Why is evolution important in cancer and what mathematics should be used to treat cancer?
Focus on drug resistance

Rebecca Chisholm, *Jean Clairambault*[†], Tommaso Lorenzi, Alexander Lorz, Benoît Perthame, Camille Pouchol, Emmanuel Trélat

Mamba INRIA team & Laboratoire Jacques-Louis Lions, UPMC, Paris

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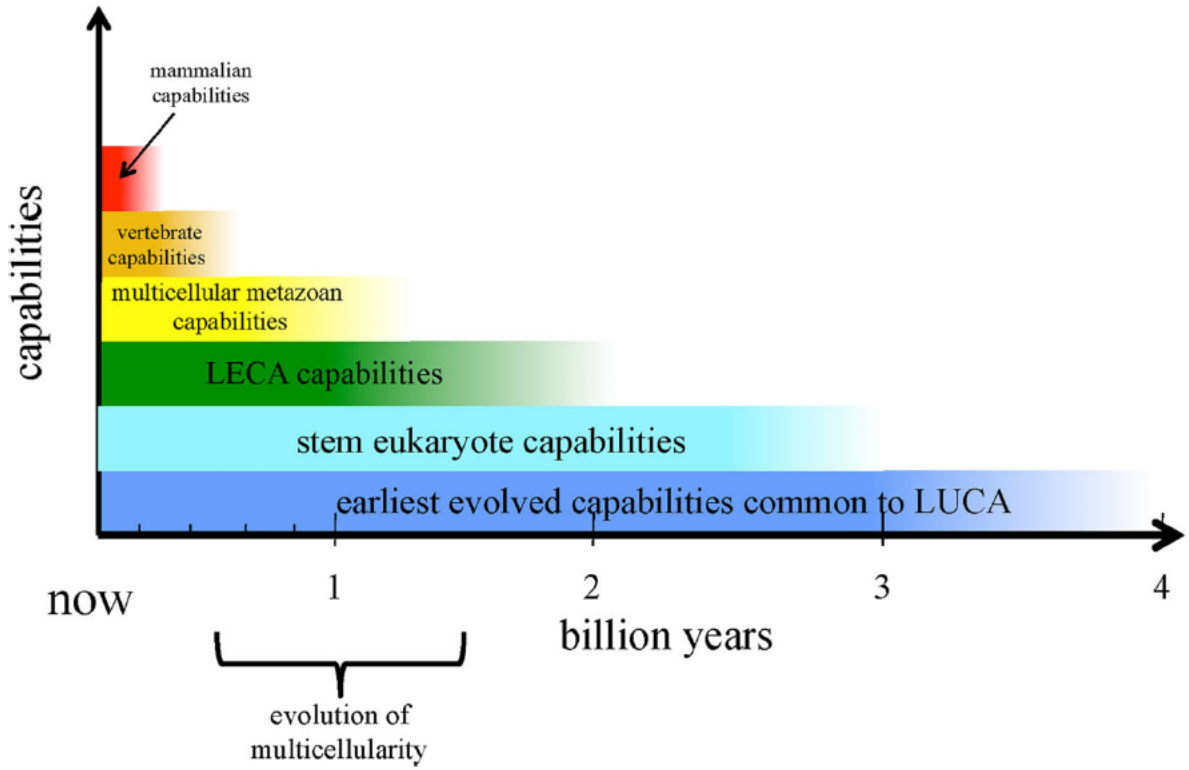
[†] *http://who.rocq.inria.fr/Jean.Clairambault/Jean_Clairambault_en.html*

Summary

- Intra-tumour heterogeneity, i.e., between-cell phenotypic variability within cancer *cell populations*, is a condition of evolution towards drug resistance in tumours.
- Slow genetic mechanisms of 'the great evolution' that has designed multicellular organisms, together with fast reverse evolution on smaller time windows, at the scale of a human disease, may explain transient or established drug resistance.
- Plasticity in cancer cells, i.e., epigenetic 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 have the advantage of being compatible with optimal control methods for the theoretical design of optimised therapeutic protocols involving combinations of cytotoxic and cytostatic (and possible epigenetic) treatments.

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

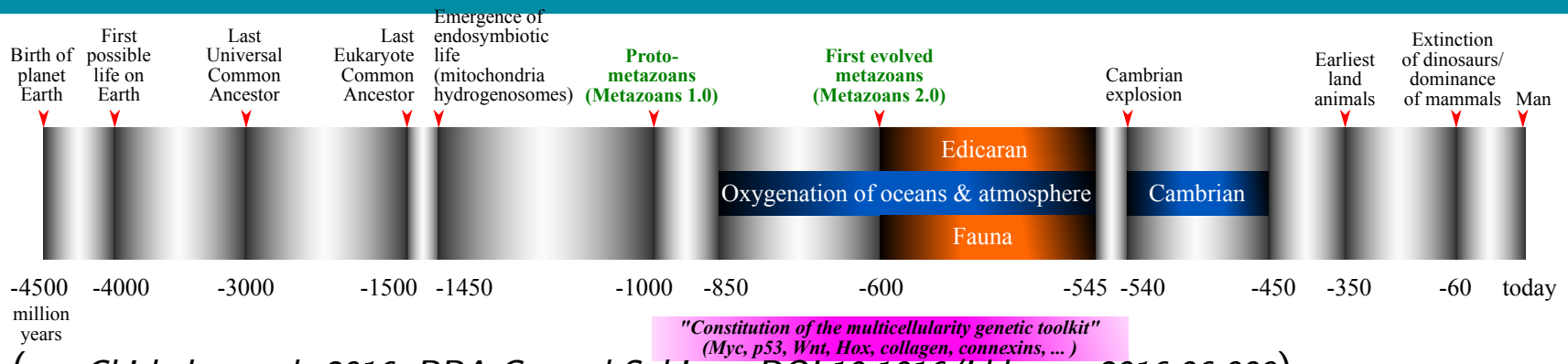
"Nothing in biology makes sense except in the light of evolution" (Th. Dobzhansky, 1973)



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

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

A possible evolutionary framework: the atavistic hypothesis of cancer (2)



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

- The genes that have appeared in the process of development to multicellularity are precisely those that are altered in cancer (Domazet-Lošo & Tautz 2011, Davies & Lineweaver 2011)
- Is there an order in evolution of multicellularity, from 1) proliferation+apoptosis to 2) cell differentiation+division of work, and to 3) *epigenetic control* of differentiation and proliferation? *[Reverse phylogenetic order observed in AML]*
- Reconstituting the phylogeny of an ancient 'multicellularity toolkit' should shed light on the robustness or fragility of genes that have been altered in cancer
- Attacking cancer on proliferation is precisely attacking its robustness. It would be better to attack its weaknesses (e.g. absence of adaptive immune response)

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

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

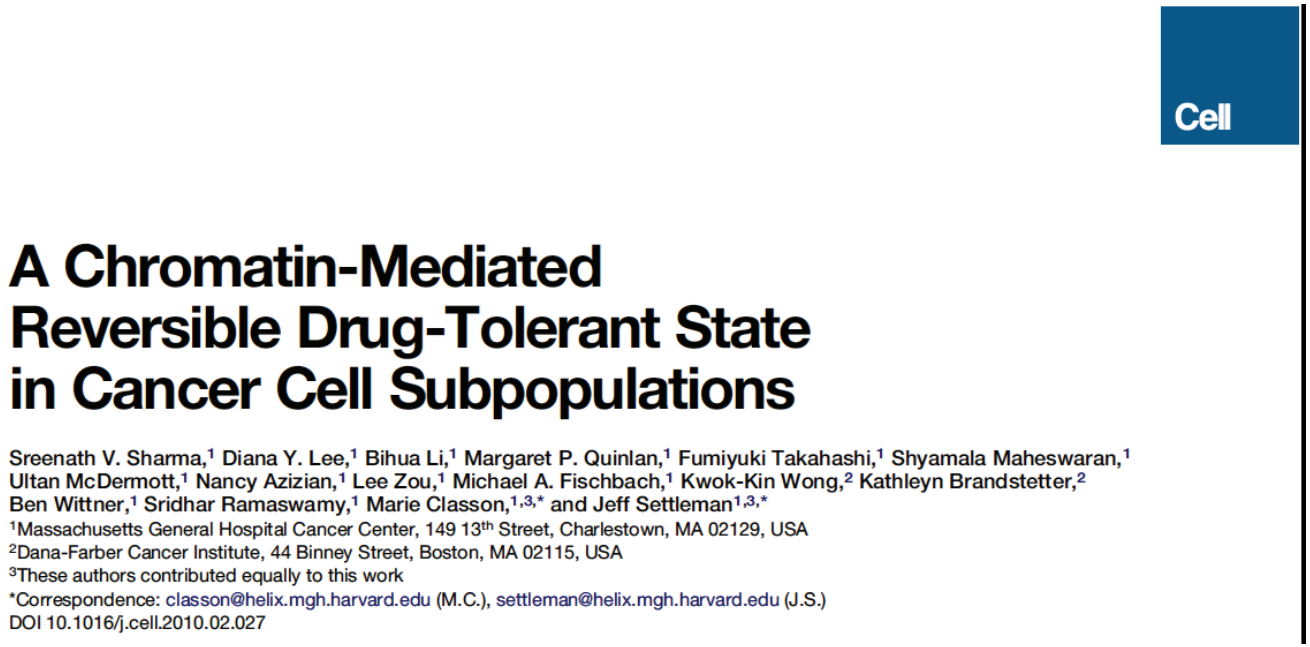
- We bear in our genomes many attempts of species evolution since billions of years; dead-end tracks ('unused attractors' in S. Huang and S. Kauffman's version of the Waddington landscape) have been silenced (e.g., by epigenetic enzymes, resulting in evolutionary barriers in this landscape), but are still there

- In cancer, global regulations are lost, differentiation is out of control, so that local proliferations without regulation overcome; sophisticated *adaptive epigenetic mechanisms* *are present, not controlling proliferation, but serving it* (by stochastic exoression of so-called *cold* genes? cf. Wu et al. PNAS 2015)

- Primitive forms of cooperation between specialised cells in a locally organised multicellular collection (tumour), with plasticity between them, may be present, exhibiting coherent intratumoral heterogeneity, and escaping external control

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

Evolution towards resistance assessed experimentally: Reversible drug resistance of cancer cells in a Petri dish



Cell

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

Sreenath V. Sharma,[1] Diana Y. Lee,[1] Bihua Li,[1] Margaret P. Quinlan,[1] Fumiyuki Takahashi,[1] Shyamala Maheswaran,[1] Ultan McDermott,[1] Nancy Azizian,[1] Lee Zou,[1] Michael A. Fischbach,[1] Kwok-Kin Wong,[2] Kathleyn Brandstetter,[2] Ben Wittner,[1] Sridhar Ramaswamy,[1] Marie Classon,[1,3,*] and Jeff Settleman[1,3,*]
[1]Massachusetts General Hospital Cancer Center, 149 13th Street, Charlestown, MA 02129, USA
[2]Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115, USA
[3]These authors contributed equally to this work
*Correspondence: classon@helix.mgh.harvard.edu (M.C.), settleman@helix.mgh.harvard.edu (J.S.)
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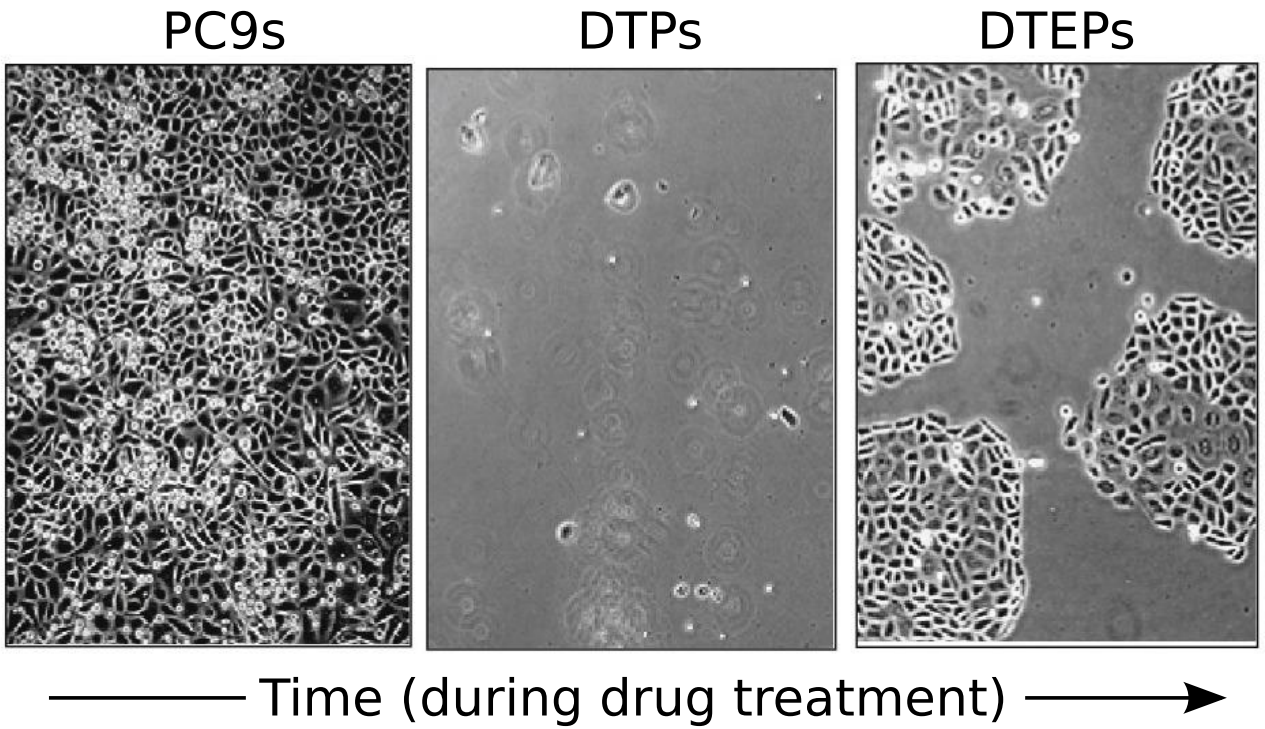
- Motivation for math: to account for biological observations of a reversible drug-resistant phenotype in cancer cell populations, *Sharma et al., Cell 2010*
- Underlying hypothesis: epigenetic modifications affect differently survival and proliferation potentials in cancer cell populations exposed to high drug doses
- Our model: 2 traits, x, stress survival potential ($\sim$ resistance to apoptosis) and y, proliferation potential ($\sim$ cell division cycle enhancement), both reversible
- A PDE model and an agent-based (AB) model both account for the observed behaviour of the cancer cell population exposed to the drug

See *Chisholm et al. Cancer Research 2015*

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

- Population of PC9 (NSCLC) cells under high doses of drugs (e.g., gefitinib)
- 99.7% cells die, .3% survive in this maintained hostile drug environment: DTPs
- In the same hostile environment, 20% of DTPs resume proliferation: DTEPs
- Total drug resensitisation is obtained by drug withdrawal after 9 doubling times for DTPs, and 30 to 90 doubling times, depending on the drug, for DTEPs
- Inhibition of epigenetic enzyme KDM5A blocks emergence of DTPs (*precisely: provokes rapid death of both DTPs and DTEPs, not affecting PC9s*)



(Sharma et al., Cell 2010)

2D continuous phenotype-structured PDE model

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

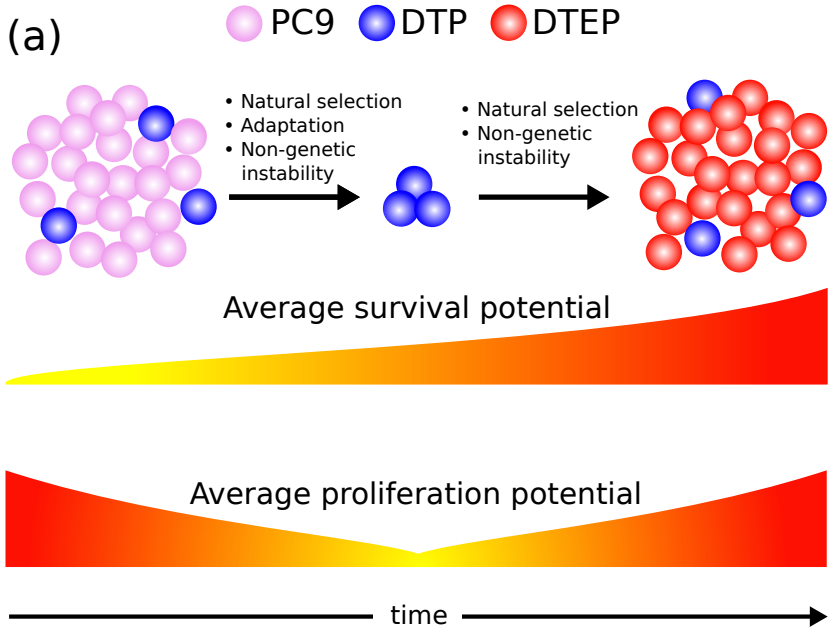
- Population density of cells $n(x, y, t)$ with phenotypic expression (x, y) at time t satisfies

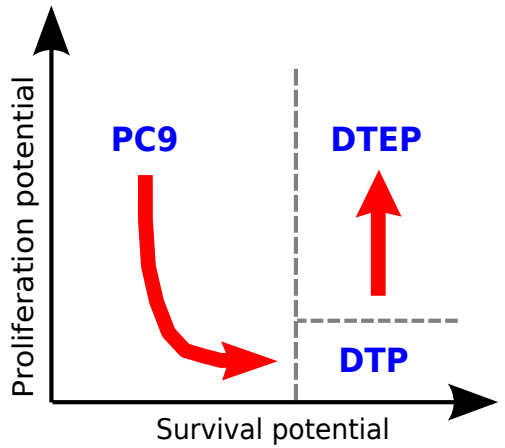
$$\frac{\partial n}{\partial t}(x,y,t) + \underbrace{\frac{\partial}{\partial y}\Big(v(x,c(t);\bar{v})n(x,y,t)\Big)}_{\text{Stress-induced adaptation of the proliferation level}} = \underbrace{\Big[p(x,y,\varrho(t)) - d(x,c(t))\Big]n(x,y,t)}_{\text{Non local Lotka-Volterra selection}} + \underbrace{\beta\Delta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

- $\varrho(t) = \int_0^1 \int_0^1 n(x,y,t)\,dx\,dy$, $p(x,y,\varrho(t)) = (a_1 + a_2 y + a_3(1-x))(1-\varrho(t)/K)$ and $d(x,c) = c(b_1 + b_2(1-x)) + b_3$
- The drift term w.r.t. proliferation potential y represents possible (if $v \neq 0$) 'Lamarckian-like', epigenetic and reversible, adaptation from PC9s to DTPs
- $v(x,c(t);\bar{v}) = -\bar{v}c(t)H(x^* - x)$ where $t \mapsto c(t)$ is the drug infusion function
- No-flux boundary conditions

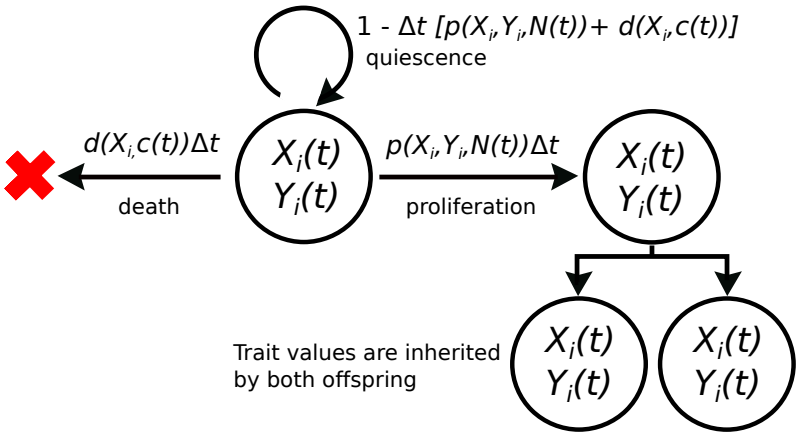
(Chisholm et al., Cancer Research 2015)

Agent-based model (ABM)





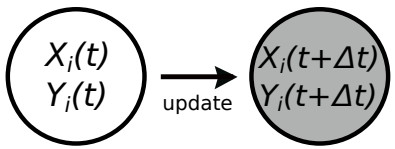
(a) Each cell i undergoes either proliferation, death or remains quiescent:



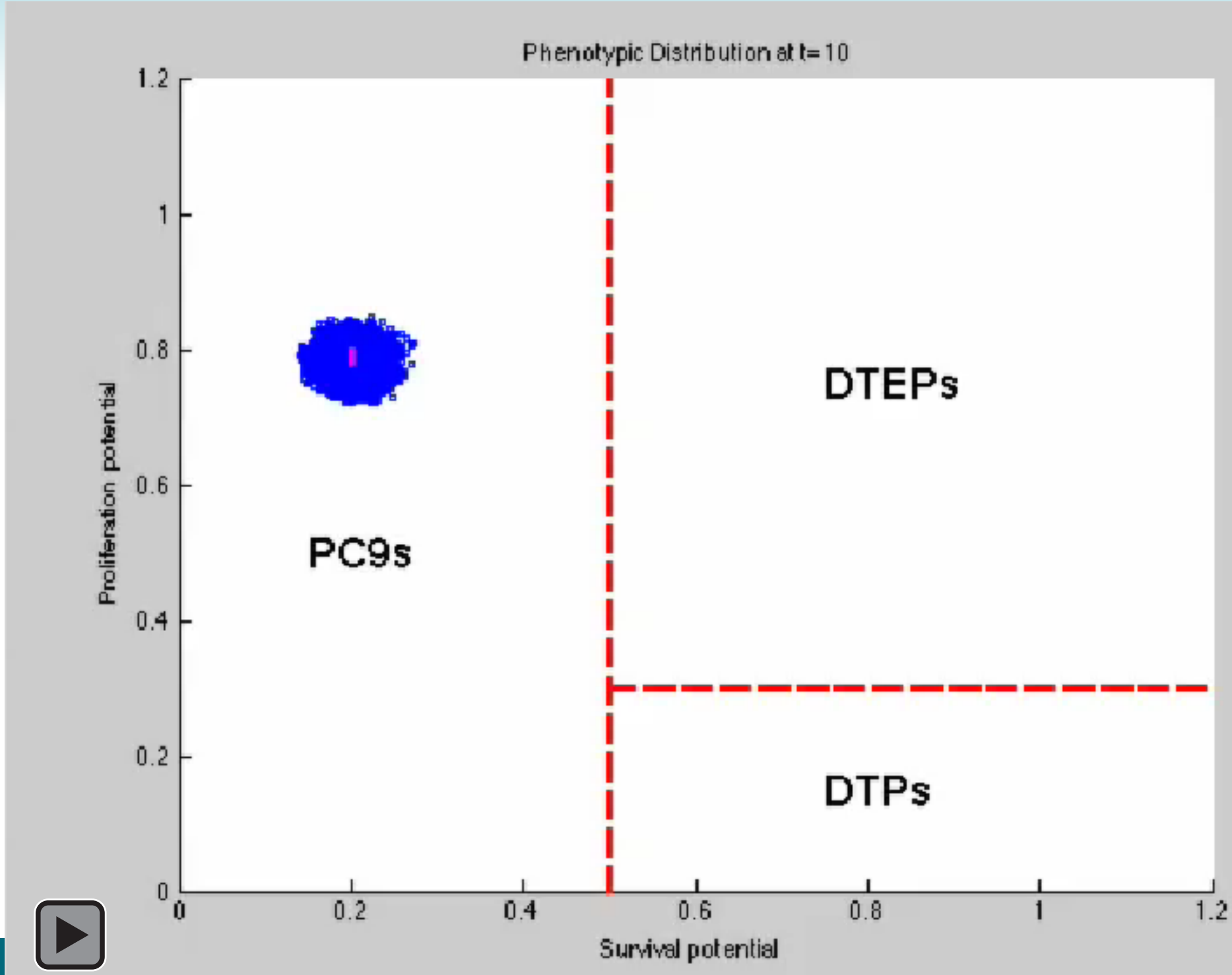
(b) Each cell i updates its trait values according to the discretised SDEs:

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

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

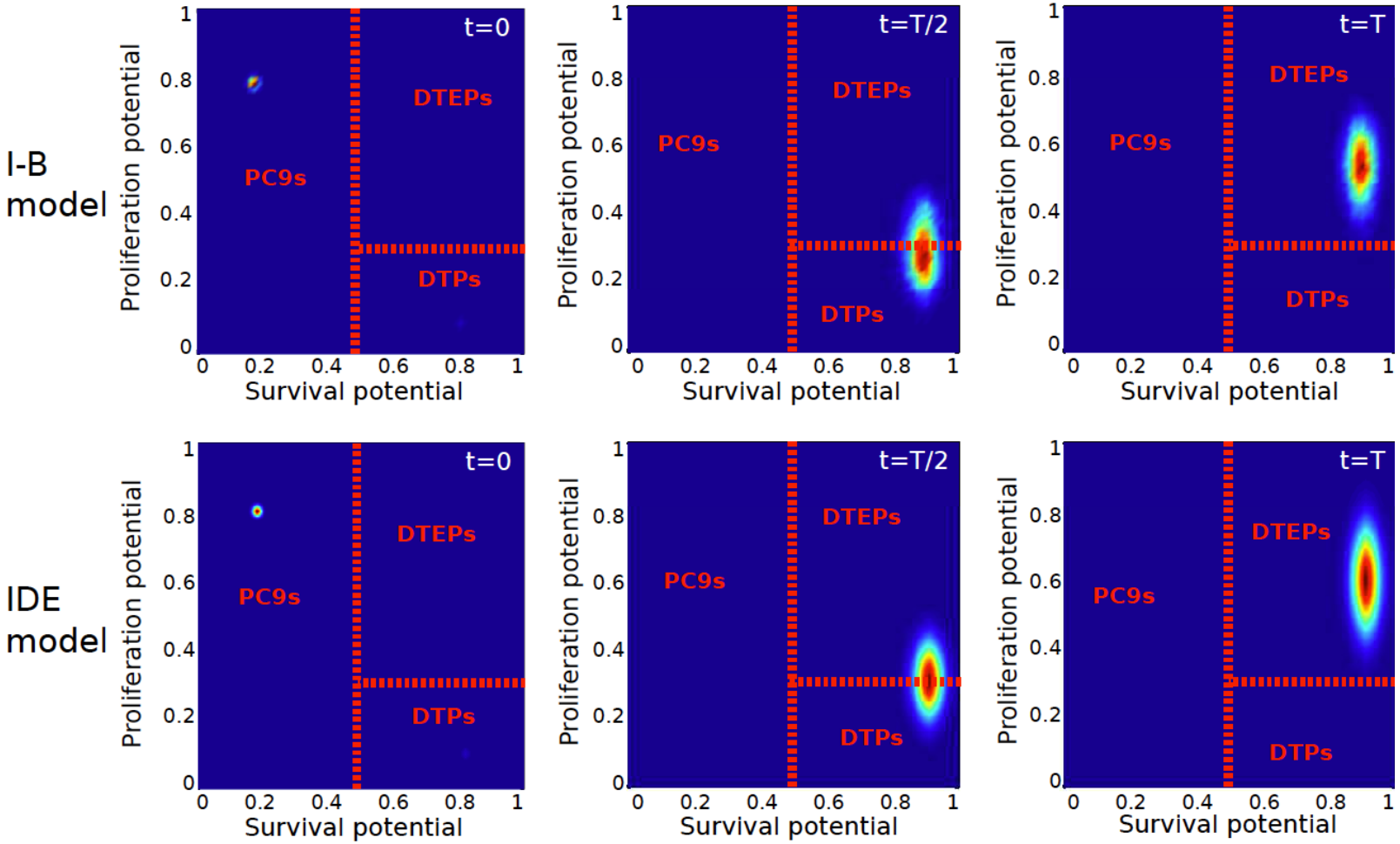


(Chisholm et al., Cancer Research 2015)


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

AB model and IDE model recover phenotype dynamics

During drug treatment (here, PC9s and DTPs present initially)



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

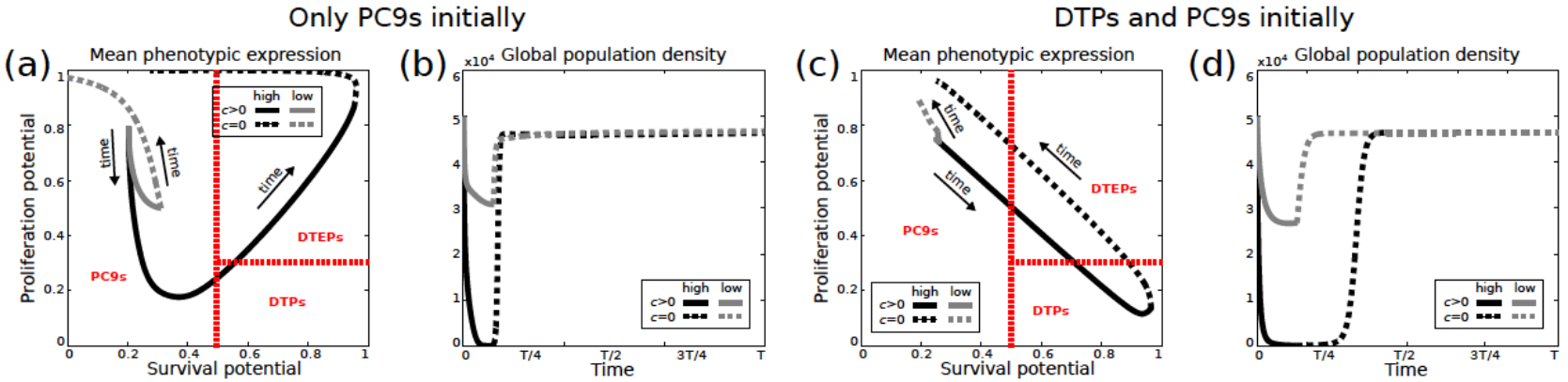
(Chisholm et al., Cancer Research 2015)

AB model and IDE model recover phenotype dynamics

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

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)

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

(Chisholm et al., Cancer Research 2015)

Use IDE model to address 3 questions

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

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

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

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

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

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

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

(Chisholm et al. Cancer Research 2015)

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

- Both mathematical models (AB, IDE) reproduce the main experimental observations

- To see the transient appearance of the DTPs during high-dose drug therapy:
  - If there are **some DTPs** present initially, model explanation requires only
    - non-genetic instability
    - selection
  - If **no DTPs** are present initially, model explanation requires interplay between
    - stress-induced adaptation
    - non-genetic instability
    - selection

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

(Chisholm et al. Cancer Research 2015)

Phenotype-structured population dynamics

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

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

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

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

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

Phenotype-structured non-local Lotka-Volterra models

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

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

with

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

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

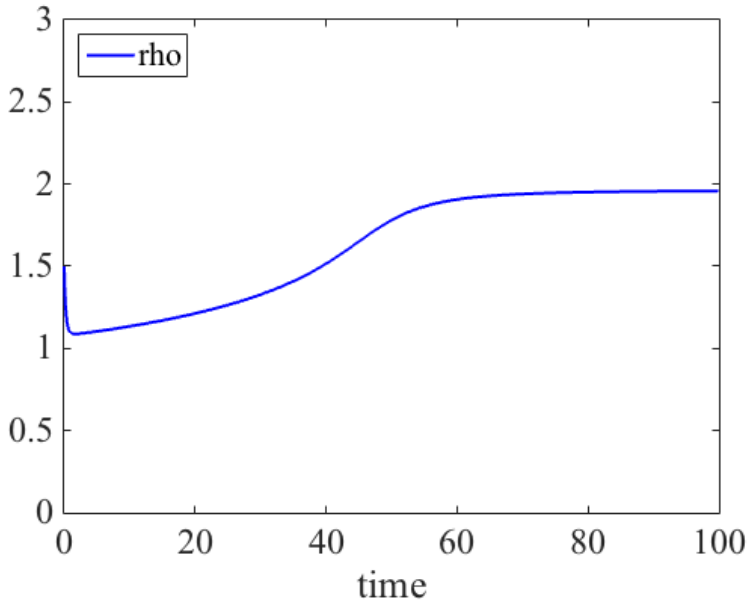
[More general settings for the growth rate $R(x,\rho(t))$, here $\big(r(x) - d(x)\rho(t)\big)$, have been studied in Benoît Perthame's book Transport equations in biology *(2007)]*

Questions: what is the asymptotic behaviour of

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

Non-local Lotka-Volterra model: convergence

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

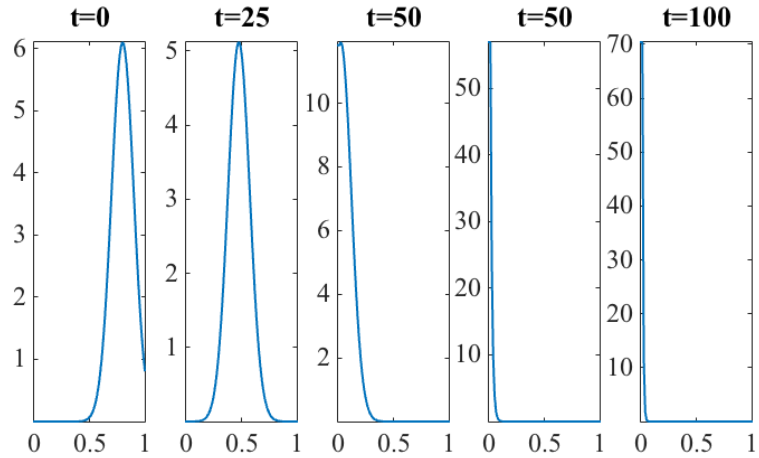


Firstly, it can be shown that
ρ converges to ρ^∞, the smallest value such that $r(x) - d(x)\rho^\infty \leq 0$ for all x in $[0, 1]$.

(Idea of proof, see Camille Pouchol's internship report: "Modelling interactions between tumour cells and supporting adipocytes in breast cancer", UPMC, September 2015, `https://hal.inria.fr/hal-01252122`*: show that* $\int_0^{+\infty} \left|\frac{d\rho}{dt}\right|_- dt < +\infty$ *and – with additional hypotheses – that* ρ *is bounded; then convergence follows.)*

Non-local Lotka-Volterra model: concentration

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



Theorem

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

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

[Proof: see Camille Pouchol's internship report: *"Modelling interactions between tumour cells and supporting adipocytes in breast cancer"*, UPMC, September 2015, `https://hal.inria.fr/hal-01252122`]

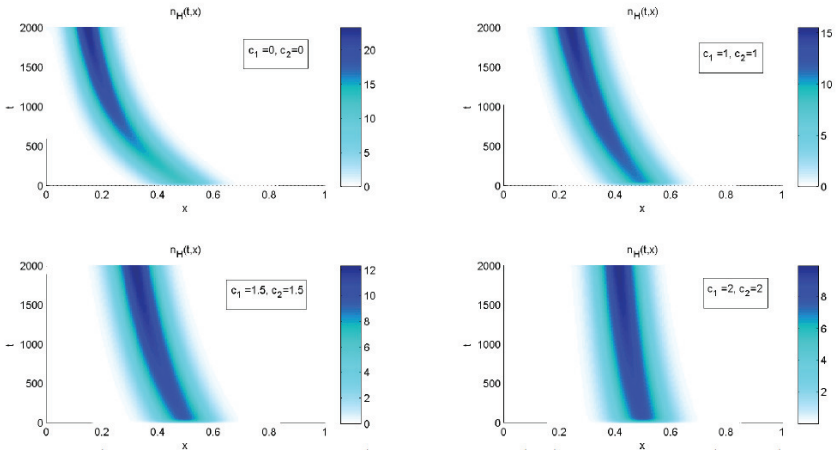
Non-local Lotka-Volterra model with 2 drugs and one (continuous scalar) resistance phenotype x

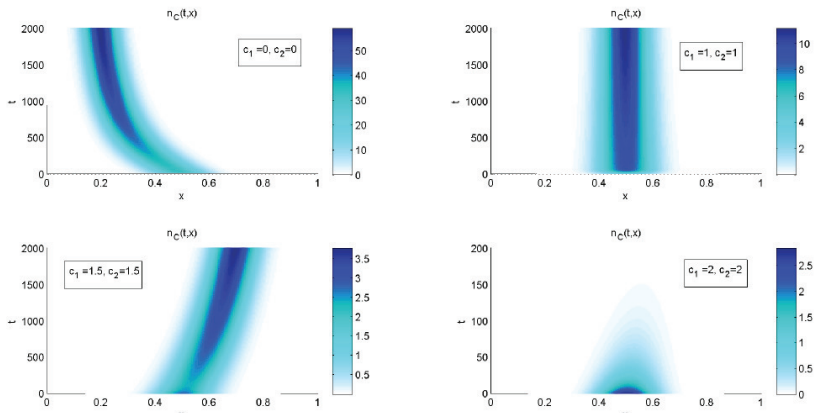
$$\frac{\partial}{\partial t} n_H(x,t) = \left[\frac{r_H(x)}{1 + k_H u_2(t)} - d_H(x) I_H(t) - u_1(t) \mu_H(x) \right] n_H(x,t)$$

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

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

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





Healthy cells: preserved

Cancer cells: eventually extinct

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

Convergence and concentration in this two-population setting, or: asymptotic behaviour with constant controls

[At the same time convergence and concentration, by using a Lyapunov functional]

Theorem

(Asymptotic behaviour theorem, no prior convergence assumed)

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

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

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

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

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

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

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

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

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

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

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

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

Find controls (u_1, u_2) minimising

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

under the additional constraints

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

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

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 $u_2 = u_2^{\max}$.

(Pouchol et al., arXiv (Dec. 2016) 1612.04698 or https://hal.archives-ouvertes.fr/hal-01416594v1)

Simulations illustrating this theorem

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

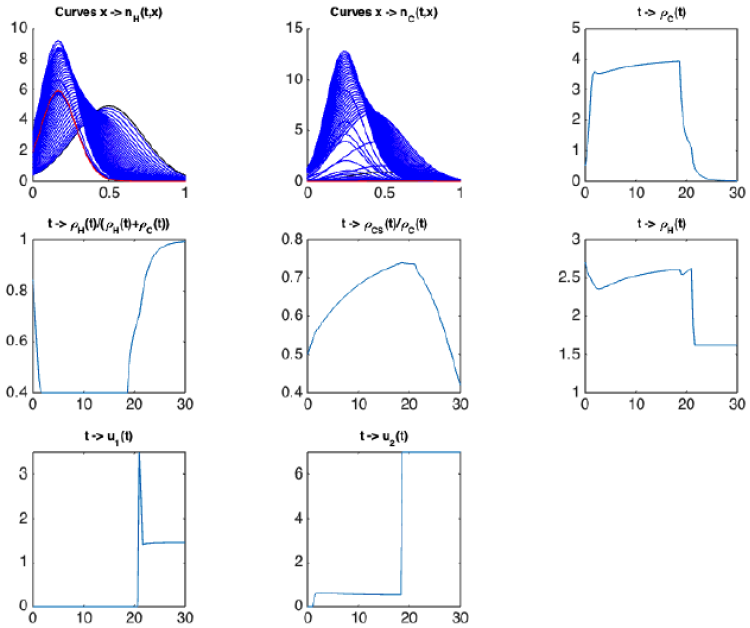


Figure 4: Simulation of (**OCP**) for $T = 30$.

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

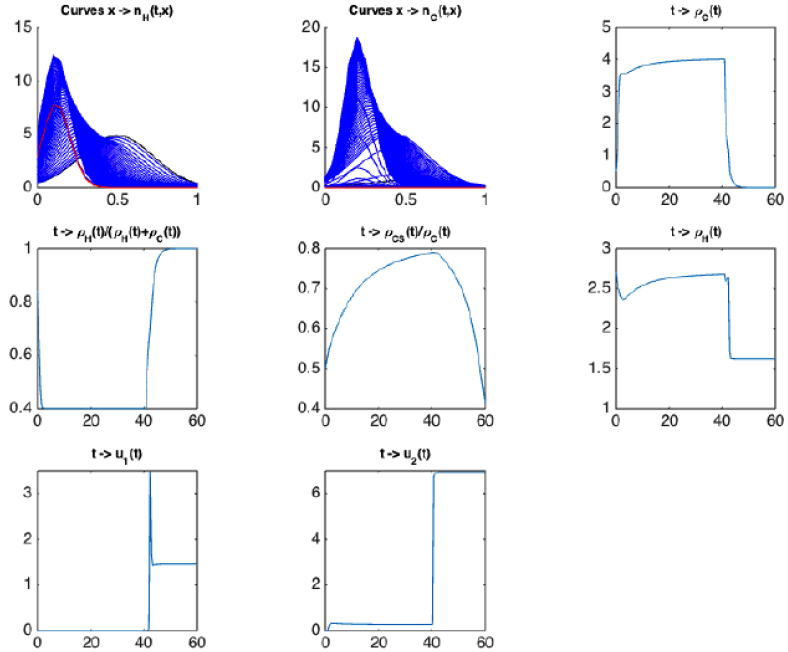
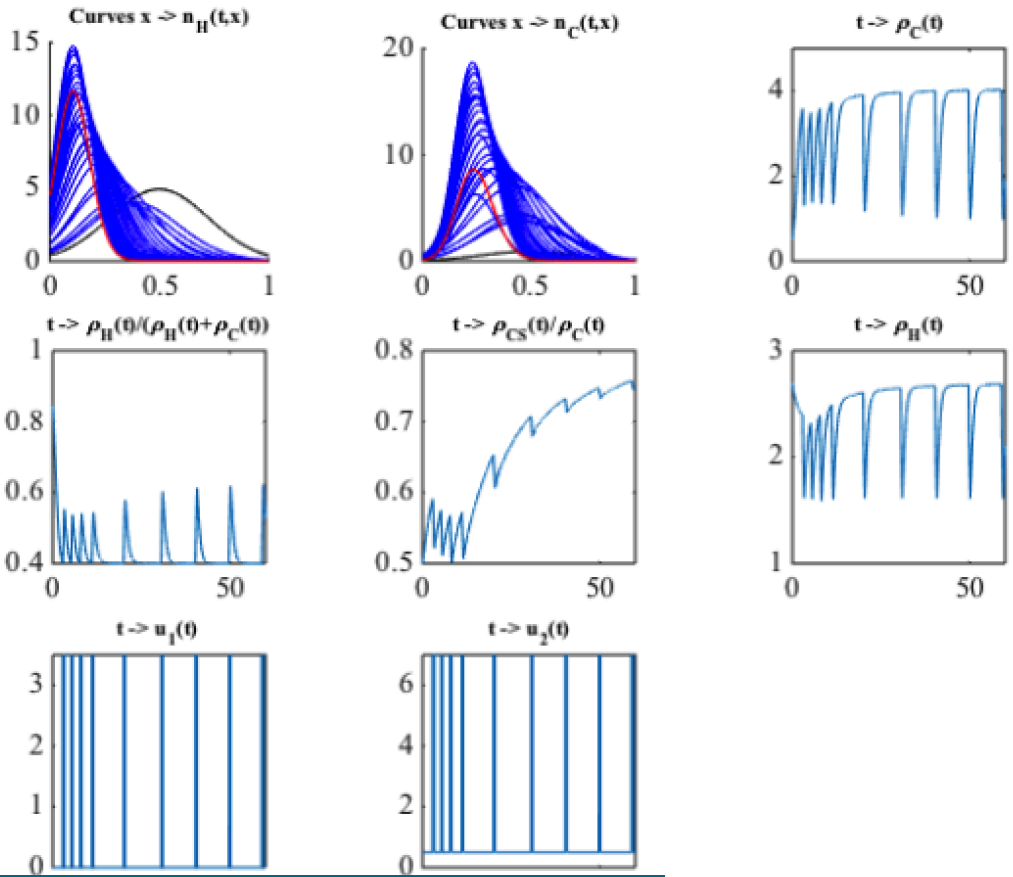


Figure 5: Simulation of (**OCP**) for $T = 60$.

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

Comparison with "almost periodic" therapeutic strategies

We mimic actual clinical settings: as long as $\frac{\rho_H}{\rho_H + \rho_C} > \theta_{HC}$, we follow the 'drug holiday' strategy by choosing $u_1 = \bar{u_1} = 0$, $u_2 = \bar{u_2} = 0.5$. Then, as long as $\rho_H > \theta_H.\rho_H(0)$, we use the maximal amount of drugs. As soon as $\rho_H = \theta_H.\rho_H(0)$, back to the drug holiday strategy. Results (note stabilised ρ_C and increasing ρ_{CS}):



Comparison with "almost periodic" therapeutic strategies

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

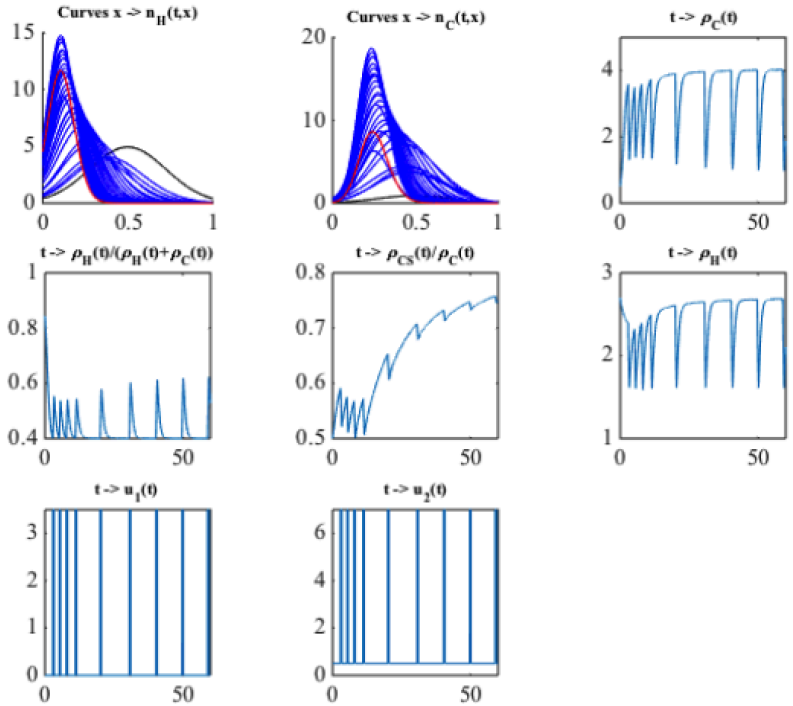


Figure 6: Quasi-periodic strategy, for $T = 60$.

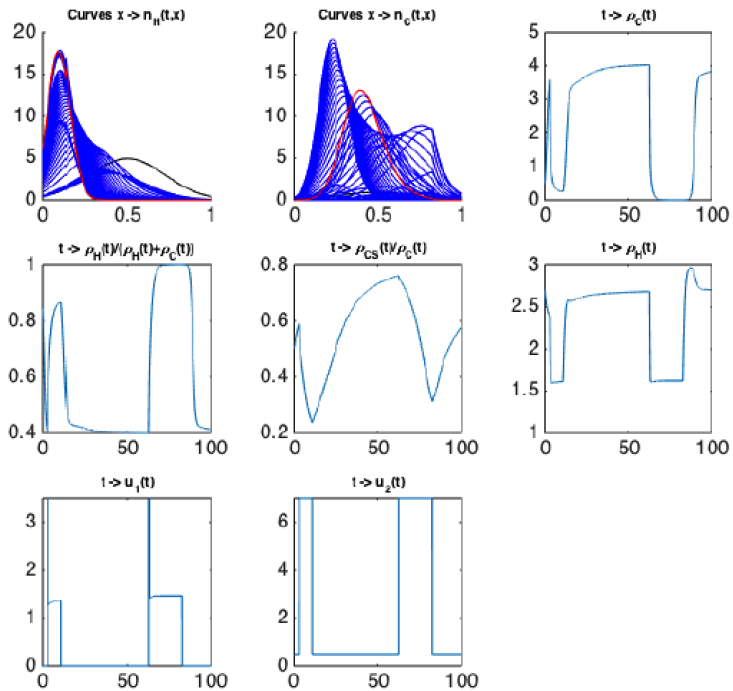


Figure 7: Second quasi-periodic strategy, for $T = 100$.

First (unsatisfying) periodic strategy: stabilisation of ρ_C only. 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 (eradication of the tumour?).

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What about space? Considering both a (1D) resistance phenotype and (1D) space in a tumour spheroid: equations

We assume that the evolution of functions n, s (nutrients), c_1 and c_2 in a 1D radially symmetric tumour spheroid ($r \in [0,1]$) is ruled by the following set of equations:

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

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

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

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

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

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

For each t, we also define $\rho(t,r) = \int_0^1 n(t,r,x)\,dx$ (local density at radius r) and $\rho_T(t) = \int_0^1 \rho(t,r)r^2\,dr$ (global density).

(Lorz et al. BMB 2015)

Tumour spheroid: simulations with constant drug doses (1)

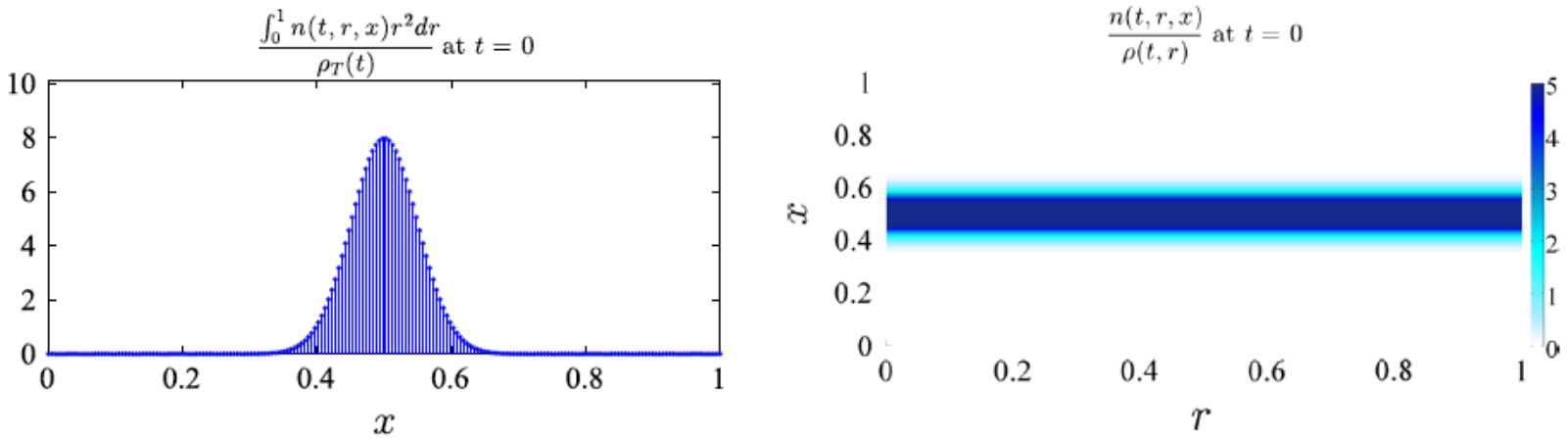
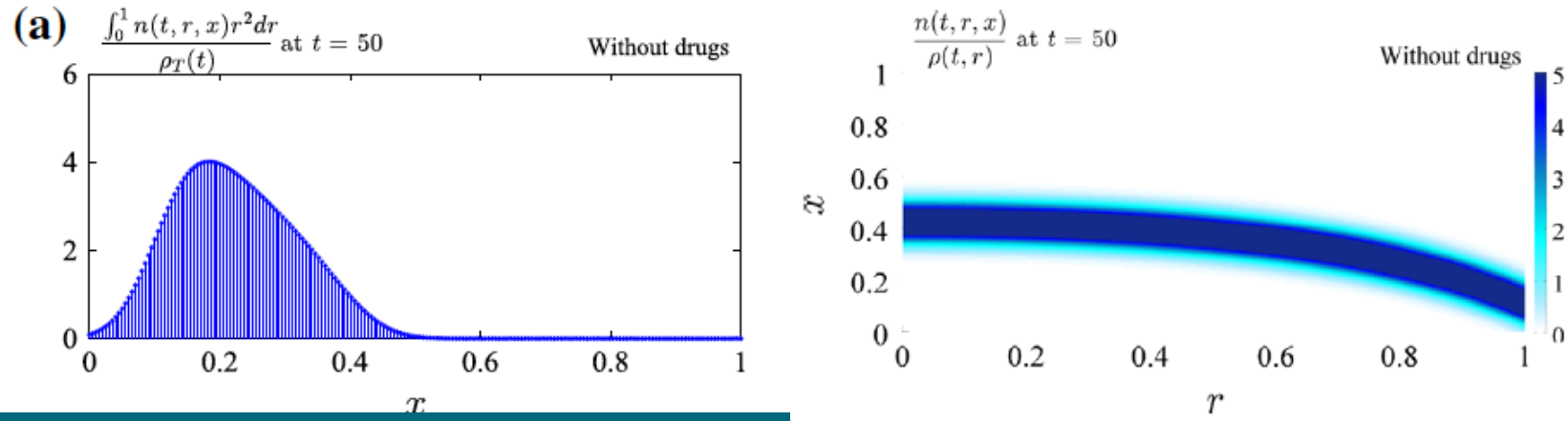
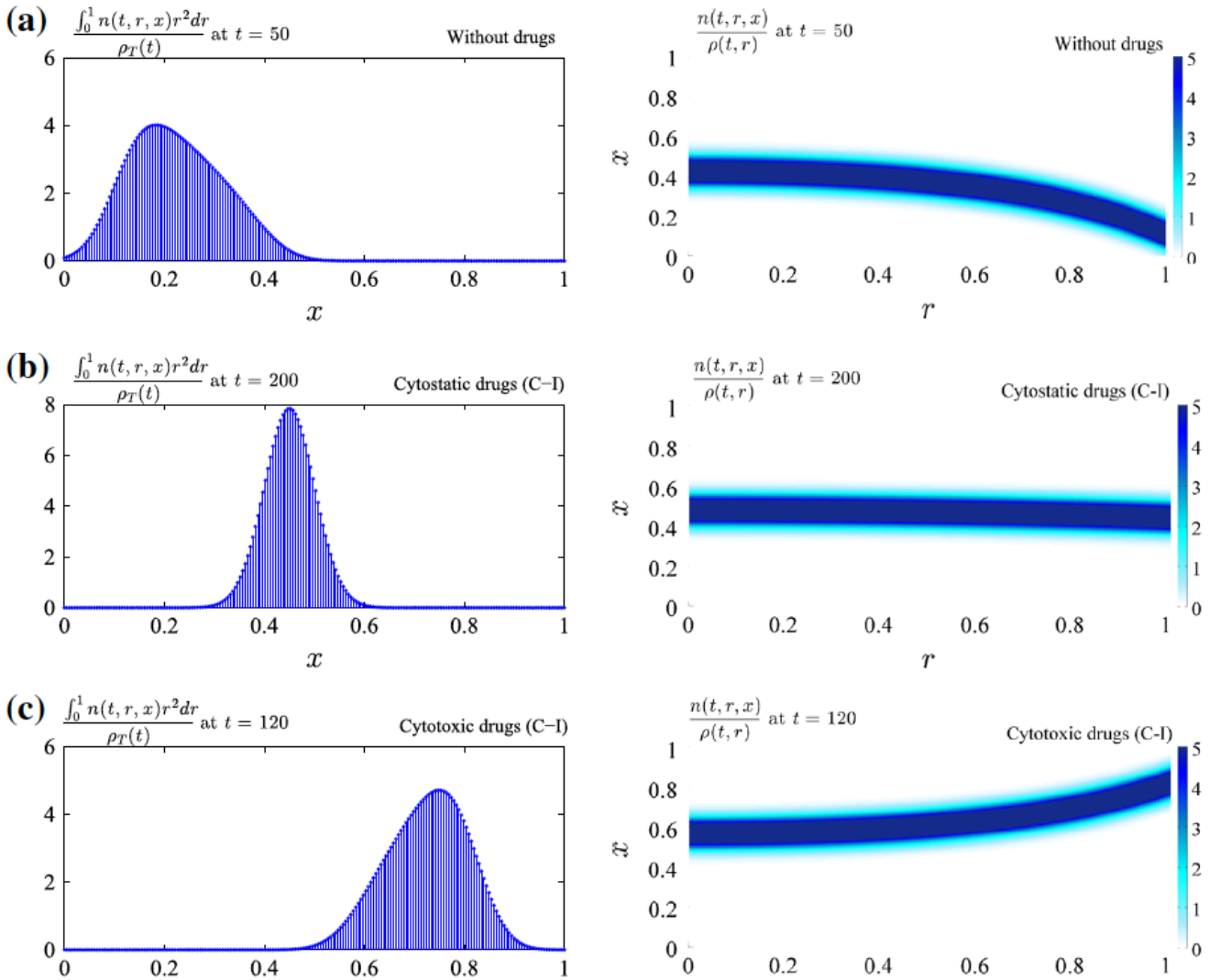


Fig. 1 Initial phenotypic distribution. Plots of $\int_0^1 n(t,r,x)r^2\mathrm{d}r/\rho_T(t)$ (*left panel*) and $n(t,r,x)/\rho(t,r)$ (*right panel*) at $t=0$. The initial cell population is almost monomorphic

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



Tumour spheroid: simulations with constant drug doses (2)



Cytotostatic c_2 has only small effects / Cytotoxic c_1 clearly induces resistance

(Lorz et al. BMB 2015)

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

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

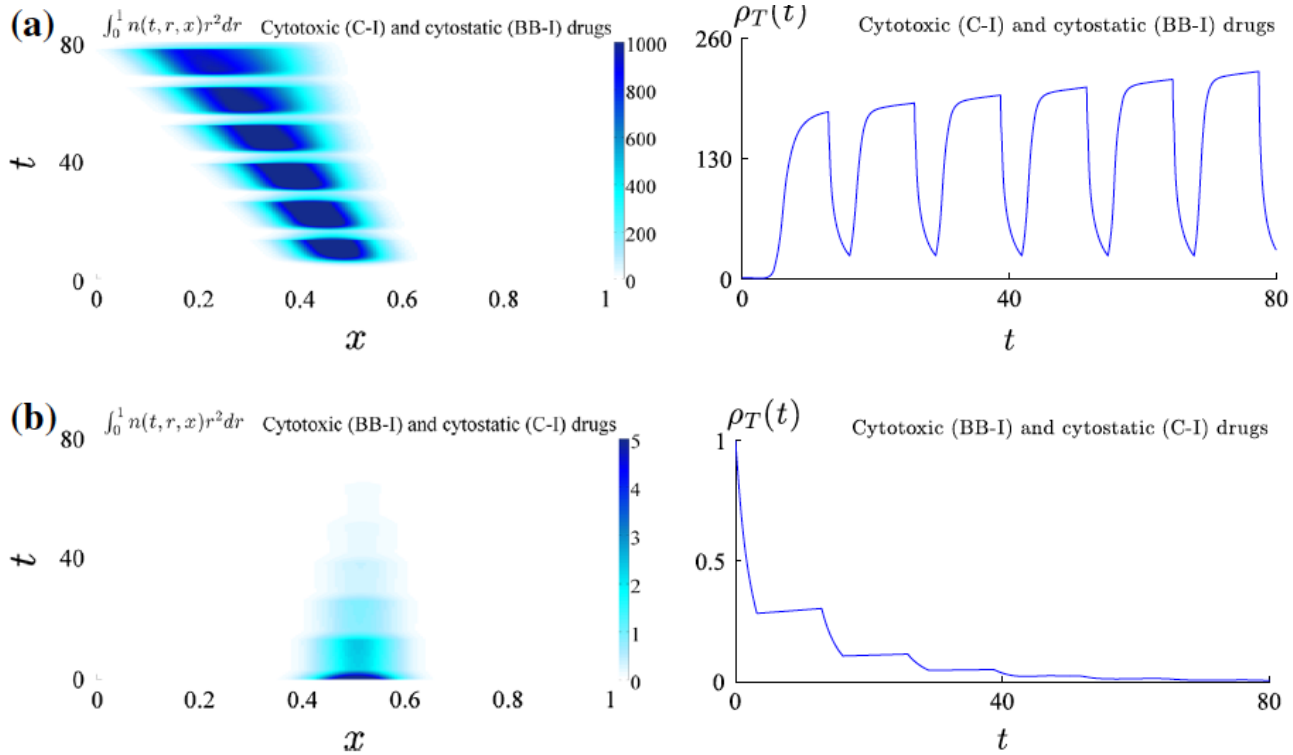


Fig. 11 **a** Cytotoxic (C-I) and cytostatic (BB-I) drugs. Plots of $\int_0^1 n(t, r, x)r^2 \mathrm{d}r$ (*left panel*) and $\rho_T(t)$ (*right panel*). Bang–bang infusion of cytostatic drugs together with constant infusion of cytotoxic drugs weakly affects the dynamics of cancer cells by comparison with the case without therapies, apart from temporary reductions of the global population density. **b** Cytotoxic (BB-I) and cytostatic (C-I) drugs. Plots of $\int_0^1 n(t, r, x)r^2 \mathrm{d}r$ (*left panel*) and $\rho_T(t)$ (*right panel*). Bang–bang infusion of cytotoxic drugs together with constant delivery of cytostatic drugs can push cancer cells toward extinction. The unit of time is days. All values are normalized with respect to the initial global population density

(Lorz et al. BMB 2015)

Back to "Why is evolution important in cancer?" Questions on *multicellularity and cancer*

- Cancer is a disease of multicellular organisms, that has been evidenced, including in fossils, in the whole animal kingdom
- Cancer *is* the failure of maintenance of a coherent (=founded on stable cellular differentiations) multicellularity, or else: encore :
- Cancer may be defined as ca loss of cohesion of tissues and organs of a same organism following failures in differentiation
- Does there exist in the construction of multicellularity a qualitative succession of new families of genes responsible for 1. proliferation and apoptosis 2. differentiation (transcription factors?); 3. epigenetic control of differentiations ? Phylogenetic scenarios of evolution of mutations in AML go in the opposite direction with increasing malignancy (Hirsch et al. Nature Comm. 2016)
- Some gene mutations predispose subjects to well-identified organ cancers: do these genes play a role in the anatomic constitution of multicellularity?
- Evolution proceeds by *tinkering* (*François Jacob*, 'Evolution and tinkering', *Science* 1977), using every possible avaible material: what in such a succession of tinkerings makes an organism viable but fragile?
- The genes that are altered in cancers are the same that serve multicellularity design (*Domazet-Lošo & Tautz 2010, Davies & Lineweaver 2011*): can we methodically collect these genes?

Questions (continued)

- What defines *a same organism* ? A 'self' that would be conserved during the sequences of differentiations that lead to the '200 terminally differentiated cell types' in Man?
- What holds together, normally without conflict, the cell types (the interferon pathways??), and what does the immune system recognises as non-self (foe rather than friend) in a cancer cell?
- Is there a relationship of such coherence with the major histocompatibility complex (MHC)? What is its primary function, if not to ensure organism cohesion (of tissues), and how does such coherence (of signals) operate?
- Can we parallel evolution of species and evolution of their immune system? Some enlightenment to collect genes active at multicellularity constitution?
- Loss of control of differentiations: do all cancers have in their evolution an epigenetic origin or compulsory step?
- Some is known of mutations in genes that control epigenetics (e.g., DNMT3A, TET2) in early leukaemogenesis, and of genes of cell metabolism (IDH1, IDH2) in cancers (AML, glioblastoma): can we propose and exemplify a standard scenario linking perturbations of metabolism / perturbations of epigenetic control of differentiations / cancers?

Questions (continued)

- Energetic metabolism of the cell, intercellular communications and cancer: appearance of gap junctions in multicellularity and perturbations physiological gap junctions, that are essential to multicellularity, in solid tumours? (*James Trosko*)

- Glycolytic vs. mitochondrial respiratory phenotypes: do cancer cells shift easily from one to the other (in other words, does a tumour practice a form of metabolic *bet hedging*?) Gravenmier et al. Bull. Math. Biol. 2017)

- What are the advantages and drawbacks of these 2 phenotypes? (efficiency of the TCA [=Krebs] cycle vs. rapidity of anaerobic glycolysis) When did appear the mitochondrial respiratory mchain a necessary condition for the establishment of reliable intercellular communications?

Questions (continued)

- Phenotypic heterogeneity of cancer cell populations in a same tumour in the case of stress response: result of primary massive de-differentiation?
- *Bet hedging* as a 'tumour strategy' to diversify its responses to deadly stress (as high doses of cytotoxic drugs) in launching different response stress in different cells? (ABC transporters, detoxication enzymes, blocking influx, DNA repair)
- Stress response through derepression of *cold genes*? Wu et al. PNAS 2015: existence of very ancient genes, constituted in a remote past of our planet, able to put at work des survival programs in a state of emergency, with *bet hedging*, in a cancer cell population?
- "Maintenance of phenotypic heterogeneity within cell populations is an evolutionarily conserved mechanism that underlies population survival upon stressful exposures." (Guler Cancer Cell 2017) Chromatin regulators as 'cold genes' aiming at maintaining a subpopopulationof resistant cells in case of extreme, life-threatening, stress?
- Role of transposable elements transposables in the maintenance of such heterogeneity? "In the context of evolution, activation, and propagation of transposable elements enables organisms to adapt to changing conditions by generating genomic diversity (...), but can also result in reduced fitness." (Guler Cancer Cell 2017)
- What is more relevant for stress response of a cell population (adaptable, as in the case of a tumour): maintain a subpopulation of all-stress resistant cells, or maintain a subpopulation of cells expressing 'cold genes' and able to launch different resistance mechanisms in different cells? (stochastically chosen?)

A few articles that open ways

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A few articles that open ways

6. *One may also consult a few personal articles*

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