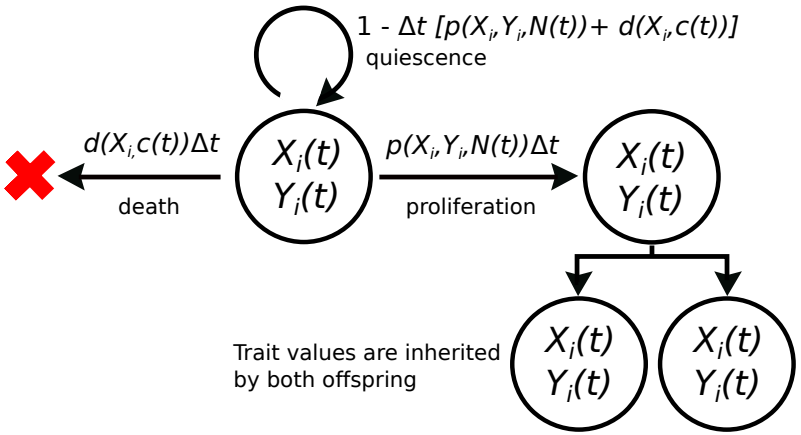
Chisholm et al., Cancer Research 2015

Agent-based model (ABM)





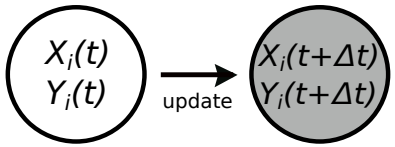
(a) Each cell i undergoes either proliferation, death or remains quiescent:



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

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

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

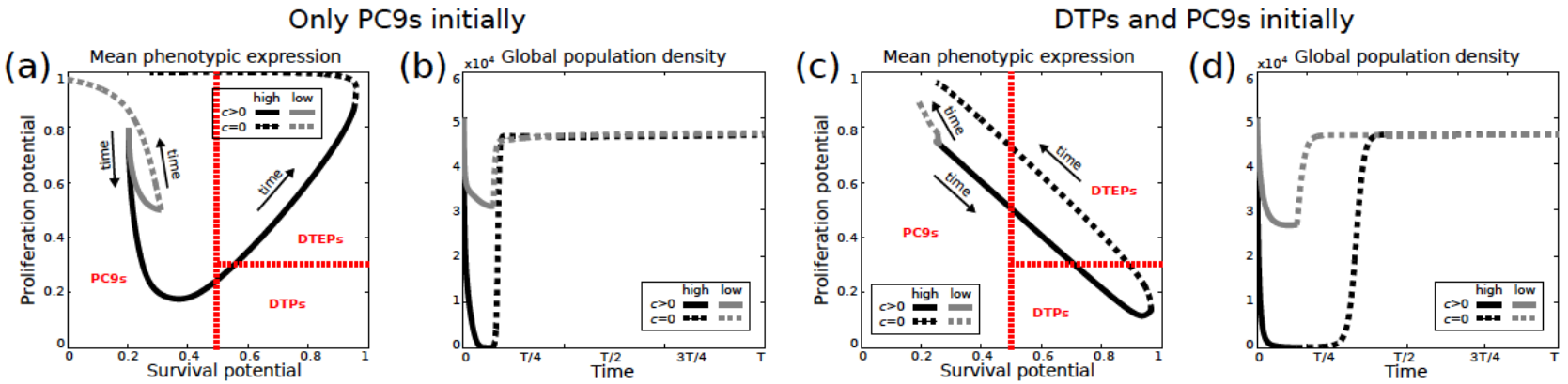


Chisholm et al., Cancer Research 2015

AB and PDE models recover the same phenotype dynamics

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

2 scenarios studied: (A) Initially no drug-tolerant cells (Lamarckian instruction)
(B) Initially a few drug-tolerant cells (Darwinian selection)

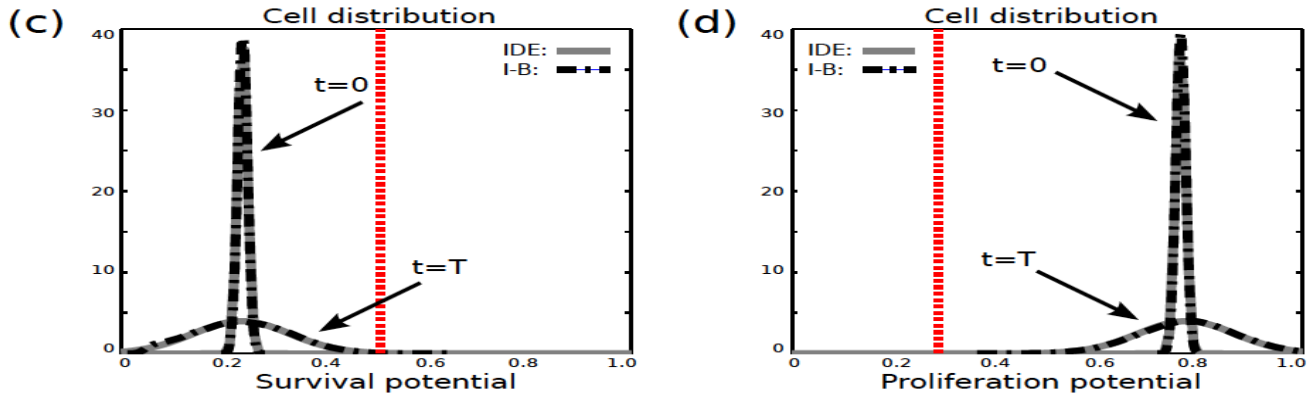


(a), (b) Only PC9s initially, adaptation on, $v \neq 0$: *'Lamarckian adaptive' scenario* (A), i.e., both Darwinian selection and Lamarckian instruction

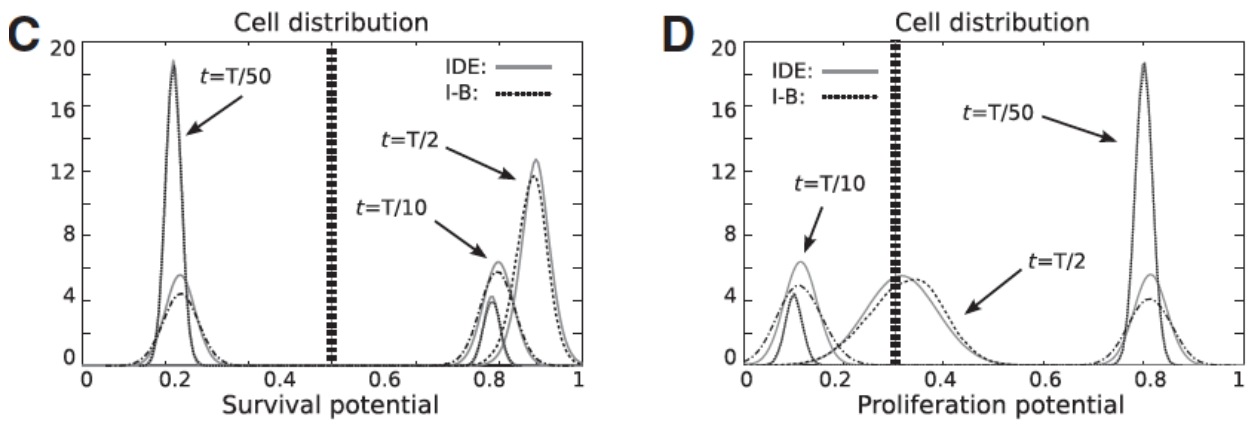
(c), (d) PC9s and DTPs initially, no adaptation, $v = 0$: *'strict Darwinian' scenario* (B), i.e., only Darwinian selection of DTPs at the expense of drug-sensitive PC9s

Chisholm et al., Cancer Research 2015

Phenotype heterogeneity in the cancer cell population



c, **d**: In the absence of treatment, the cancer cell population becomes more heterogeneous when it is left to evolve; 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-axis; (d) Projection onto the y-axis.



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

Chisholm et al., Cancer Research 2015

Use PDE model to address 3 questions

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

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

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

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

(i) (*'Darwinian' scenario (B)*: the dogma) PC9s and few DTPs initially, no adaptation ($v = 0$)

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

To make a long story short, (simulations not shown)

Q1. Always yes! Whatever the scenario

Q2. Low drug doses result in DTEPs, but no DTPs

Q3. Never! Whatever the scenario

Chisholm et al. Cancer Research 2015

Summary of simulation results on the *Sharma et al.* paper

- Both mathematical models (ABM, PDE) 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 inducing no or little drug resistance): case of metronomic therapies? Might be assessed using this model, not done yet.

Chisholm et al., Cancer Research 2015

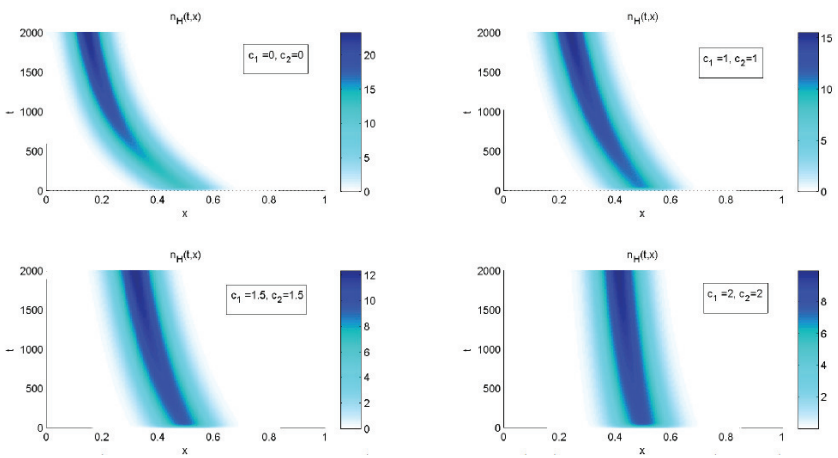
Non-local Lotka-Volterra model of treatment for 2 cell populations, 2 different drugs and 1 resistance phenotype x

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

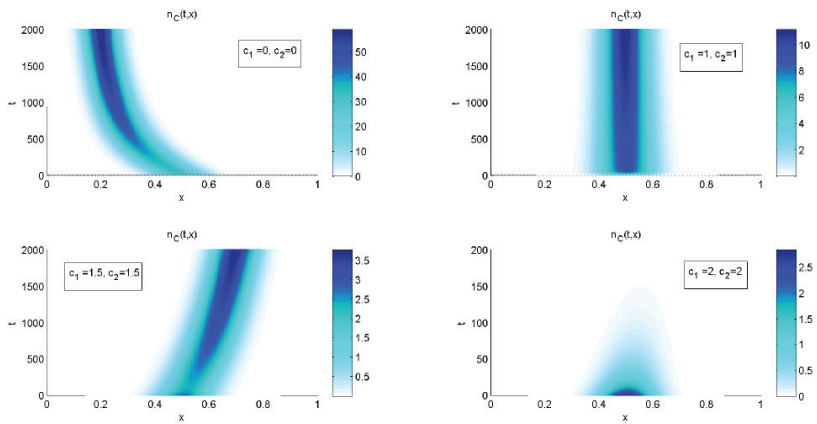
$$\textit{(Cancer cells } C\textit{)} \quad \frac{\partial}{\partial t} n_C(t,x) = \left[\frac{r_C(x)}{1 + k_C u_2} - d_C(x) I_C(t) - u_1 \mu_C(x) \right] n_C(t,x)$$

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

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



Healthy cells: preserved



Cancer cells: eventually extinct

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

Asymptotic behaviour with constant controls (=no control)

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

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

Theorem

(Asymptotic behaviour theorem)

Assume that u_1 *and* u_2 *are constant:* $u_1 \equiv \bar{u}_1$, *and* $u_2 \equiv \bar{u}_2$. *Then, for any positive initial population of healthy and of tumour cells,* $(\rho_H(t), \rho_C(t))$ *converges to the equilibrium point* $(\rho_H^\infty, \rho_C^\infty)$, *which can be exactly computed as follows.*
Let $a_1 \geq 0$ *and* $a_2 \geq 0$ *be the smallest nonnegative real numbers such that*

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

Then $(\rho_H^\infty, \rho_C^\infty)$ *is the unique solution of the invertible* ($a_{HH}.a_{CC} >> a_{CH}.a_{HC}$) *system*

$$I_H^\infty = a_{HH}\rho_H^\infty + a_{HC}\rho_C^\infty = a_1,$$
$$I_C^\infty = a_{CH}\rho_H^\infty + a_{CC}\rho_C^\infty = a_2.$$

Let $A_H \subset [0,1]$ *(resp.,* $A_C \subset [0,1]$*) be the set of all points* $x \in [0,1]$ *such that equality holds in one of the inequalities above. Then the supports of the probability measures*

$$\nu_H(t) = \frac{n_H(t,x)}{\rho_H(t)}\, dx \quad \text{and} \quad \nu_C(t) = \frac{n_C(t,x)}{\rho_C(t)}\, dx$$

converge respectively to A_H *and* A_C *as* t *tends to* $+\infty$.

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Basis of proof (constant controls): a Lyapunov functional

Firstly, the correspondence $(a_1, a_2) \mapsto (\rho_H^\infty, \rho_C^\infty)$ being bijective and controls $\bar{u_1}$, $\bar{u_2}$ being constant (omitted in the sequel), one can write the two inequalities in (a_1, a_2) as

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

with, furthermore, by definition

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

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

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

where $n_{H,C}^\infty(x)$ are measures with support in $A_{H,C}$ such that $\displaystyle\int_0^1 n_{H,C}^\infty(x)\, dx = \rho_{H,C}^\infty$, the positive constants λ_H and λ_C being adequately chosen (in fact $\lambda_H = \dfrac{1}{a_{HC}}$ and $\lambda_C = \dfrac{1}{a_{CH}}$) to make V decreasing along trajectories.

The functional V yields simultaneously convergence and concentration.

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Cell-killing strategy preserving healthy cells: optimal control problem using this 1D phenotype-structured model

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

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

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

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

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

Optimal control problem: find controls (u_1, u_2) minimising in fixed horizon T

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

under the additional constraints

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

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

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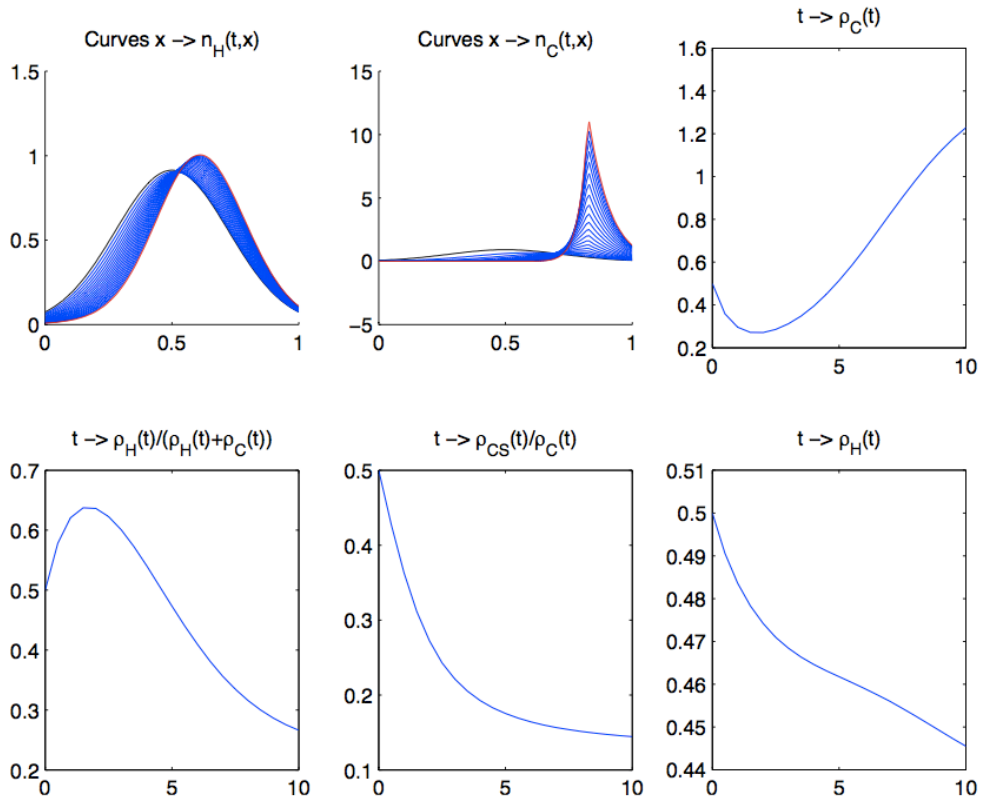
How to be deleterious by using constant doses of drugs

[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 $T = 10$ yields a seemingly 'pessimal' solution:



- 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}.

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Optimal control problem: theoretical results

Theorem
(Optimal control theorem)

The optimal therapeutic trajectory (u_1, u_2) in large time $T > 0$ consists of 2 parts:

- a long-time part, with constant controls on $[0, T_1]$, at the end of which populations have almost concentrated in phenotype (for T_1 large);
- a short-time part on $[T_1, T]$ consisting of at most three arcs, for $T - T_1$ small:
  1. a boundary arc, along the constraint $\dfrac{\rho_H(t)}{\rho_H(t) + \rho_C(t)} = \theta_{HC}$,
  2. a free arc (no constraint saturating) with controls $u_1 = u_1^{\max}$ and $u_2 = u_2^{\max}$,
  3. a boundary arc along the constraint $\rho_H(t) \geq \theta_H . \rho_H(0)$ with $u_2 = u_2^{\max}$;
- the proof uses the Pontryagin maximum principle.

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Simulations illustrating this theorem

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

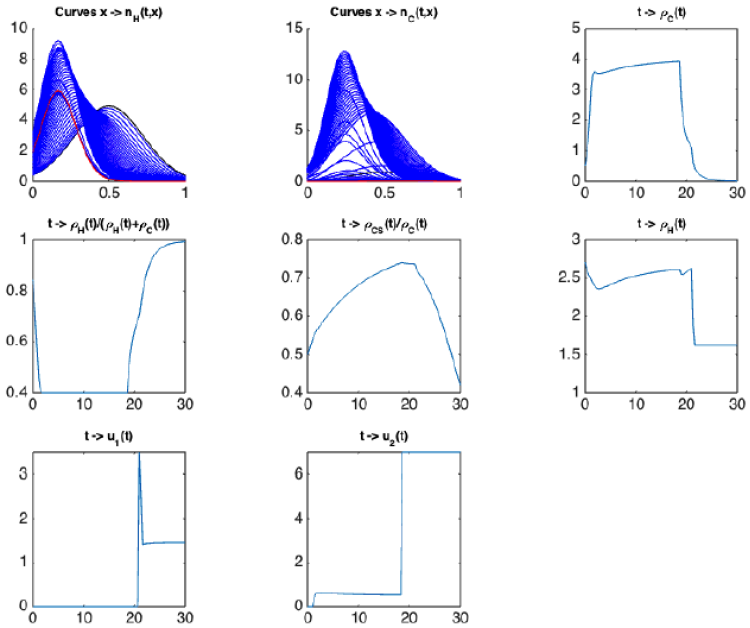

Figure 4: Simulation of (**OCP**) for $T = 30$.

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

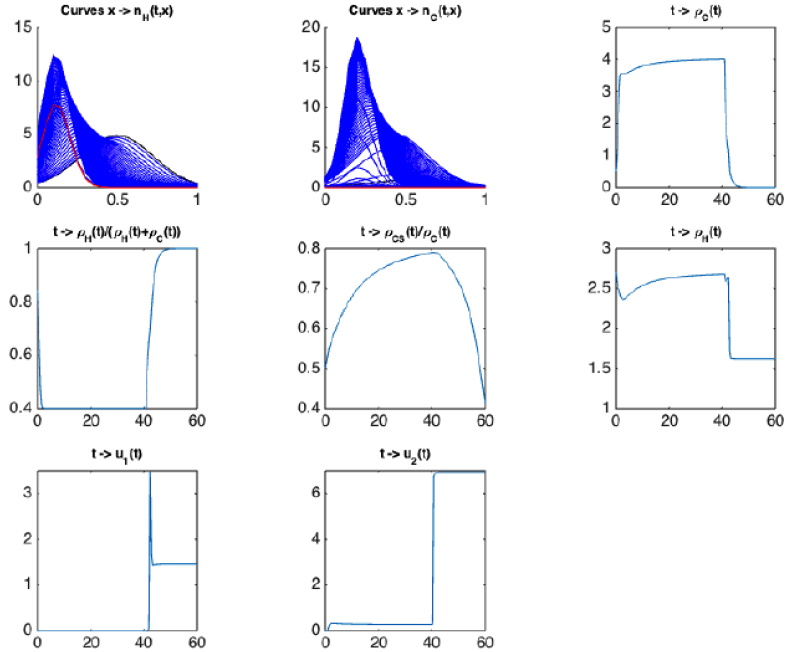

Figure 5: Simulation of (**OCP**) for $T = 60$.

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

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Interpretation

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 lower than $u_1^{\max}$, and $u_2 = u_2^{\max}$.

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).

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Comparison with "almost periodic" therapeutic strategies

1) Mimicking the clinic; 2) the same with saturation of the constraint $\rho_H = \theta_H.\rho_H(0)$

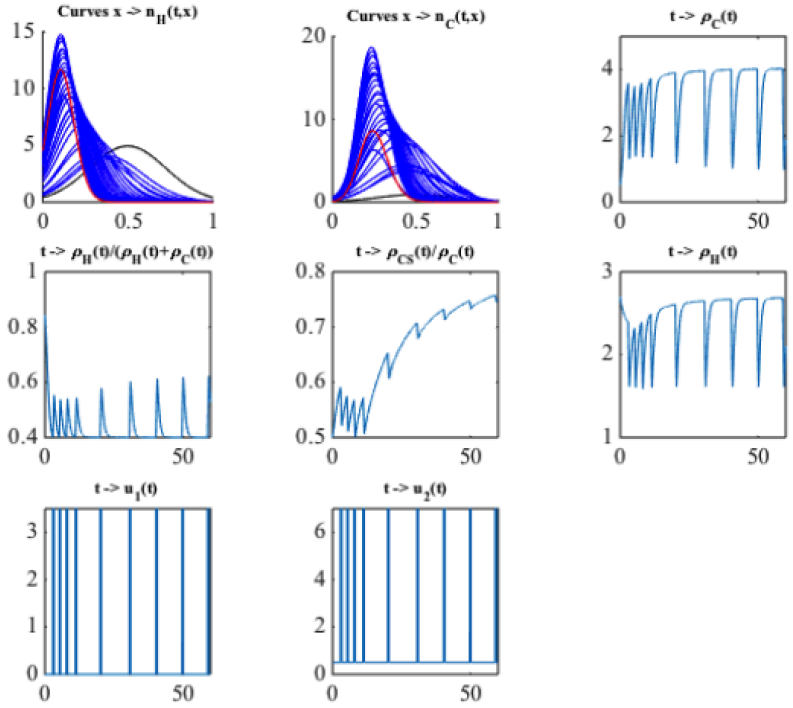

Figure 6: Quasi-periodic strategy, for $T = 60$.

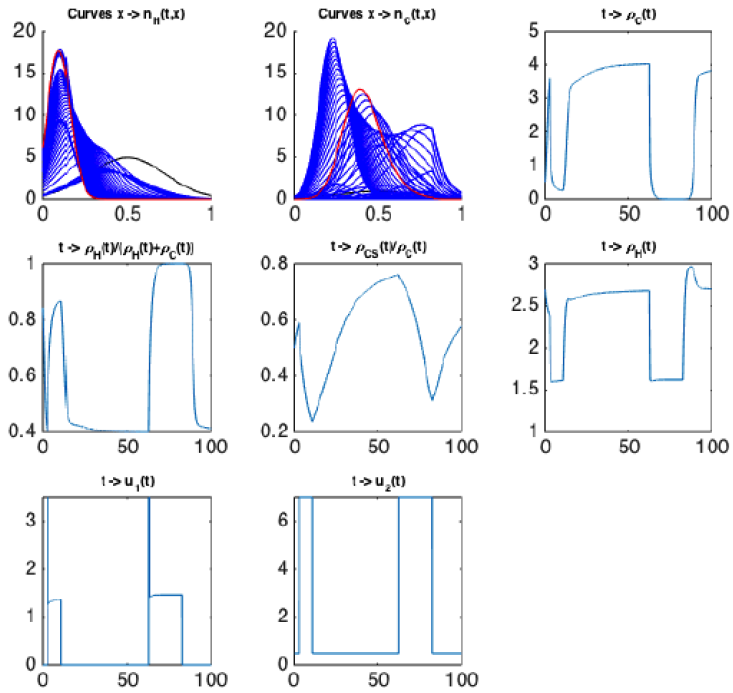

Figure 7: Second quasi-periodic strategy, for $T = 100$.

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

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Notes about the 'cooking recipes' used in the simulations (1)

In this version of the simulations (used throughout in the sequel)

$$r_H(x) = \frac{1.5}{1+x^2}, \quad r_C(x) = \frac{3}{1+x^2},$$
$$d_H(x) = \frac{1}{2}(1-0.1x), \quad d_C(x) = \frac{1}{2}(1-0.3x),$$
$$u_1^{\max} = 3.5, \quad u_2^{\max} = 7,$$

and the initial data are

$$n_H(0,x) = C_0 \exp(-(x-0.5)^2/\varepsilon), \quad n_C(0,x) = C^0 \exp(-(x-0.5)^2/\varepsilon),$$

with $\varepsilon > 0$ small (typically, we will take either $\varepsilon = 0.1$ or $\varepsilon = 0.01$), and where $C_0 > 0$ is such that

$$\rho_H(0) + \rho_C(0) = 1.$$

Notes about the 'cooking recipes' used in the simulations (2)

The closer to 1 is the variable x, the more resistant are the tumour cells. The choice (already done in *Lorz et al. 2013*) is

$$\mu_H(x) = \frac{0.2}{0.7^2 + x^2}, \quad \mu_C(x) = \frac{0.4}{0.7^2 + x^2}.$$

Note that, with this choice of functions, if we take constant controls u_1 and u_2, with

$$u_1(t) = \mathrm{Cst} = u_1^{\max} = 3.5, \qquad u_2(t) = \mathrm{Cst} = 2,$$

then we can kill all tumour cells (at least, they decrease exponentially to 0), and no optimisation is necessary.

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Notes about the 'cooking recipes' used in the simulations (3)

The environment variables $I_{[H,C]}(t)$ defined by

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

and

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

have been chosen such that

$$a_{HH} = 1, \quad a_{CC} = 1, \quad a_{HC} = 0.07, \quad a_{CH} = 0.01, \quad k_H = 0.01, \quad k_C = 1,$$

which means in particular that in the limiting logistic terms in the model, intraspecific competition is overwhelmingly higher than interspecific competition, i.e., cell growth is mainly limited by access to resources, and very little by frontal competition between cancer and healthy cells, a choice done on biological grounds (*cancer cells and healthy cells are not thriving on the same metabolic niche, e.g., aerobic vs. glycolytic metabolisms*). As a consequence, as in classical Lotka-Volterra models with competition, the choice of these parameters will lead in the simulations to asymptotic coexistence of the two species, healthy and cancer, in a non trivial equilibrium state.

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Cellular stress-launched de-differentiation signals?

- Cellular stress is a cell state in which a cell threatened by a deadly environmental insult (drugs, UV radiations, hypoxia, etc.) launches a variety of response signals, with internal or external destination (*Nedelcu & Michod 2020*).

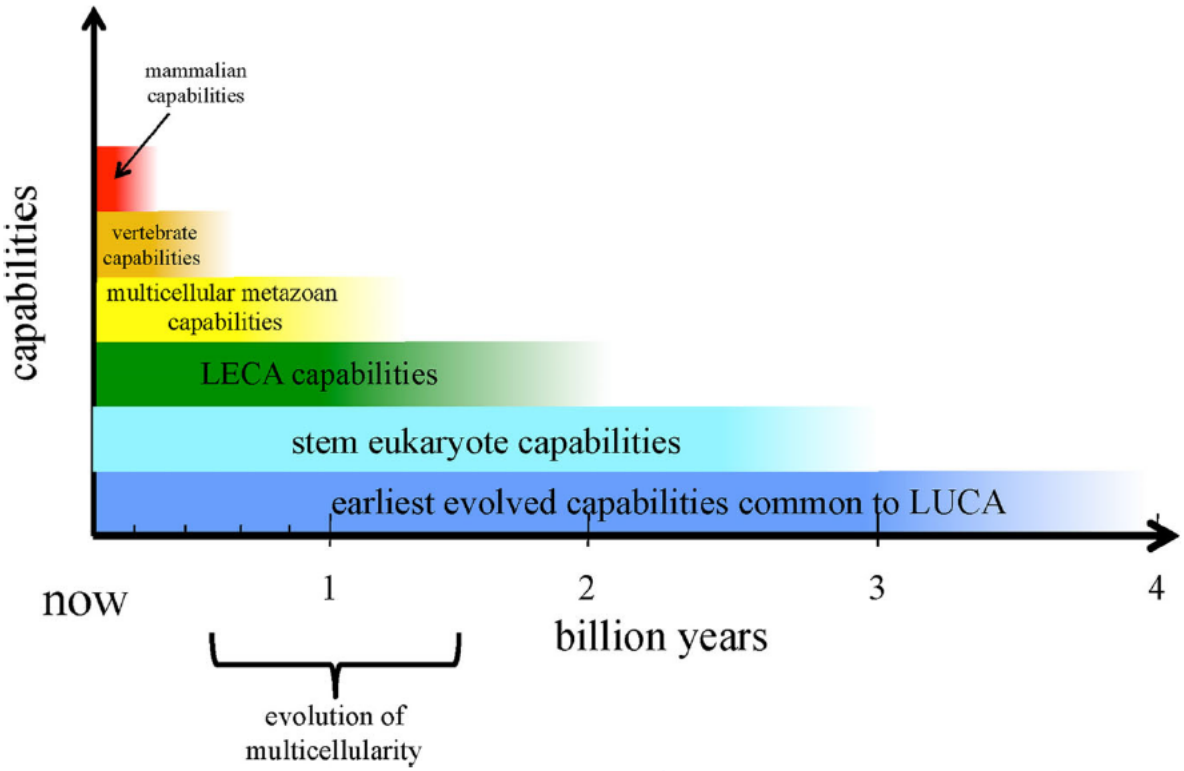
- It has been proposed that under extreme stress (Multiple Myeloma exposed to doxorubicin, *A. Wu et al. PNAS 2015*), cancer cells overexpress so-called 'cold genes', i.e., (very ancient) genes that are never substituted, thus being possible testimonies of 'a form of life adapted to high fitness under extreme stress', as the expression of these genes coincides with the rapid emergence of a subpopulation of MM cells resistant to doxorubicin.

- Could the expression of such 'cold genes', launched by a de-differentiation stress signal sent to the chromatin, be, or secondarily result in unmasking, thanks to the plasticity of cancer cells, the expression of diverse (with *bet hedging, Nichol 2016*) ancient genes, dating back to unicellular ancestors that were able to resist extreme stress conditions on our planet?

- This speculation refers to the so-called 'atavistic theory of cancer' (*Davies, Lineweaver and Vincent 2011*), according to which a tumour is a very primitive state of multicellularity, unable to lead to a cohesive multicellular organism by lack of a coherent development program, and nevertheless trying to put at work the bases of multicellularity (division of work, i.e., cooperativity between cells on different tasks, motility, plasticity in developmental stages) for its own benefit.

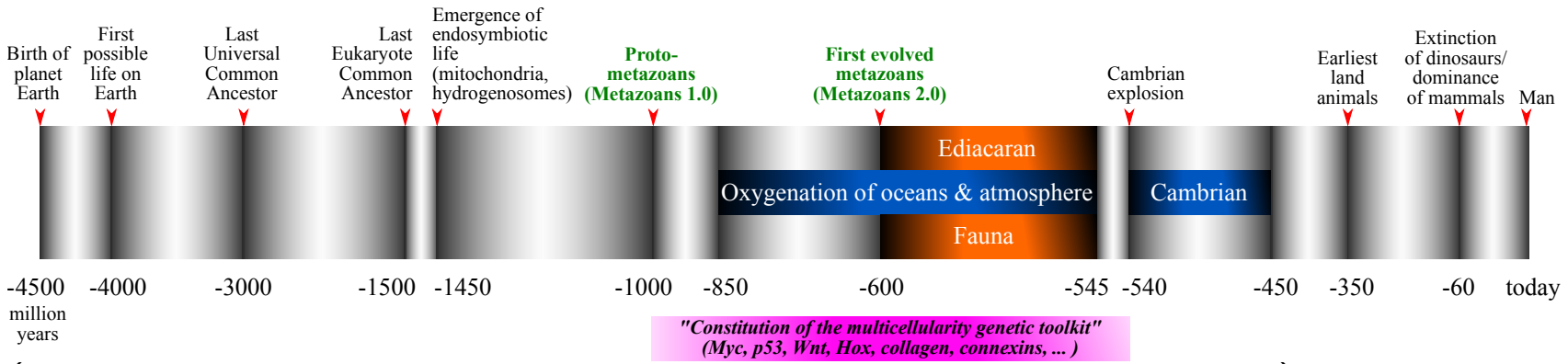
Reverse evolutionary framework (*billion year-term view* for multicellular organisms): the atavistic theory of cancer (1)

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



"Cancer: more archeoplasm than neoplasm" (Mark Vincent, 2011) More references: *Boveri: 'Zur Frage der Entstehung der maligner Tumoren' 1914, Israel JTB 1996, Davies & Lineweaver Phys Biol 2011, Vincent Bioessays 2011, Lineweaver, Davies & Vincent Bioessays 2014, Lineweaver et al. 2020, 2021, Bussey et al. PNAS 2017, Cisneros et al. PLoS One 2017, Trigos et al. PNAS 2017, Trigos et al. BJC 2018, Trigos et al. eLife 2019 and bioRxiv 2023*

Reverse evolutionary framework (*billion year-term view* for multicellular organisms): the atavistic theory 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 development of multicellularity are those that are altered in cancer: phylostratigraphic analyses by Domazet-Lošo & Tautz 2010; multicellularity vs. unicellularity gene investigations by Trigos *et al.* 2017, 2018, 2019 show overexpression of unicellularity genes and underexpression of multicellularity genes in cancer.

- Evolution order: 1) proliferation + contact inhibition to 2) cell differentiation + division of work, and to 3) achieved *epigenetic control* on differentiation and proliferation? (reverse mutation order in AML, *Hirsch Nature Comm. 2016*).

- Attacking cancer on proliferation is precisely attacking its robustness. It is better to attack its weaknesses: absence of protecting immune system in tumours.

Functional arguments in favour of the atavistic theory

- Physiologically in wound healing, and in cancer cells as a way to gain motility or viability (EMT, persister cells), changes of phenotypes in cells appear as a *temporary reversal* to more adapted ones to challenging situations: wound, cancer invasion by metastases, resistance to life-threatening treatments.

- However, such changes of phenotypes do not revert cells to a complete unicellularity state, and furthermore these changes are themselves reversible: the physiological wound healing program is abandoned when the healing is complete (*the axololotl*), in epithelial cancers EMT is reverted to MET in metastatic niches, and persister cells in Petri dishes apparently disappear, reverting the cancer cell population to their initial (main) phenotype when a life-threatening drug is withdrawn (*Sharma et al. Cell 2010*).

- Which naturally leads to consider, in particular by analogy with the physiological wound healing case, that plasticity of cancer cell populations is not all of the scenery, but an essential component of it, which must be completed by the possibility of a *primitive state of control of cohesion* within cancer cell populations, making them amenable to very coarse homeostasis principles, likely by minimisation of global energy costs.

- Such coarse homeostasis principles might be constitutive of the primitive state of multicellularity *Metazoa 1.0*, in the terminology of Davies and Lineweaver.

What role for the atavistic theory in mathematical models of cell population dynamics under treatment?

- Although the atavistic theory is not needed to introduce plasticity as a structure variable to describe heterogeneity in cell populations, healthy and cancer, it provides fundamentals for characterising these two different cell populations when taking into account drug resistance and unwanted toxic side effects.

- Indeed, cancer cell populations are not just disorganised colonies of cells. They are in particular able to show organised at the population level, successful reactions to treatments by the emergence of thriving persister cells (*Sharma et al. Cell 2010*, modelled in *Chisholm et al. Cancer Research 2015*).

- The atavistic theory gives a clear rationale for such emergence: choosing, by necessity to resist deadly changes coming from the environment, in the genome of Metazoa 1.0 the available genes, normally silenced, to be expressed to develop enough viability (yielding new or hitherto masked cell types, possibly by bet hedging of phenotypes) in view of tolerance to treatments in cancer cells.

- Similarly, the existence of coarse cooperativity in cancer cell populations (e.g., *Marusyk et al. Nature 2014, Tabassum & Polyak Nature Rev. Cancer 2015*), another testimony of rough multicellular organisation in cancer, supported by the atavistic theory, is a further challenge to design mathematical models, not only of cheating between defectors and cooperators, but also of coarse cooperation within organised cancer cell populations.

The atavistic theory and 'philosophy of cancer'

- Do 'philosophers of cancer' need the atavistic theory? Apparently not, as no mainstream publications refer to it, whereas the opposition between the dominating somatic mutation theory (SMT) and the less accepted tissue organisational field theory (TOFT, *Soto & Sonnenschein*) is often reported (e.g., by *Marta Bertolaso*, for whom cancer is a disruption of control on both differentiations and proliferation), while the atavistic theory usually is not.

- However, did seismologists need the hypothesis of continental drift, proposed by Alfred Wegener in 1912 to predict seisms? Apparently, they were reluctant to admit it, until it gave rise to plate tectonics, which is now accepted since the late sixties and is an essential theory in seismology.

- Although direct observations are more feasible on our planet than on ancient genomes, observations by *Domazet-Lošo & Tautz 2008, 2010* on comparison by stratigraphic analyses of genomes, and by a team in Australia by *Trigos et al. 2017, 2018, 201*) on the allocation of unicellularity vs. multicellularity characteristics to genes constituting modern genomes, may represent new tracks in compared evolution and in genetics to assess the atavistic theory.

- By investigating mechanisms of the emergence of multicellularity and of possible phenotype bet hedging in cancer *and by designing mathematical models based on structured equations*, we may contribute to shed light on the dynamics of the cancer disease and propose unexplored ways so far of therapeutic control.

In summary

- Heterogeneity of cancer cell populations and plasticity of individual cancer cells, that make cancer tissues adaptable to changing conditions in the tumour micro-environment, in particular under drug pressure, are crucial traits to be taken into account in mathematical models of cancer growth and therapy.

- Continuous trait-structured models, with or without added space, either based on relatively hidden functional phenotypes that determine cell population fates, such as viability, fecundity, plasticity, motility, or on well-identified, specific or relevant measurable gene or protein expression levels, can achieve this goal.

- Furthermore, the continuously developing atavistic theory of cancer (Davies & Lineweaver 2011, and others), that, marginal though it may seem, is presently more and more considered among evolutionary biologists of cancer, gives a biological and genetic rationale to support and guide this mathematical task.

- Such mathematical models, in particular those based on well-studied non-local Lotka-Volterra equations, including built-in trait-dependent targets for treatments impinging on them, are amenable to optimisation and theoretical optimal therapeutic control, and could be transfered to actual clinical settings.

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Hard problems still lying ahead of us

- (Atavistic theory) Helping identify a limited number of crucial *reversal* steps of physiological animal development from normal, cohesive organisms to abnormal local, tumour-like, organisation.
- Modelling *phenotype divergence* (possibly resulting in bet hedging in tumours) *together with intratumoral cooperation between cell subpopulations*, and how to act on them by drugs or other means to prevent it in cancer therapeutics.
- Modelling *blockade of uncontrolled plasticity* and re-establishing control on it.
- Modelling and understanding the interactions of *cancer stem-like cells* with the micro-environment and understanding how drugs affect such interactions.
- Modelling interactions between cancer cells and the immune response (cellular or soluble, *inflammatory* or not, efficacious or adverse) and how to guide them.
- Modelling *non-cell killing, redifferentiating therapies*, such as ATRA in Acute Promyelocytic Leukaemia (AML3), and their optimal combination with cytotoxic therapies (e.g., anthracyclins).
- Developing *methods of detection and optimal control in cancer therapeutics*, making use of realistic means of action on cancer cell populations, taking into account their impact on healthy cells, and *transfering them to clinical settings*.