From mathematical modelling of cancer cell plasticity to philosophy of cancer

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Joint work at LJLL with Rebecca Chisholm, Tommaso Lorenzi, Alexander Lorz, Benoît Perthame, Camille Pouchol, Emmanuel Trélat

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

Cancer puzzle: beyond intracellular signalling pathways

- Cancer is a disease of multicellular organisms: it makes little sense (except for monogenic cases: CML, APL,...) to search for its *determinants* in single cells
- Cancer is a localised loss of coherence between cells and tissues in a multicellular organism: loss of control on cell differentiations, prior to enhanced proliferation
- The atavistic hypothesis of cancer by Davies, Lineweaver and Vincent (2011) proposes an evolutionary origin for cancer cell populations
- Between-species phylostratigraphic analyses associate genes of multicellularity and genes altered in cancer: Domazet-Lošo & Tautz (2010)
- What is coherence/cohesion within/between cells and tissues in an organism? Why and how is it disrupted in cancer?

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Motivations from, and focus on, drug resistance in cancer

- Slow genetic mechanisms of 'the great evolution' that has designed multicellular organisms, together with fast reverse evolution on smaller time windows, at the scale of a human disease, may explain transient or established drug resistance.
- Intra-tumour heterogeneity, here meant as between-cell phenotypic variability within cancer *cell populations*, w.r.t. a given relevant trait, is a relevant setting to represent continuous evolution towards drug resistance in tumours.
- Plasticity in cancer cells, i.e., epigenetic propension to reversal to a (stem-like?) de-differentiated status, and resulting adaptability of cancer cell populations, makes them amenable to resist abrupt drug insult as extreme stress response.
- Reversible plasticity is captured by mathematical models that incorporate between-cell heterogeneity by making use of continuous phenotypic variables.
- Such models are compatible with optimal control methods for the design of therapeutic strategies involving combinations of cytotoxic and cytostatic drugs

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Plan of the talk

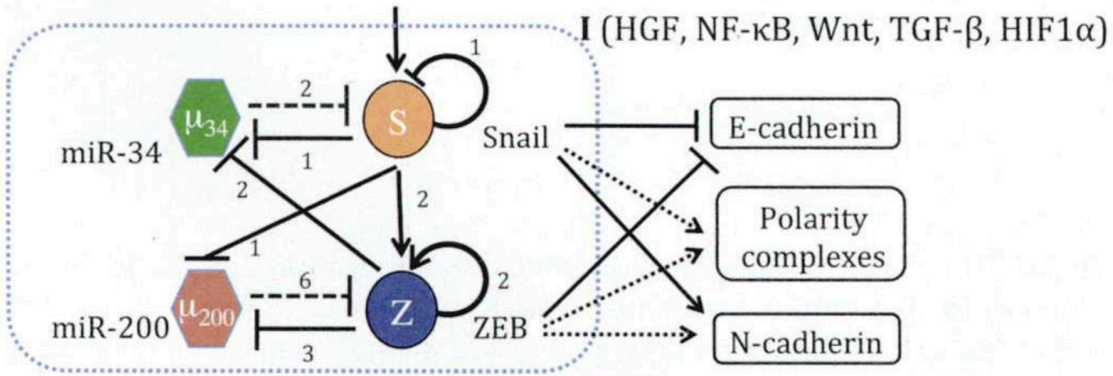
1. Facts about cell plasticity in cancer
2. Modelling cell population plasticity by structured equations
3. Sources of cell plasticity in cancer
4. What is a multicellular organism and how is it 'de-unified'?
5. Possible consequences for therapeutics in oncology

1. Facts about cell plasticity in cancer

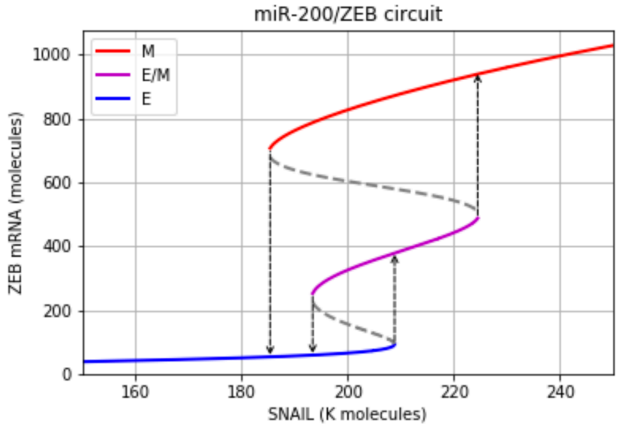
- Major concerns in oncology: drug resistance and metastases
- Established drug resistance as evolutionary rescue
- Experimental evidence of cell plasticity in cancer
- Genetic mutations and/or phenotypic switches

Pitfalls of therapy: side effects, drug resistance, metastases

- Unwanted side effects of drug treatments on healthy tissues *must be taken into account in a therapeutic optimisation perspective*, however healthy cell populations are terminally differentiated and are not concerned by plasticity.
- Genetically established drug resistance in cancer cell populations, due to mutations, may be favoured by non-genetic adaptations that are manifestations of cancer cell plasticity: persistence and tolerance to drug treatments.
- Metastases, generalising an initially localised disease to the whole organism, rely on the reversible eptithelial to mesenchymal transition (EMT/MET), a typical manifestation of non genetic cancer plasticity.



(MY Lu et al., PNAS 2013)



(J. Guilberteau, Inria Report 2020)

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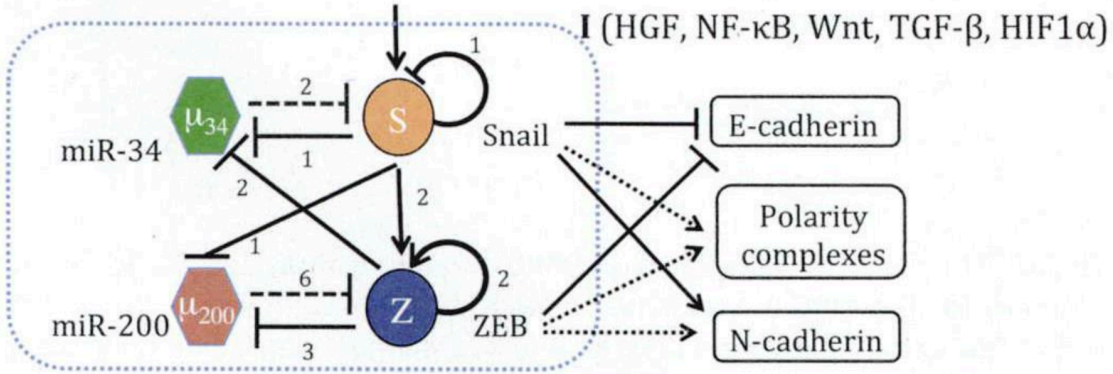

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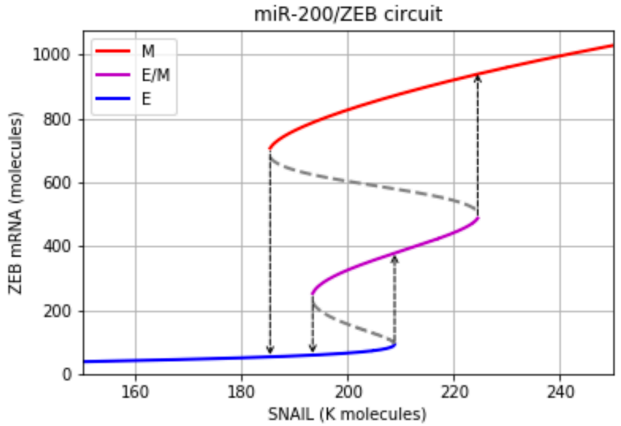

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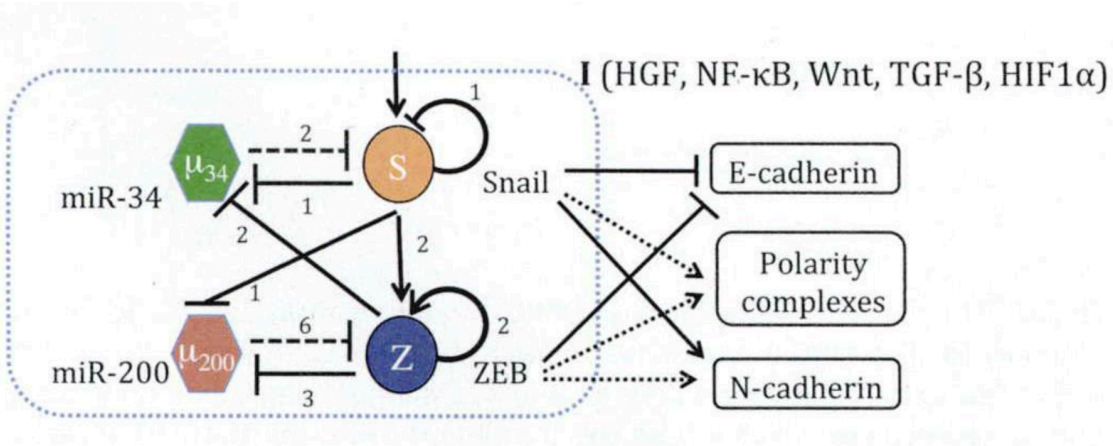

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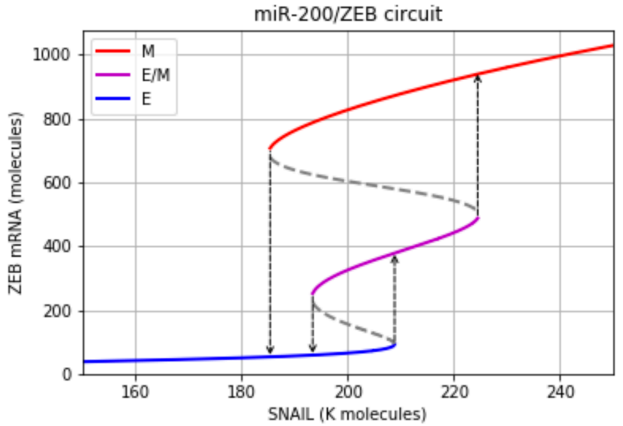

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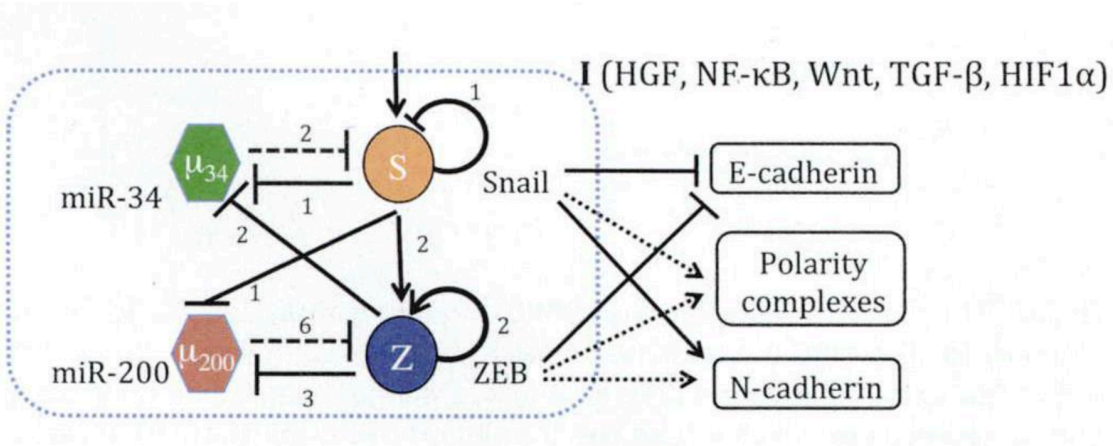

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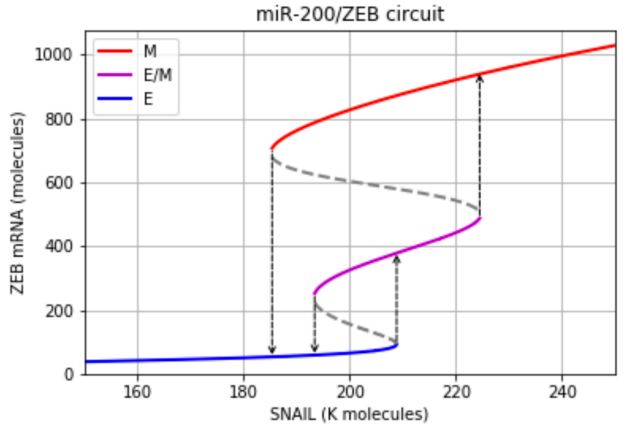

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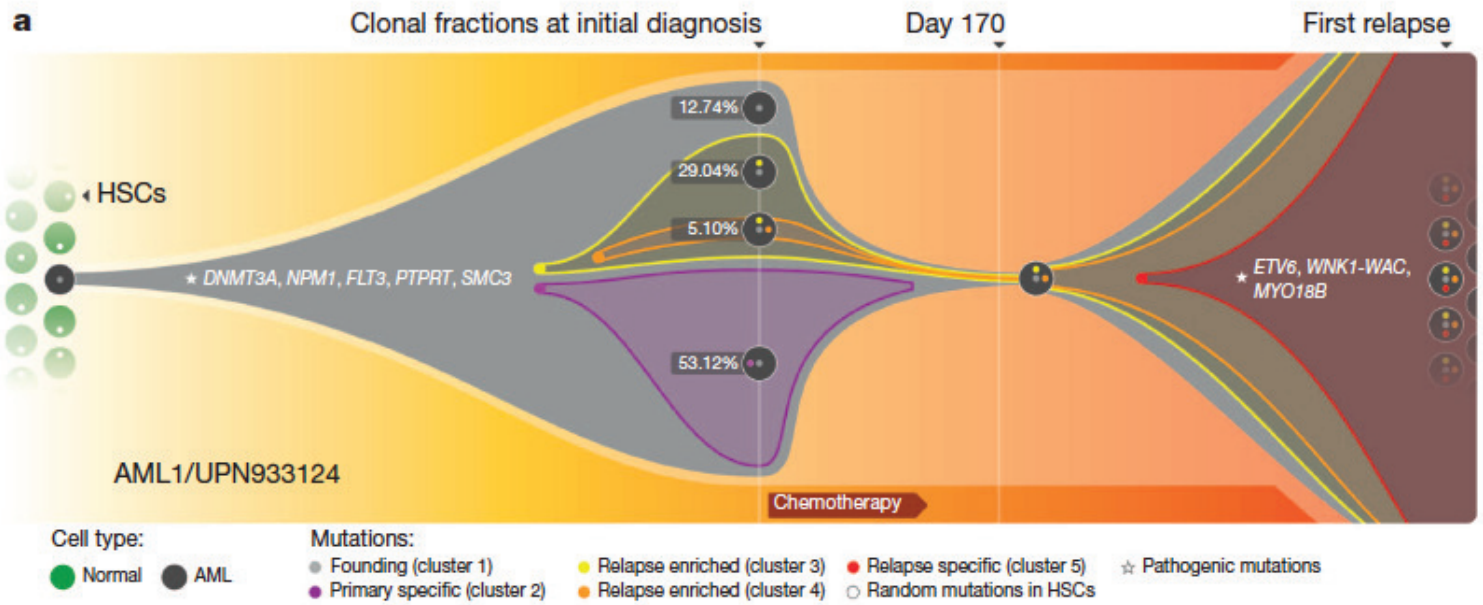
Established drug resistance as evolutionary rescue

- Animal genome (of the host to cancer) is rich and amenable to adaptation scenarios that may recapitulate developmental scenarios - thus resulting in insufficient cohesion of the ensemble - that were normally abandoned in the process of evolution towards stable metazoa (*Davies & Lineweaver 2011*).
- In cancer cell populations, enhanced heterogeneity with enhanced proliferation results in a high phenotypic or genetic diversity of proliferating clonal cell subpopulations.
- Drug therapies may be followed, after initial success, by relapse due to selection of a resistant clone: in the words of evolutionary biology, an evolutionary rescue.



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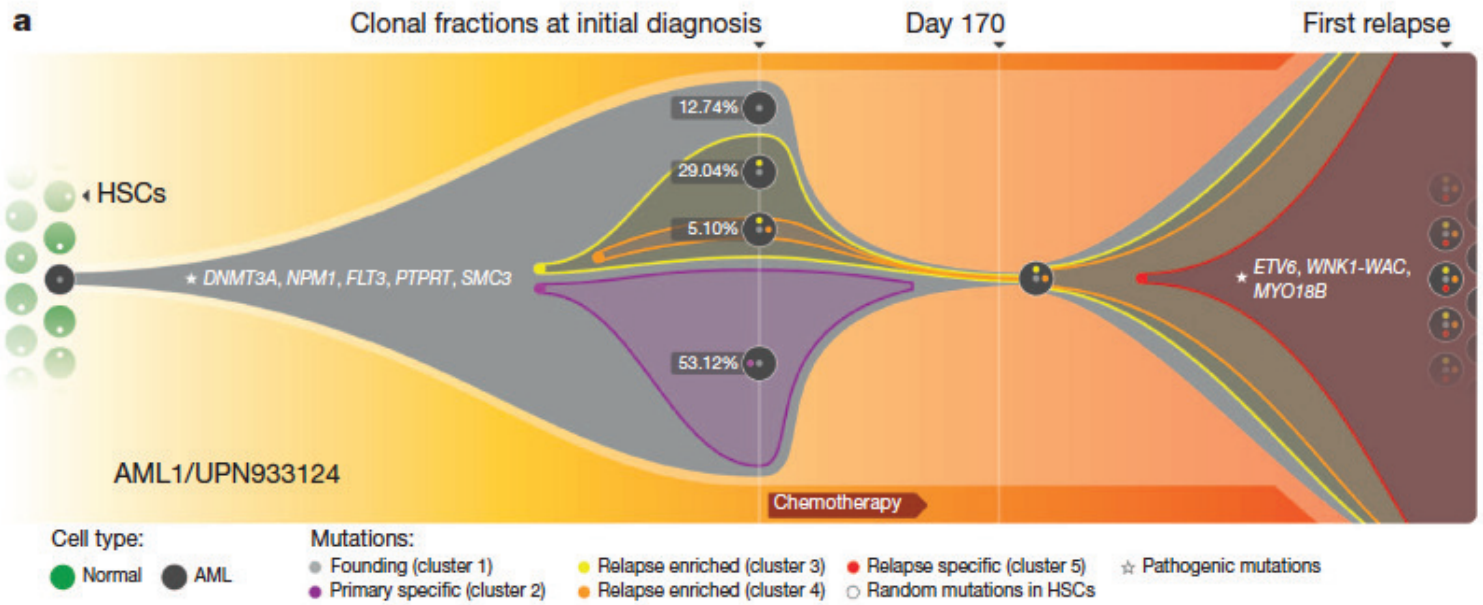

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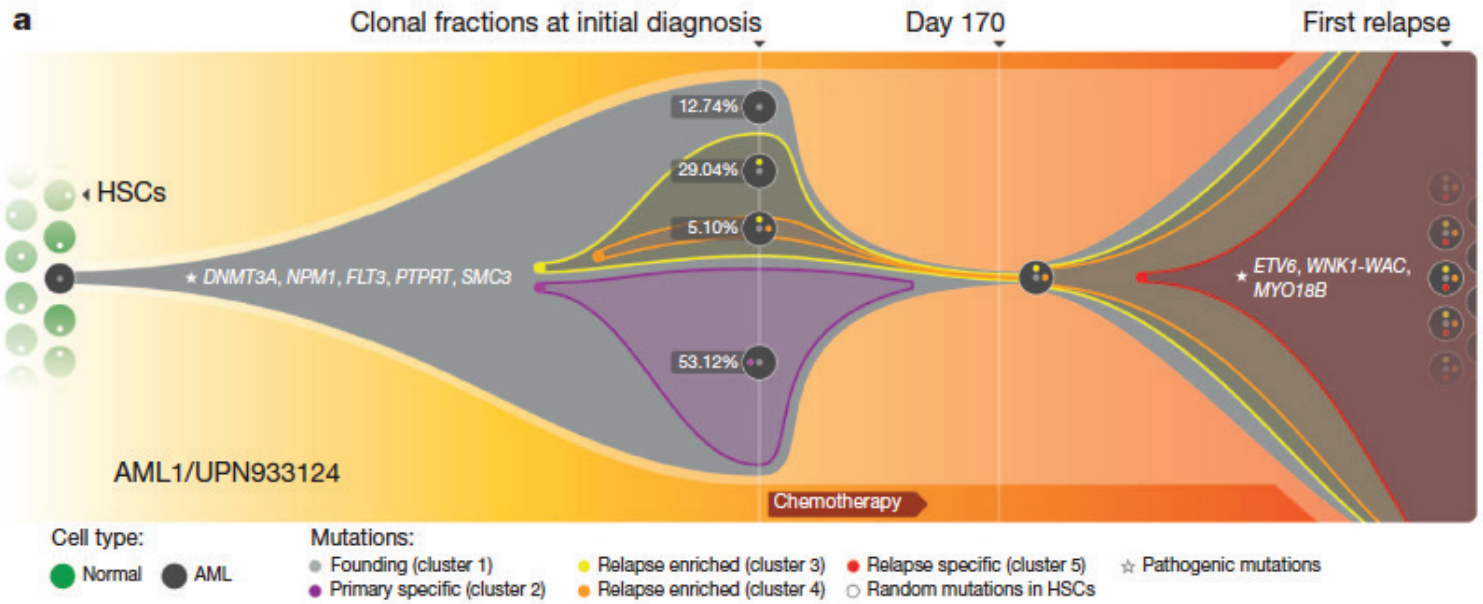

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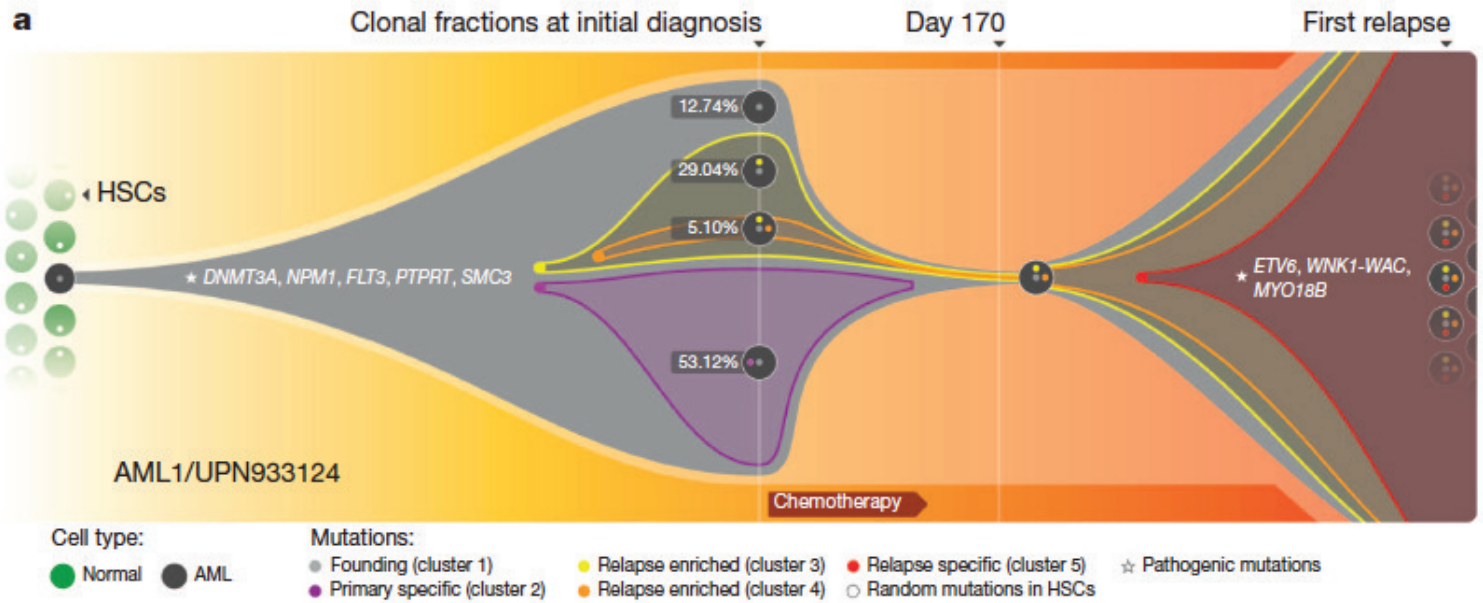

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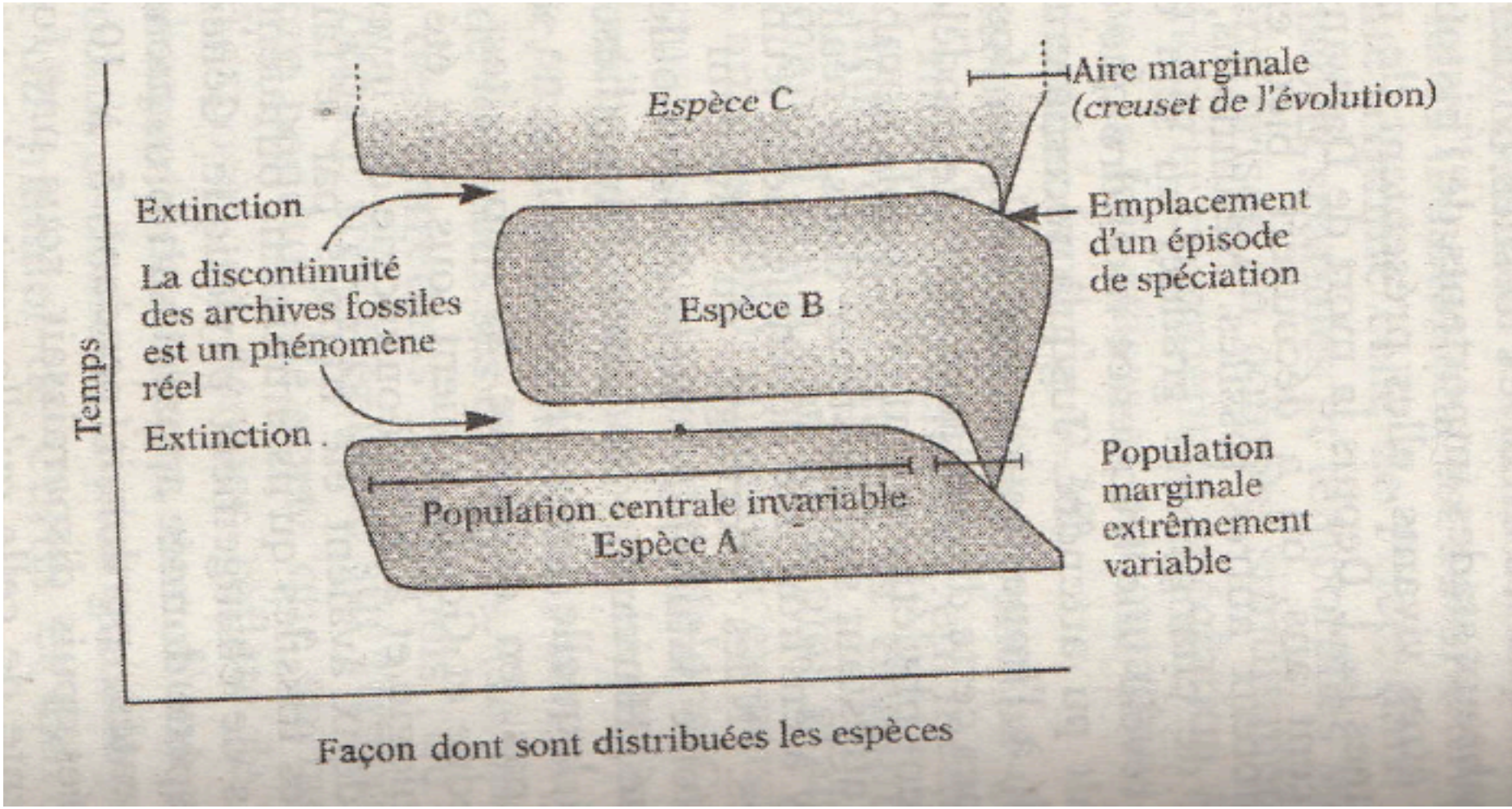
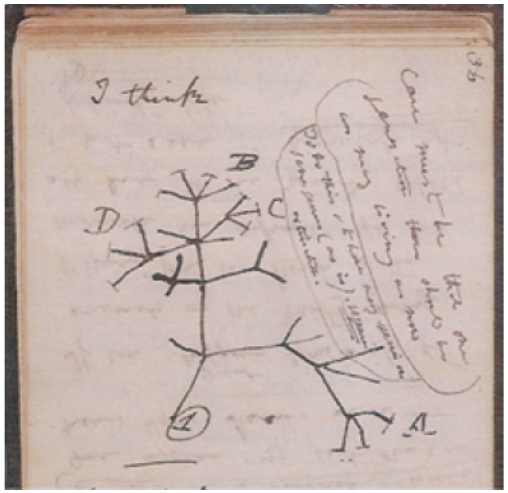
[My vision of evolutionary rescue: PW Price and SJ Gould]

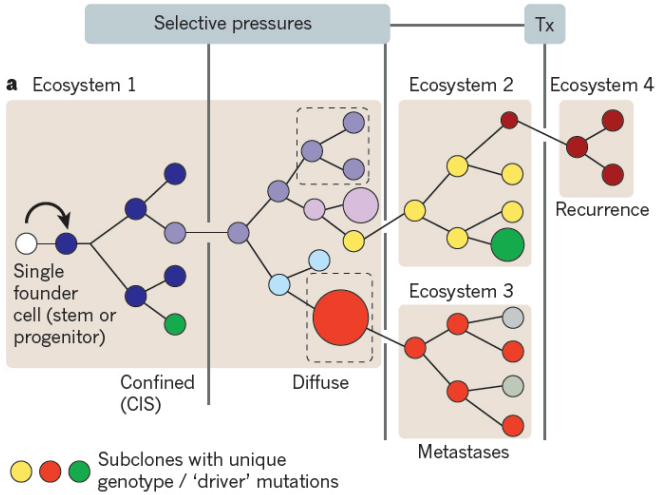


(SJ Gould 'Punctuated equilibrium' 2007, citing PW Price 'Biological evolution' 1996)

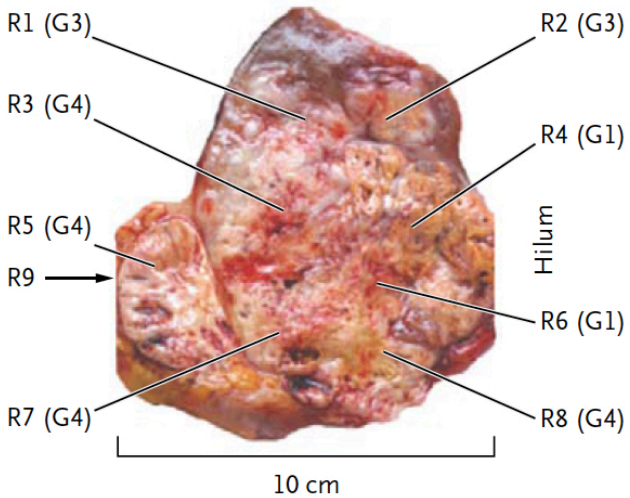
Mutations and evolutionary branching in solid tumours

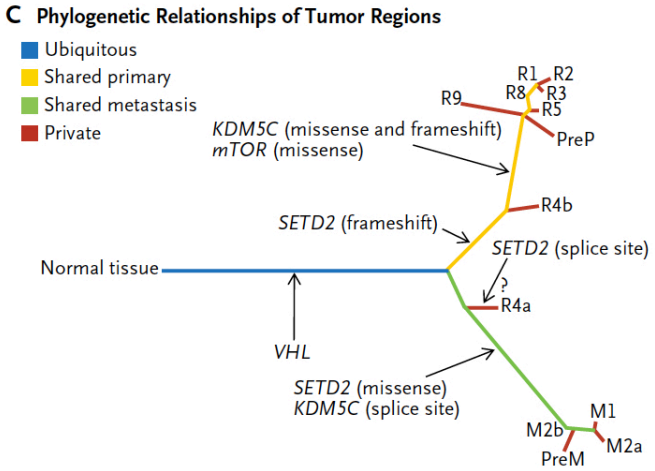


Darwin's notebook 1837



Maley & Greaves Nature 2012

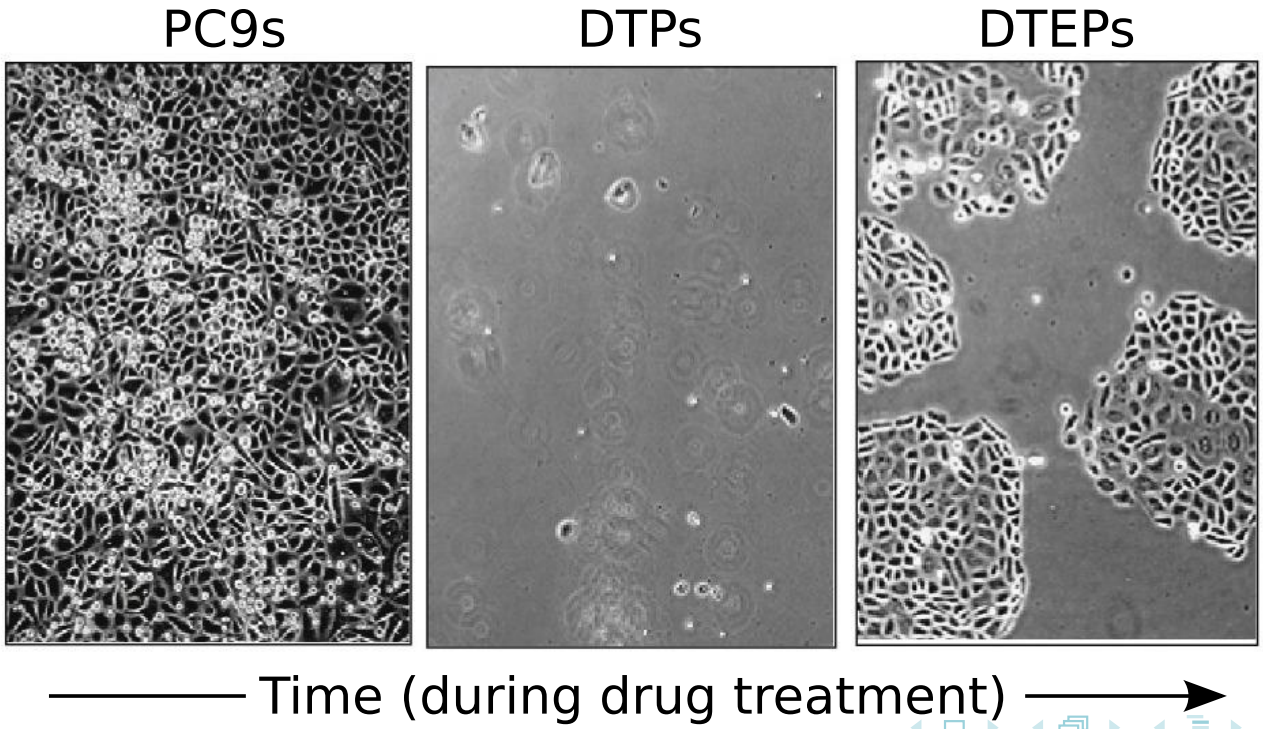




Gerlinger et al. NEJM 2012

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



(Sharma et al. Cell 2010)

Genetic mutations or phenotypic switches?

- EMT/MET and *drug persistence* (if a *prolonged* drug-insensitive subpopulation can be identified), or *drug tolerance* (if the whole population is concerned by *transient* treatment escape), are non-genetic adaptive, *reversible* mechanisms that rely on environment-induced phenotypic switches.
- Whereas drug resistance relies on established, *irreversible*, genetic mutations.
- However, cannot prolonged tolerance induce generalised stable persistence, that itself may promote (by selection on genetically instable cells) irreversible drug resistance by mutations?
- Indeed, it has been reported that epigenetic silencing by methylation makes single nucleotide C to T mutations on the DNMT3A locus highly probable, entraining in turn more epigenetic alterations *(You & Jones, Cancer Cell 2012)*.

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2. Modelling cell populations by structured equations

- Adaptive mechanisms in drug persistence or tolerance
- Phenotype-structured cell population dynamic models
- Adaptive dynamics: cell population asymptotic behaviour
- Optimal control therapeutic strategies in oncology

Structured cell population model: *cell-functional* variables

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

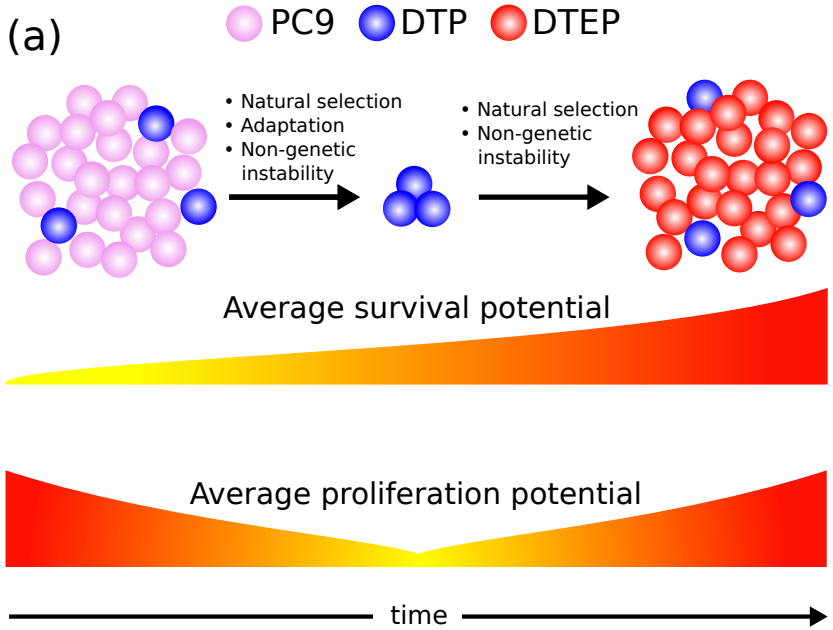
- Population density of cells $n(x, y, t)$ with phenotypic expression (x, y) at time t satisfies

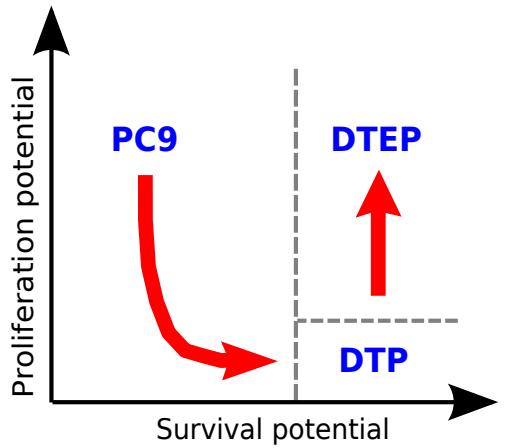
$$\frac{\partial n}{\partial t}(x,y,t) + \underbrace{\frac{\partial}{\partial y}\Big(v(x,c(t);\bar{v})n(x,y,t)\Big)}_{\text{Drift=stress-induced adaptation of the proliferation level}} = \underbrace{\Big[p(x,y,\varrho(t)) - d(x,c(t))\Big]n(x,y,t)}_{\text{Non local Lotka-Volterra selection}} + \underbrace{\beta\Delta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

- $\varrho(t)=\int_0^1\int_0^1 n(x,y,t)\,dx\,dy$, $p(x,y,\varrho(t))=(a_1+a_2y+a_3(1-x))(1-\varrho(t)/K)$ and $d(x,c)=c(b_1+b_2(1-x))+b_3$
- The drift (=advection) term w.r.t. proliferation trait y represents possible (if $v \neq 0$) 'Lamarckian', 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

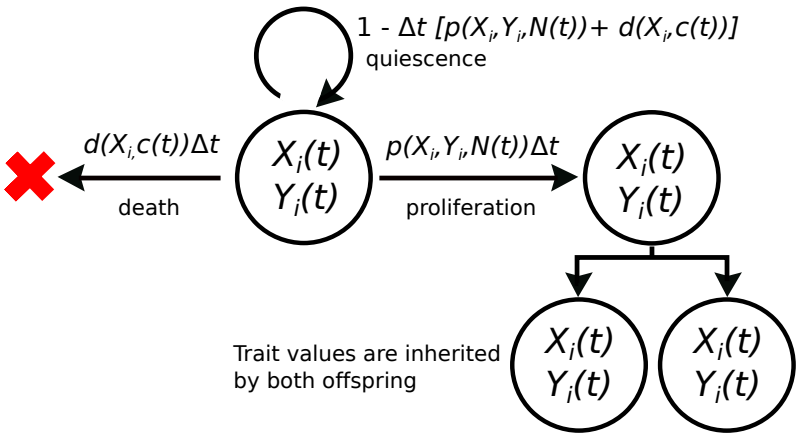
Chisholm et al., Cancer Research 2015
(inspired by Sharma et al. Cell 2010)

Same framework using an agent-based model (ABM)





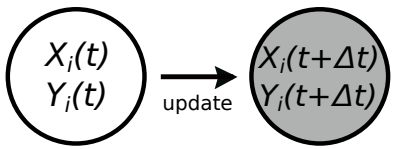
(a) Each cell i undergoes either proliferation, death or remains quiescent:



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

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

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

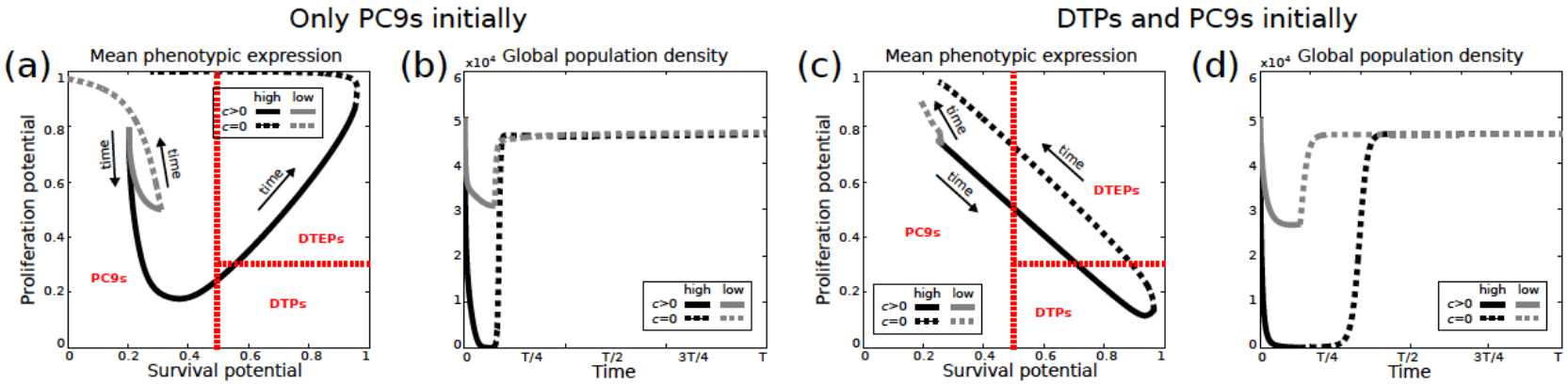


Chisholm et al., Cancer Research 2015

Resensitisation after drug washout is in the model

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

Two scenarios: Lamarckian adaptation, or sheer Darwinian selection of the fittest

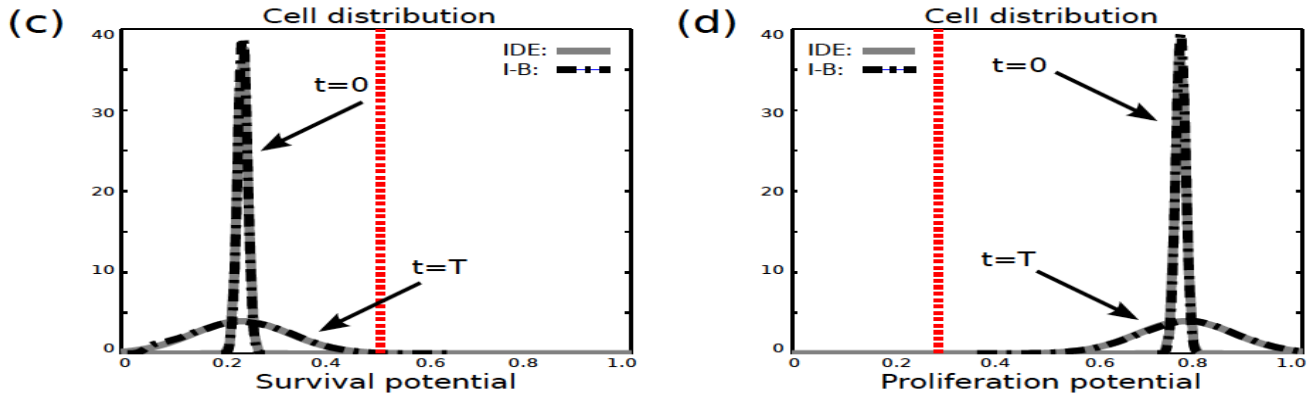


(a), (b) Only PC9s (no DTPs initially), adaptation on ($v \neq 0$): *'Lamarckian' scenario*

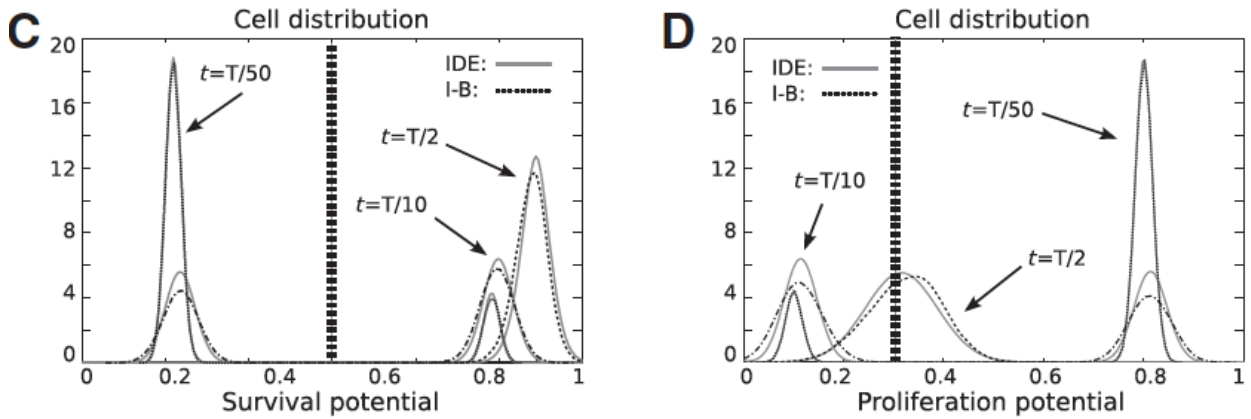
(c), (d) PC9s and DTPs initially, no adaptation ($v = 0$): *'Darwinian' scenario*
(sheer selection of the fittest = DTPs, supposed to be present in the initial population)

Chisholm et al., Cancer Research 2015

Phenotype heterogeneity in the cancer cell population



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 (or AB) model to address 3 questions

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

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

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

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

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

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

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

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

Q3. Never! Whatever the scenario

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

- Both mathematical models (AB, IDE) reproduce the main experimental observations
- To see the transient appearance of the DTPs during high-dose drug therapy:
  - If there are **some DTPs** present initially, model explanation requires only
    - non-genetic instability
    - selection
  - If **no DTPs** are present initially, model explanation requires interplay between
    - stress-induced adaptation
    - non-genetic instability
    - selection

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

Phenotype-structured adaptive dynamics to represent the fates of heterogeneous cell populations

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

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

- (2) Distribution of the trait in the population and its asymptotics

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

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

Adaptive dynamics: cell population asymptotic behaviour

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

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

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

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

with

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

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

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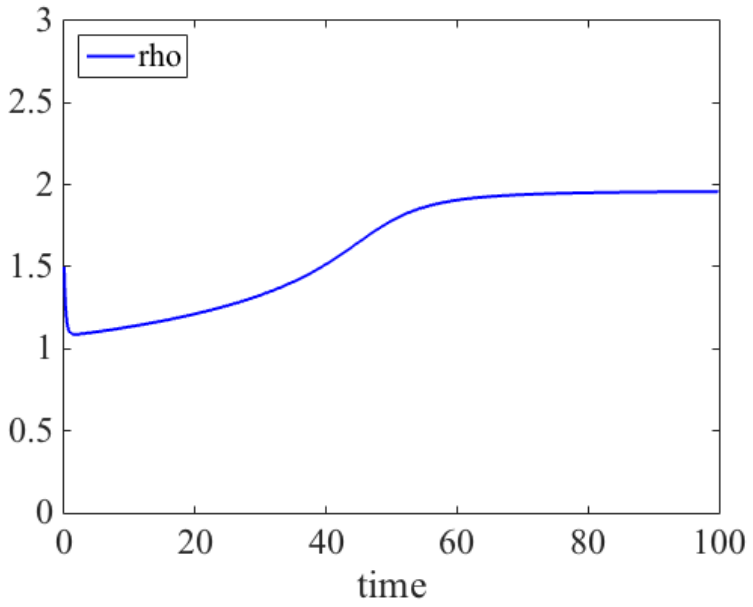
Non-local Lotka-Volterra 1D model: convergence in time

Convergence (one-population case): plot of $t \mapsto \rho(t)$



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

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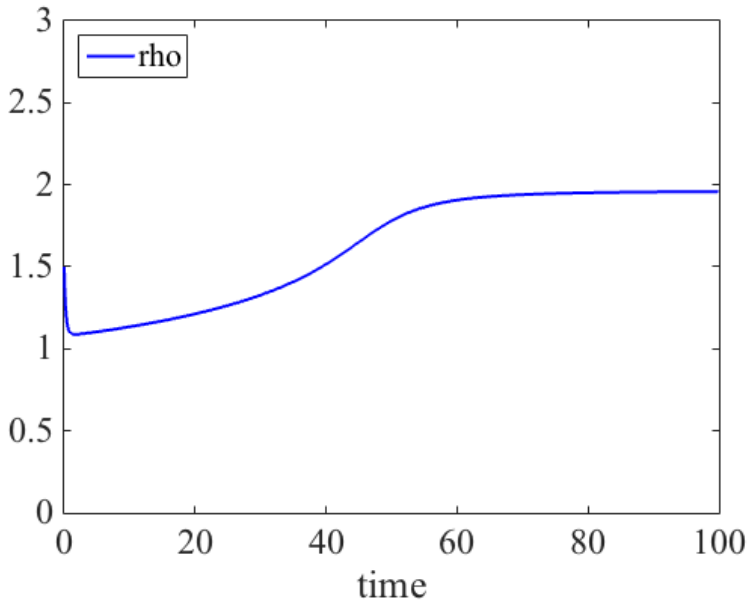

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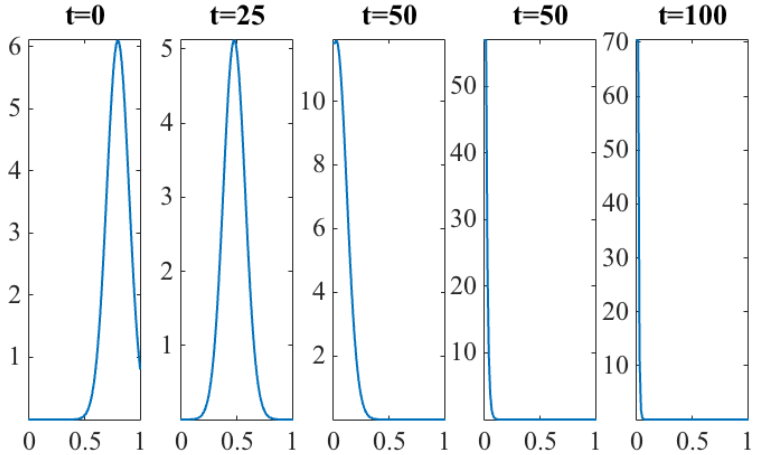
Non-local Lotka-Volterra 1D model: concentration in x

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



Theorem

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

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

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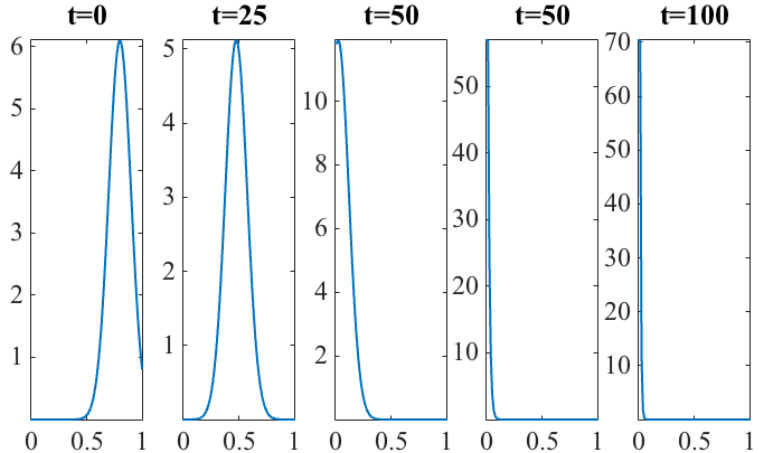

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Non-local Lotka-Volterra 1D model: convergence and concentration using a Lyapunov functional

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

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

one can show that

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

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

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3. Sources of cell plasticity in cancer

- Physiological framework: development and tissue repair
- Loss of control on differentiations in cancer
- Cancer stem cells? Not necessarily needed
- Cellular stress: launching de-differentiation signals?

Physiological framework: 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 obviously of epigenetic nature), 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.

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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
- In castration-resistant 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.

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Cancer stem cells? Not necessarily needed

- Cancer stem cells have been proposed to be at the origin of cancers. This may be so for a number of cancers, however not for all of them.

- In particular, cancer may occur at intermediate stages of differentiation, as in the case of acute myeloid leukaemia in the old French-American-British (FAB) classification. For instance, reestablishing impaired differentiation by ATRA at the promyelocytic stage (AML3, aka APL) of myeloid differentiation cures the disease (where are the leukaemic *stem* cells?)

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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 (drug, hypoxia, reactive oxygen species, radioisotopes) launches a variety of response signals, with internal or external destination. Noteworthy is the high potential of cellular stress to induce cell differentiation: see *Wagner et al. Bioessays 2019, Nedelcu & Michod Bioessays 2020*.

- Under extreme stress (Multiple Myeloma exposed to doxorubicin, *A. Wu et al. PNAS 2015*), cancer cells overexpress 'cold genes', i.e., ancient genes that are never substituted, thus being 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 resistant cancer cells.

- Could the expression of these '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 ancient genes, dating back to unicellular ancestors that were able to resist extreme stress conditions on our planet, such as toxic molecules, UV radiations, hypoxia, hyperacidity, etc.?

- This speculation refers to the so-called 'atavistic theory of cancer' (Davies, Lineweaver and Vincent 2011), according to which cancer 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 launch the bases of multicellularity (cooperativity, motility, plasticity) for its own benefit.

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- Under extreme stress (Multiple Myeloma exposed to doxorubicin, *A. Wu et al. PNAS 2015*), cancer cells overexpress 'cold genes', i.e., ancient genes that are never substituted, thus being 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 resistant cancer cells.

- Could the expression of these '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 ancient genes, dating back to unicellular ancestors that were able to resist extreme stress conditions on our planet, such as toxic molecules, UV radiations, hypoxia, hyperacidity, etc.?

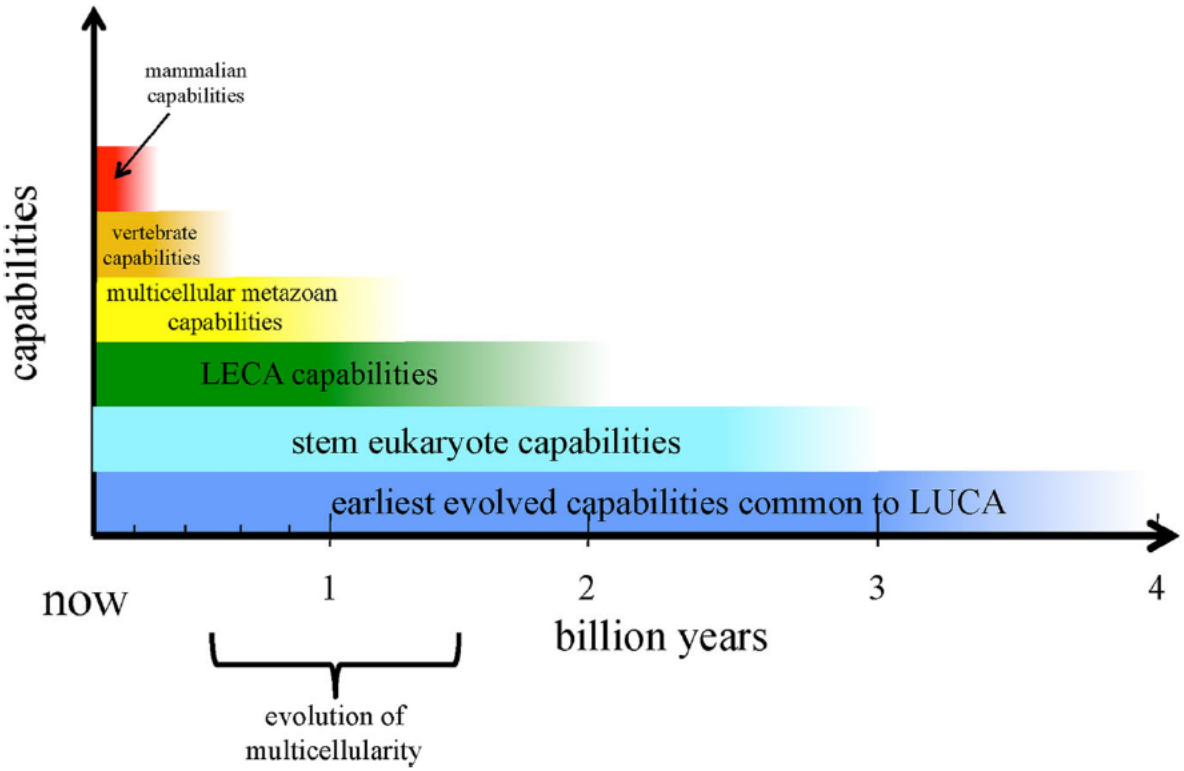
- This speculation refers to the so-called 'atavistic theory of cancer' (Davies, Lineweaver and Vincent 2011), according to which cancer is a very primitive state of multicellularity, unable to lead to a cohesive multicellular organism by lack of a coherent development program, and nevertheless trying to launch the bases of multicellularity (cooperativity, motility, plasticity) for its own benefit.

4. How may a multicellular organism be 'de-unified'?

- Evolution of multicellular organisms: atavistic theory of cancer
- The metaphoric Waddington epigenetic landscape
- A complementary metaphor: the wickerwork basket
- What sort of disruptions may elicit cancer?

A possible 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)



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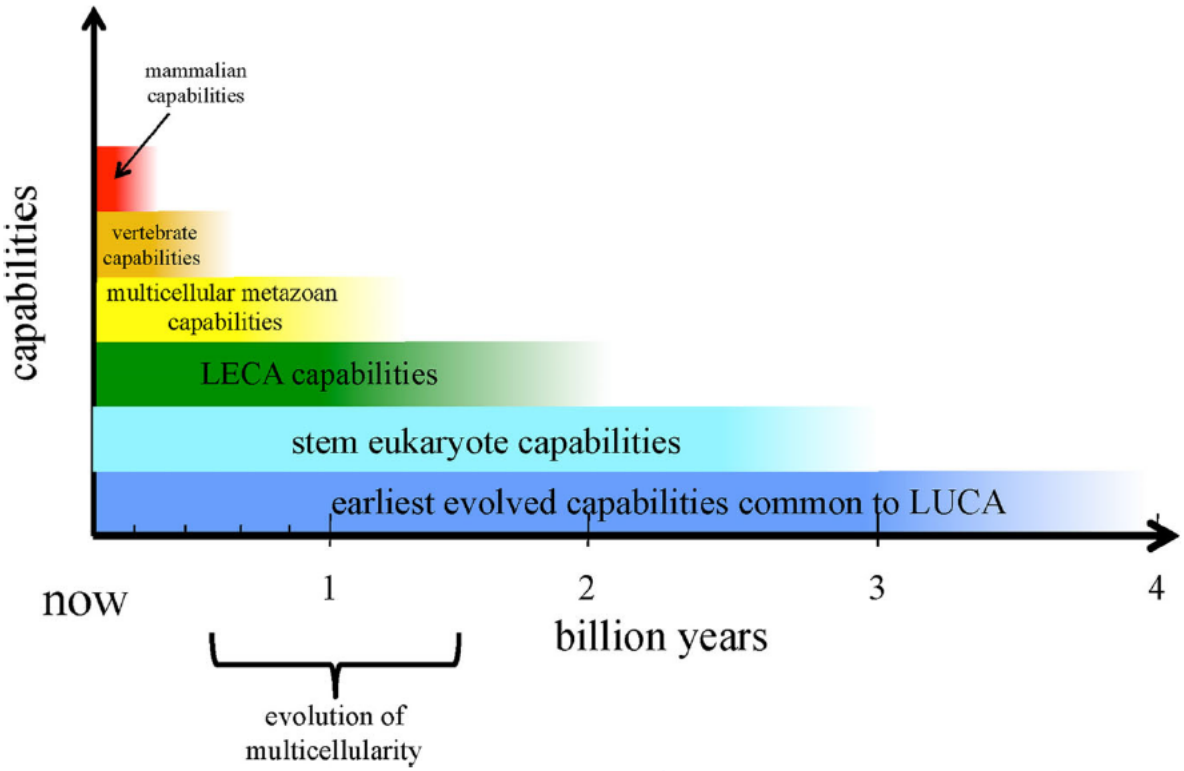

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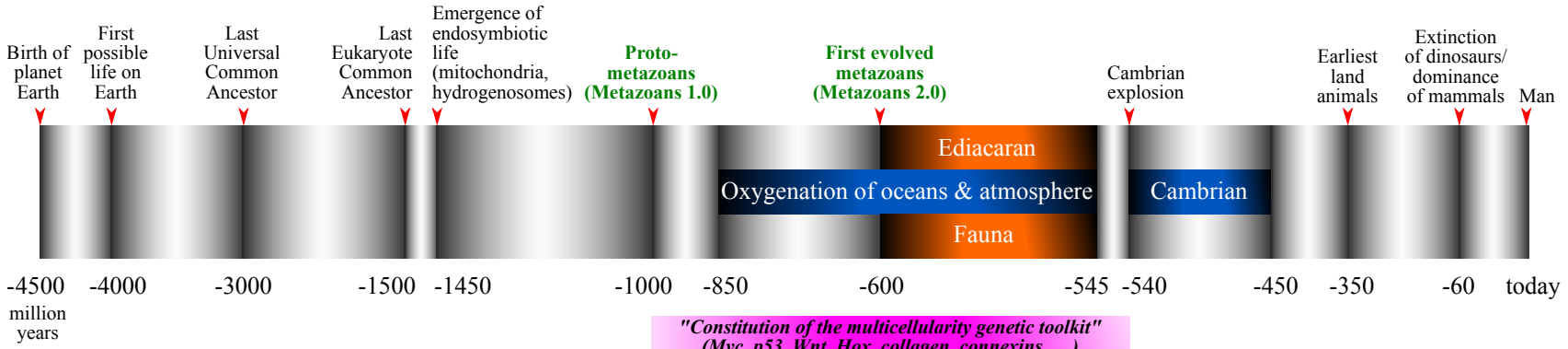
A possible evolutionary framework (*billion year-term view* for multicellular organisms): the atavistic theory of cancer (2)



(*see Chisholm et al. 2016, BBA General Subjects DOI:10.1016/j.bbagen.2016.06.009*)

- The genes that have appeared in the development to multicellularity are those that are altered in cancer (as shown in phylostratigraphic analyses by Domazet-Lošo & Tautz 2010; investigated by Trigos *et al.* 2017, 2018, 2019)
- In order, in evolution, from 1) proliferation+apoptosis to 2) cell differentiation + division of work, and to 3) *epigenetic control* of differentiation and proliferation? (reverse mutation order w.r.t. *Hirsch et al. Nature Comm. 2016*)
- Reconstituting the phylogeny of this 'multicellularity toolkit' should shed light on the robustness or fragility of genes that have been altered in cancer.
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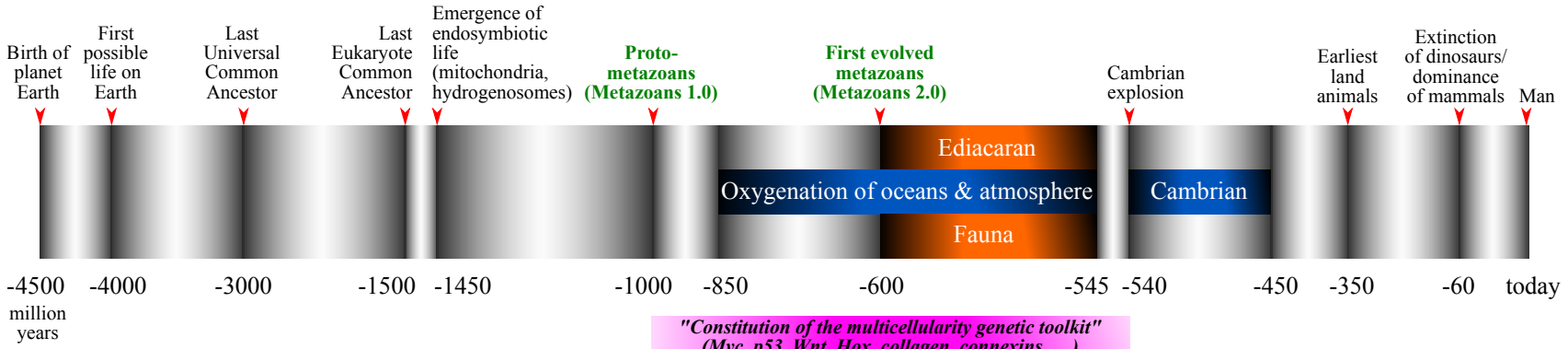

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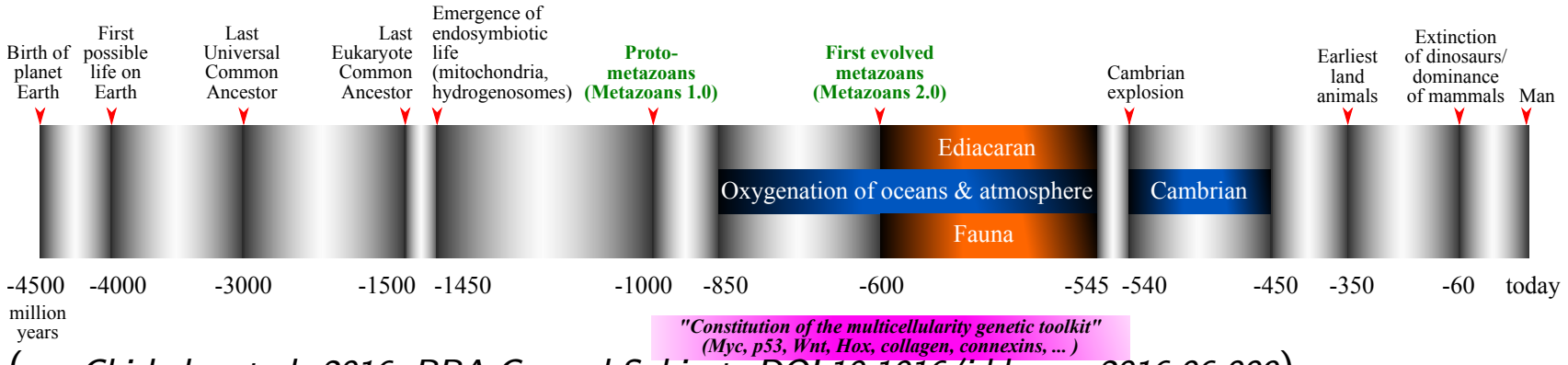

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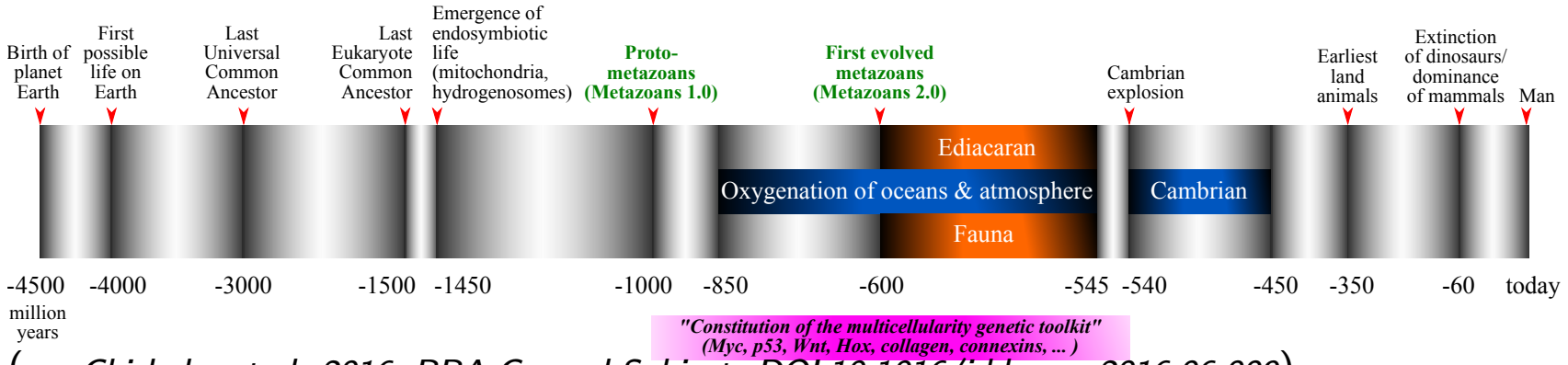

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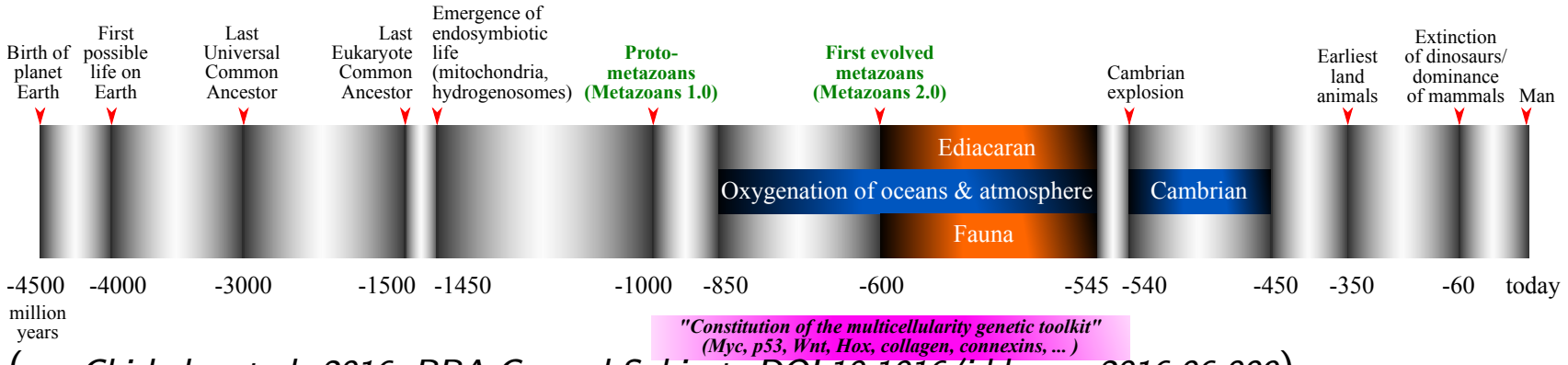

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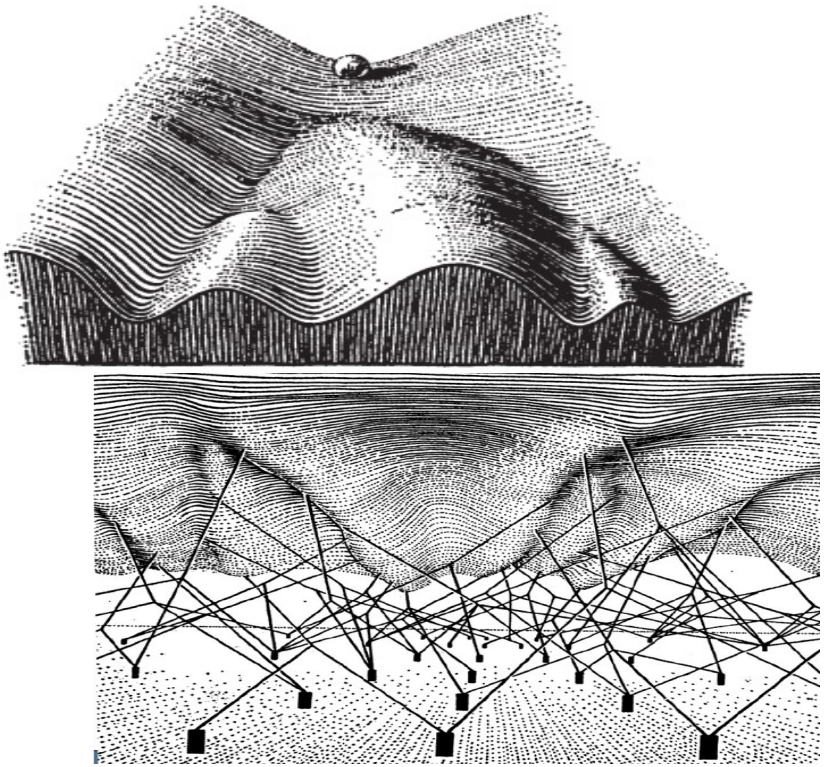
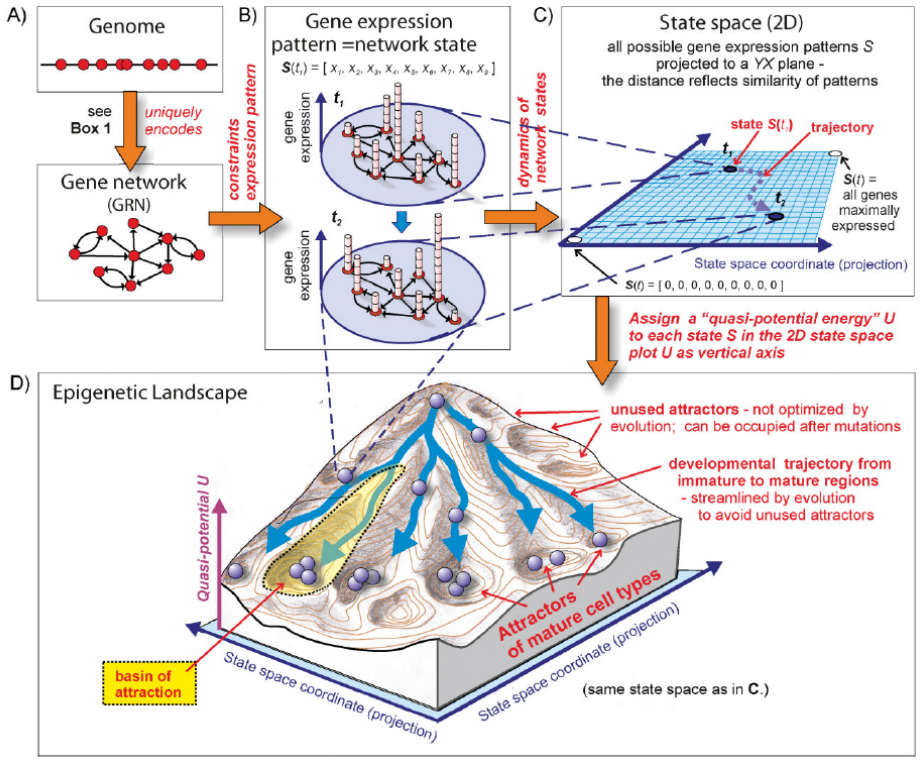
Another evolutionary framework (*life-term view*): revisiting the Waddington epigenetic landscape

The classic Waddington landscape ("The strategy of genes", 1957) for cell differentiation



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



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

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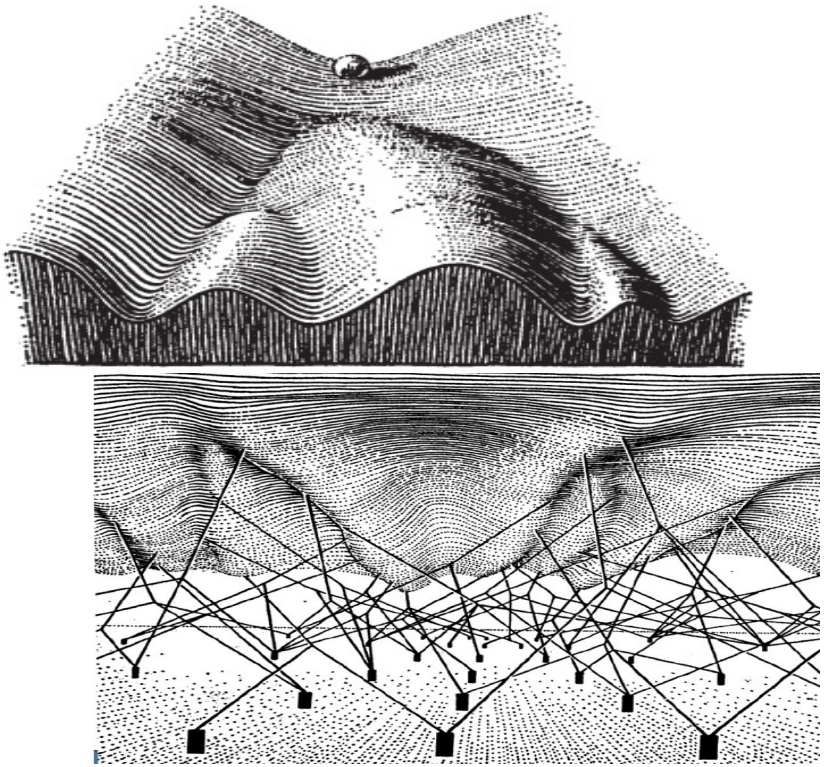

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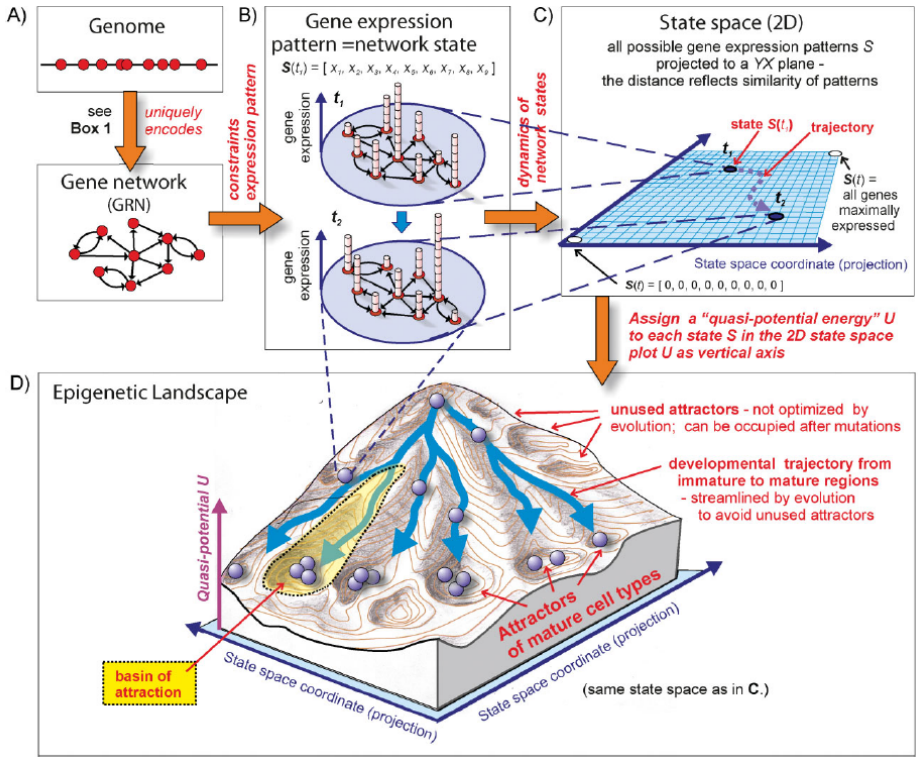

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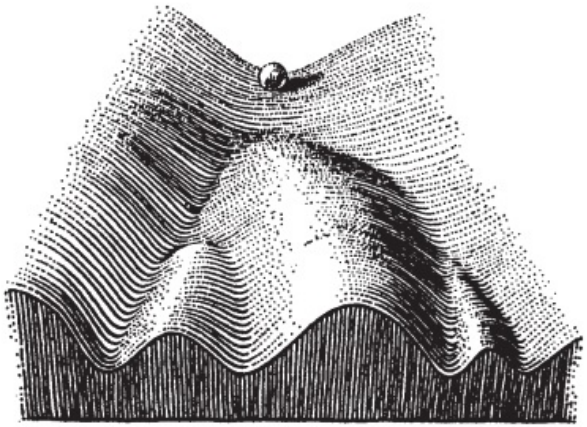
Milestones to reconstruct the global differentiation landscape



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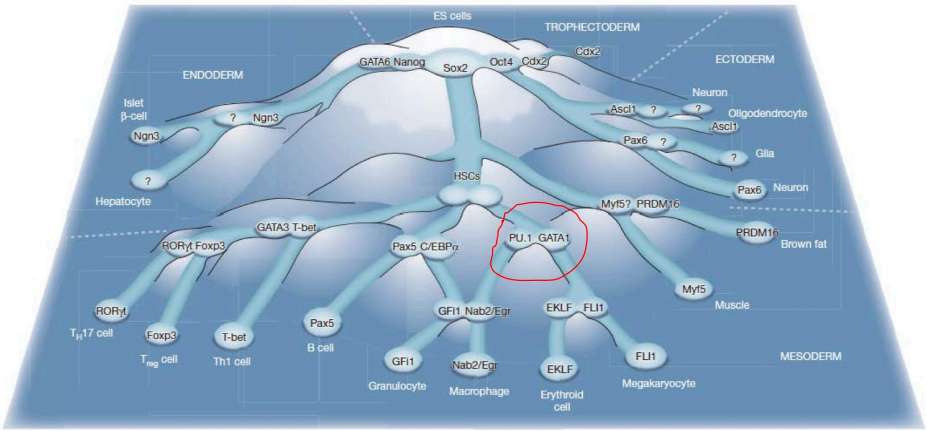
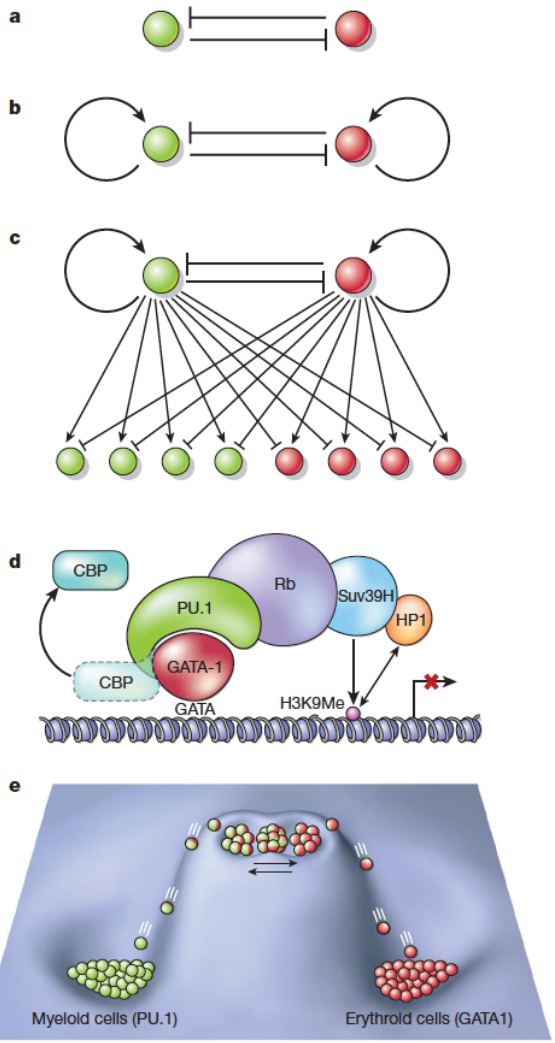


Stem cell fate: modern version by Tariq Enver *(ASH meeting 2011)*



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

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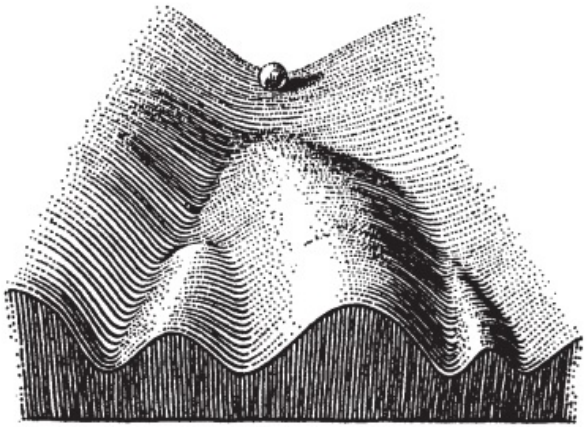

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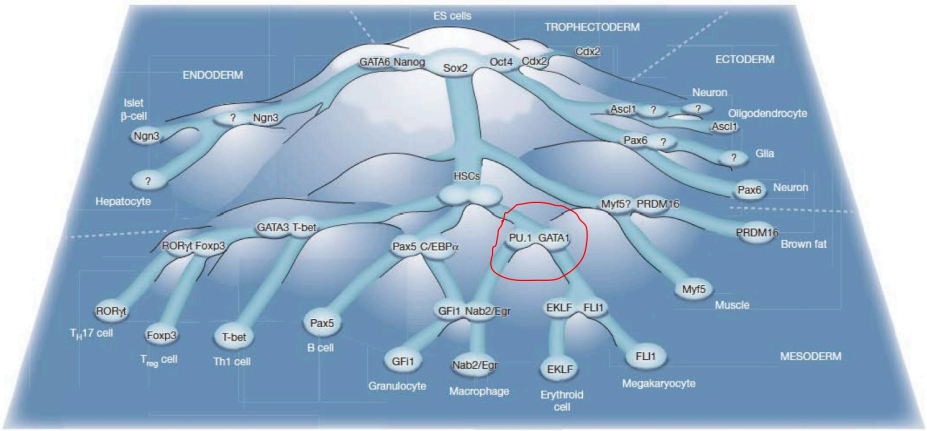

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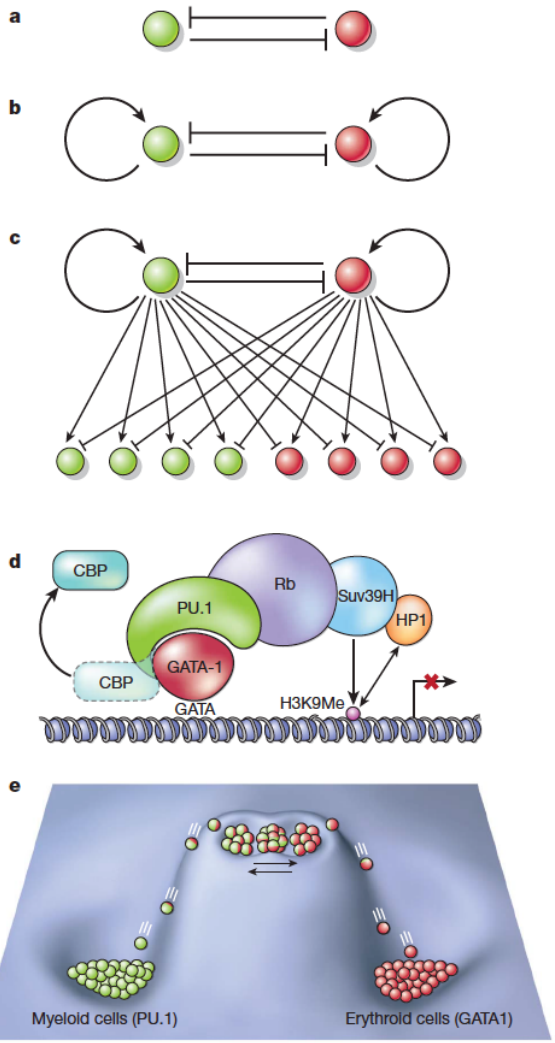

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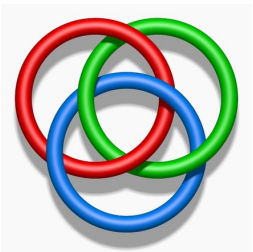
Differentiation control by what? Making a multicellular organism, another metaphor: the wickerwork basket

The base is the body plan. The fibres are the cell differentiation trees. The rim of tips is where are the terminally differentiated cells. Intertwining the trees/twigs that stem from the base is the work of between-fibre connections, part of a proposed extended vision of the immune system, the *cohesion watch*. These 3 elements: (1) body plan, (2) differentiation trees and twigs and (3) *cohesion watch* make sense only together in a *Brunnian structure*, better known in the most elementary case as a Borromean knot.



What sort of disruptions may elicit cancer?

- Fibres may be fragile and break. For instance, in the case of acute myeloid leukaemia (AML), differentiation in the haemopoietic tree is blocked at different possible stages.Then immature cells accumulate at these stages and invade surrounding structures (bone marrow, then blood).

- This illustrates the fact that cancer may primarily be due to a loss of control on differentiations in a most abrupt way: blockade, by breaking of a differentiation fibre, a rough pathology of the vertical *cohesion watch* (along fibres).

- More commonly, loss of control on differentiations may be due to impaired connections between fibres. When neighbouring differentiation trees are not clearly determined (as in the case of histological poor separation between oesophagus and stomach epithelia, or duct and endometrium epithelia), then immature cells may develop and proliferate, uncontrolled.

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Modelling *bet hedging* in cancer cells using a 3D cell-functional phenotype for population heterogeneity?

- What is more relevant for cellular stress response of a cell population (adaptable, as in the case of a tumour): maintain a subpopulation of all-stress resistant cells, or maintain a subpopulation of *plastic* cells expressing 'cold genes' (*Wu et al. PNAS 2015*), able to launch different resistance mechanisms in different cells? (About cellular stress-induced differentiation, see also *Wagner et al. Bioessays 2019, Nedelcu & Michod Bioessays 2020*.)

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

- Two conflicting phenotypes x and y, and a third one coding for cell pasticity, θ.

$$\partial_t n + \nabla \cdot \{V(x,y,\theta,D)\, n - A(\theta)\nabla n\} = n\{r(x,y,\theta) - d(x,y,\theta)\rho(t) - \mu(x,y,\theta,D)\}$$

- More generally, model for evolution in cell populations structured according to *conflicting* phenotypes x and y only bound by a constraint like $C(x,y) \leq k$? (adhesivity/motility, fecundity/motility, germinal/somatic) yielding either a homogeneous population of hybrid cells, or a heterogeneous cell population of two sticking together subpopulations separately maximising each phenotype. *Is not the latter choice at the origin of multicellularity in eucaryote cell populations, admitting that tumours constantly reinvent multicellularity?*

(*with F.E. Alvarez Borges, work underway*)

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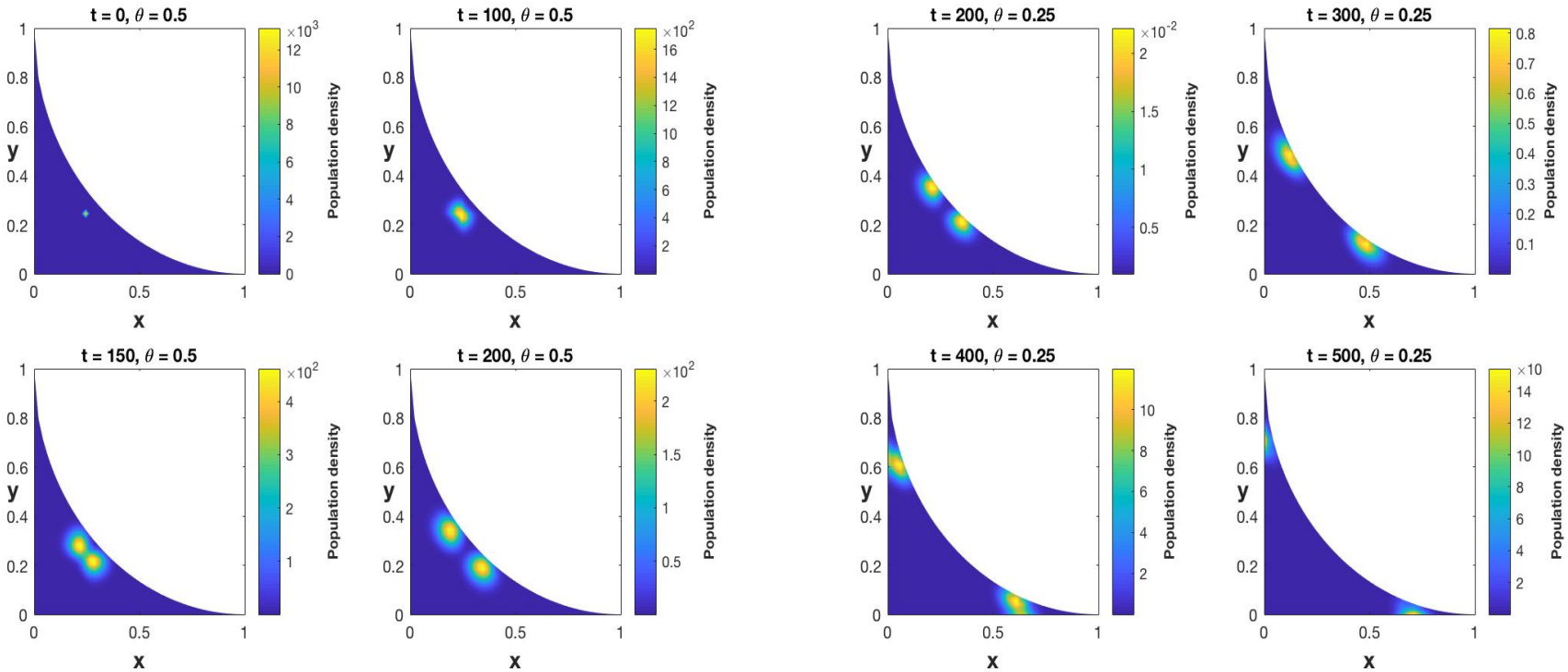
Illustrations for this 3 cell-functional trait equation

With $(x, y) \in \Omega = \{(x-1)^2 + (y-1)^2 > 1\}$, no drug D, growth and death rates as:
$r(x, y, \theta) = 1_{\{y>x\}} e^{-(0.1-x)^2-(0.9-y)^2} + 1_{\{x \geqslant y\}} e^{-(0.1-y)^2-(0.9-x)^2}$, $d(x, y, \theta) = \frac{1}{2}$.
Diffusion matrix and advection term:

$$A(\theta) = \begin{pmatrix} (\theta+1)10^{-6} & 0 & 0 \\ 0 & (\theta+1)10^{-6} & 0 \\ 0 & 0 & 10^{-6} \end{pmatrix},$$

$$V(x, y, \theta) = (-\theta y 10^{-3}, -\theta x 10^{-3}, -\theta(x+y)10^{-3}).$$



Simulations by F. E. Alvarez Borges

5. Possible consequences for therapeutics

- Insufficiency of single cell-based molecular therapies
  (intracellular pathways do not make a cell population)
- Cell-killing strategies: cytotoxics and cytostatics
  (the cannonade and the siege)
- Adding immunotherapies
  (increasing the power of the immune police forces)
- Reestablishing lost control connections between cell lineages?
  (cells to speak to their neighbours towards an understanding)

Insufficiency of single cell-based therapies

- Cancer exists as a disease only in multicellular organisms. Searching for impaired intracellular signalling pathways for blocking or enhancing them by externally imposed molecules (targeted therapies) may lead to transient or partial successes, however if control of differentiation is still impaired in some part of the organism, the disease is likely to recur.

- And this is indeed what is often observed in clinical settings, maybe because tumours are so heterogeneous that a drug that seems efficacious on a typical cell of the cancer under treatment, may be ineffective on a deviant cell of the same tumour, either because it has developed mutational resistance, or, even without mutations, because it has developed a drug-tolerant phenotype.

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Cell-killing strategies (*the cannonade*): optimal control problem, phenotype-structured IDE model

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

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

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

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

Find controls (u_1, u_2) minimising

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

under the additional constraints

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

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

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Asymptotic behaviour with constant controls

Following an argument by P.-E. Jabin & G. Raoul (*J Math Biol 2011*), convergence and concentration can be proved at the same time by using the Lyapunov functional

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

Theorem

(Asymptotic behaviour theorem, generalising to 2 populations the 1D case)
Assume that u_1 and u_2 are constant: $u_1 \equiv \bar{u}_1$, and $u_2 \equiv \bar{u}_2$. Then, for any positive initial population of healthy and of tumour cells, $(\rho_H(t), \rho_C(t))$ converges to the equilibrium point $(\rho_H^\infty, \rho_C^\infty)$, which can be exactly computed as follows.

Let $a_1 \geq 0$ and $a_2 \geq 0$ be the smallest nonnegative real numbers such that

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

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

$$I_H^\infty = a_{HH}\rho_H^\infty + a_{HC}\rho_C^\infty = a_1,$$
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Let $A_H \subset [0,1]$ (resp., $A_C \subset [0,1]$) be the set of all points $x \in [0,1]$ such that equality hold in one of the inequalities above. Then the supports of the probability measures

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

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

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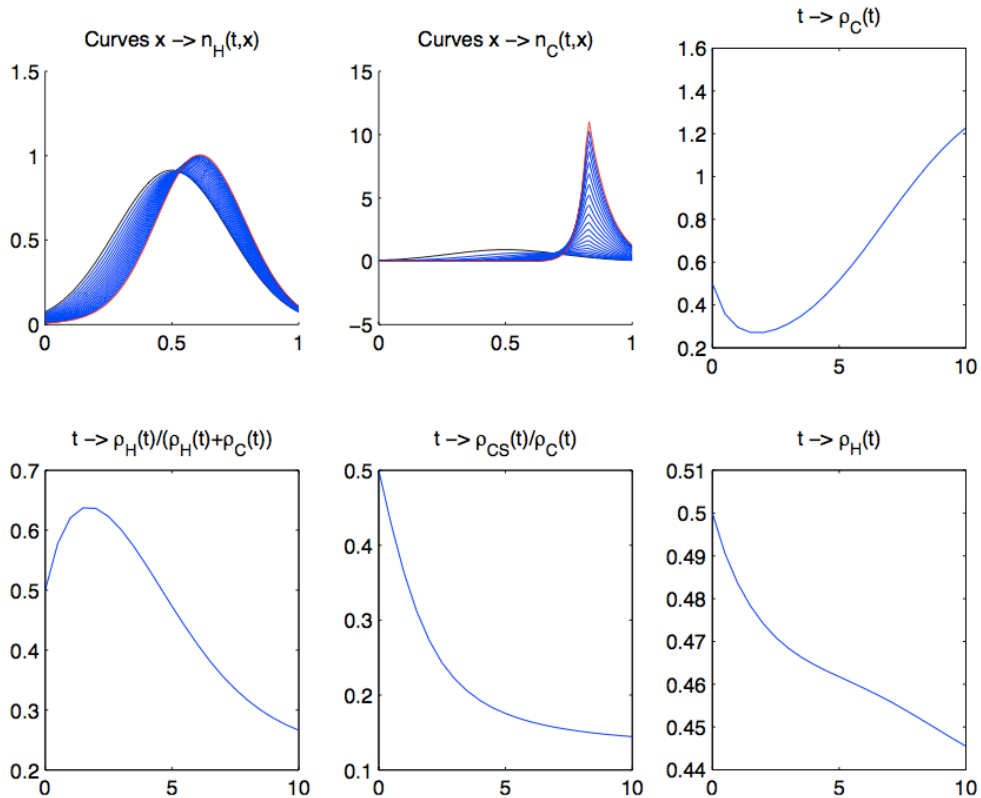
How to be deleterious by using constant doses of drugs

[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}: the *pessimal* strategy.

Optimal control problem: theoretical results

Theorem

(Optimal control theorem)

Under these conditions, the optimal trajectory in large time $T > 0$ consists of 2 parts:

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

Simulations illustrating this theorem

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

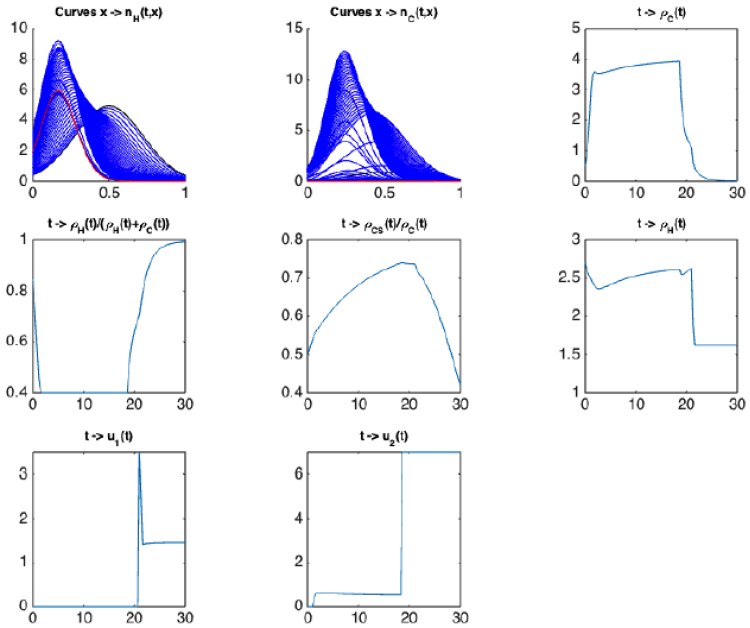


Figure 4: Simulation of (**OCP**) for $T = 30$.

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

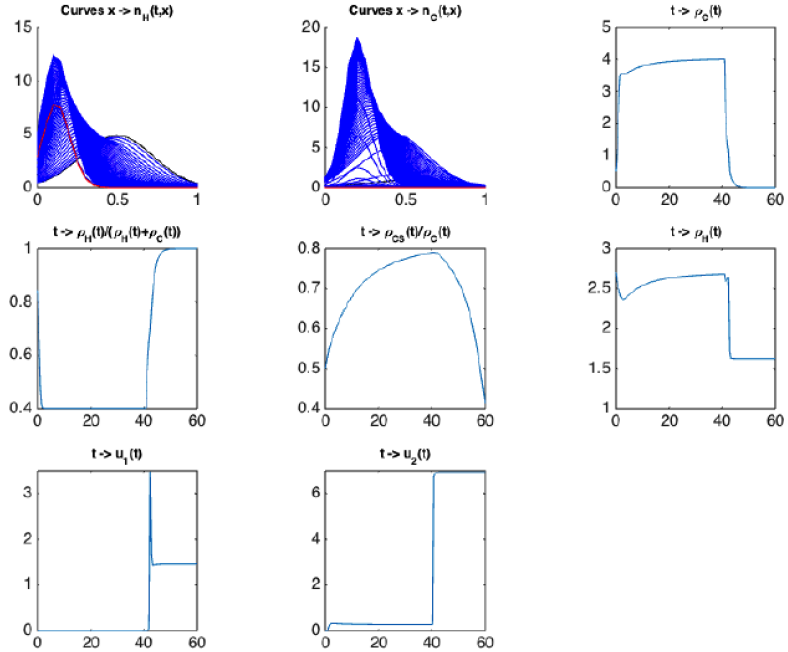


Figure 5: Simulation of (**OCP**) for $T = 60$.

Note that this strategy (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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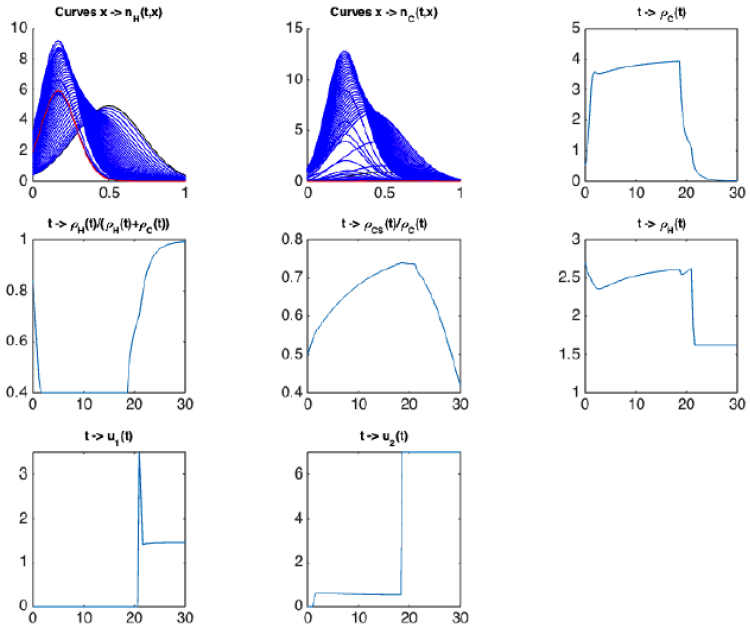

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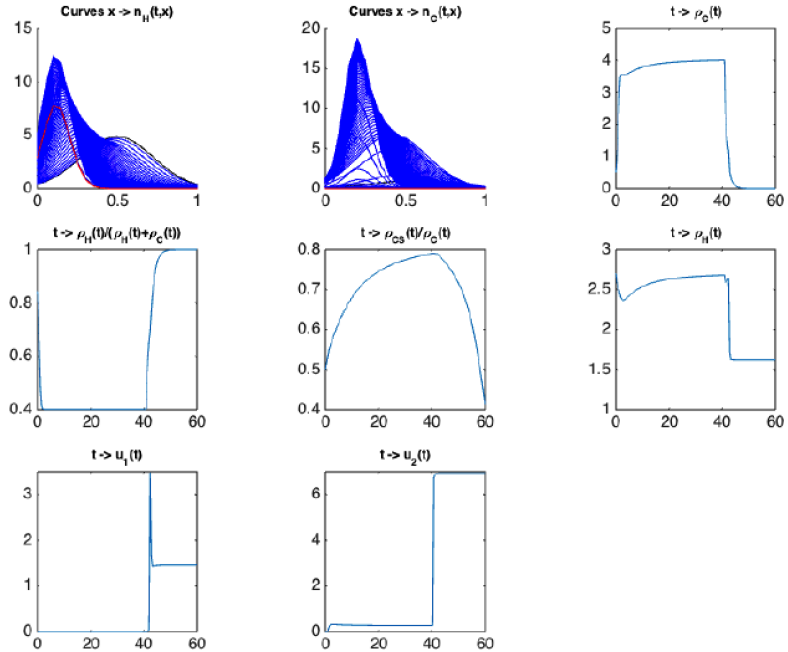

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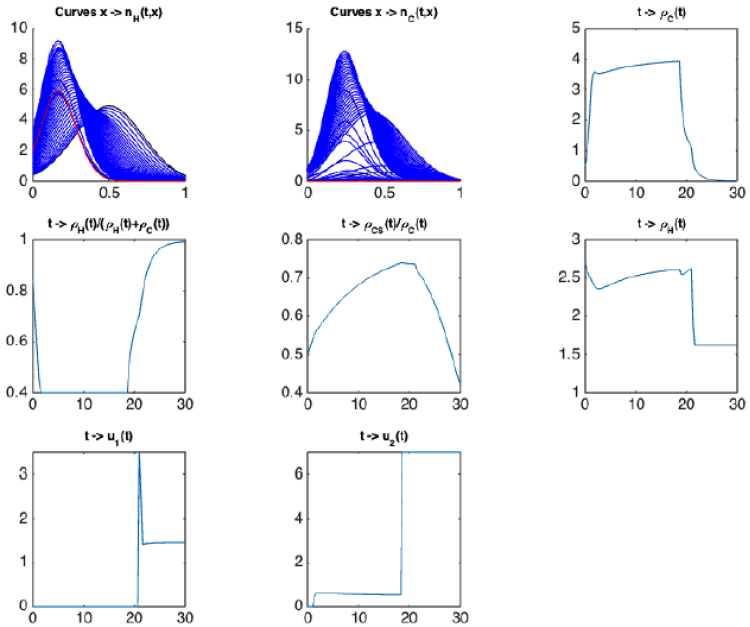

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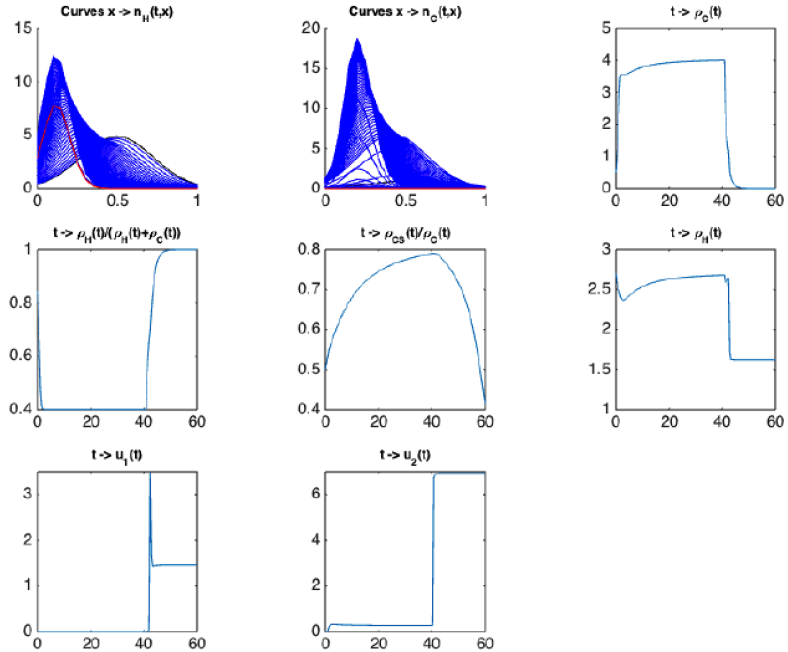

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Immunotherapies (*reinforcing the killing power of the immune cell police*)

- The immune cells (T-lymphocytes; dendritic cells; B-lymphocytes that diffuse immunoglobulins; monocytes and macrophages) are the part of the immune system in charge of eliminating external pathogens and deviant cells that are not recognised as part of the *self*. They are the immune police.

- Immune checkpoint inhibitors (ICIs): anti-CTLA4, anti-PD1, anti-PDL1 molecules, reinforce their power, boosting their action on tumour cells when they become too weak to kill them, due to tumour immunoescape.

- Although able to cure some cancers that were until recently out of reach (in particular cases of melanoma), their success is limited (about 20% of complete cures, the remaining 80% consisting of partial response, no effect and even sometimes tumour hyperprogression, with poor understanding of these failures, except at times uncontrolled immune cell response.

- CAR T-cells have also achieved remarkable cures (ALL, B-cell lymphomas), however with the same limitations: boosting the power of the immune police may have unexpected and unpredictable counter-productive effects (e.g. CRS).

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A simple structured model for ICI therapy (work underway)

Structure variables:
x, a malignancy trait (stemness-like, in tumour cells)
y, an anti-tumour aggressiveness trait (in competent lymphocytes)

$n(t,x)$ cancer cells, $\ell(t,y)$ competent lymphocytes at tumour site, $p(t,y)$ lymphocytes differentiated and amplified in lymphoid organs, $\chi(t)$ APC-borne message from tumour cells, $ICI(t)$ immune checkpoint inhibitor therapy

$$\begin{cases} \dfrac{\partial n}{\partial t}(t,x) = [r(x) - d(x)\rho(t) - \varphi(t)]n(t,x) \quad [+\beta \dfrac{\partial^2 n}{\partial x^2}(t,x)], \\ \dfrac{\partial \ell}{\partial t}(t,y) = p(t) - \dfrac{\nu(y)}{1+ICI(t)}(1+\rho(t))\ell(t,y), \\ \dfrac{\partial p}{\partial t}(t,y) = \alpha\chi(t,y)p(t,y) - kp^2(t,y), \end{cases}$$

where

$$\rho(t) = \int_0^1 n(t,x)dx, \quad \sigma(t) = \int_0^1 \ell(t,y)dy,$$

$$\varphi(t) = \int_0^1 \psi(y)\ell(t,y)dy, \quad \chi(t,y) = \int_0^1 \omega(x,y)n(t,x)dx.$$

Work underway with Z. Kaïd

Future prospects: reformatting the cohesion watch? (*reinforcing concord between stromal cells towards a common goal, serving the health of the whole organism?*)

- Admitting the necessary existence, within the immune system seen as *what sticks cells together* in a multicellular organism, of a *cohesion watch*, firstly virtual as principles of coherence within the genetic developmental programme launched by fecundation, then material as a set of cohesive intercellular connections within the organism constituted by self-organisational development, it remains to us to identify it.

- This should lead us to investigate intercellular connections during development, i.e., during the first stages of embryogenesis that yield the body plan, and later during the following steps in which functionally defined trees (the great physiological functions of the organism) stem from the body plan. These connections should be conserved in some way in the adult multicellular organism to ensure its cohesion (maintenance). Understanding them as generic elements of a global unifying system, part of the immune system, might be a help to recognise them.

- Then finding ways to enhance them, possibly but not necessarily by molecular therapies, would be the next step to design non-cell killing anticancer therapies, a goal that is still far ahead of us, but not unreachable.

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By way of conclusion

- To find new therapeutic tracks for fighting the cancer disease, one can make use of existing (cell-killing) therapies, however one has to optimise their use.

- Immunotherapies are no exceptions to this proposition, as they are also cell-killing therapies. They may be optimally combined with chemotherapies and targeted therapies, provided that their pitfalls are well enough identified to design optimal combinations, which does not seem to be the case so far (and to the best of my knowledge, we still have not understood the reasons of the successes and failures of William Coley's founding experiments in cancer immunotherapy, more than a century ago).

- This situation should invite us to better understand what a multicellular organism is (limiting ourselves to the metazoan, i.e., animal case), what its cohesion consists of, how it is altered in cancer, and how such cohesion could be reinforced by enhancing intercellular connection means. Mere speculations? Not necessarily only. At least having such prospects in mind might help us to give sense to upcoming new observations and possibly reinterpret old ones.

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