CATMO 2020 Workshop on Cancer Adaptive Therapy Models

Plasticity in cancer cells and emergence of drug-induced drug resistance: what consequences for therapeutics?

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Virtual meeting, December 7-10, 2020

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

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Cancer puzzle: beyond intracellular signalling pathways

- Cancer is a disease of multicellular organisms: save for known molecular events (CML, APL, Ewing sarcoma), there are no *determinants* of cancer in a single cell
- Cancer is a localised *loss of cohesion* between cells and tissues in a multicellular organism: loss of control on differentiations, prior to uncontrolled proliferation
- The atavistic hypothesis of cancer by Davies, Lineweaver and Vincent (2011) sets a *reverse* evolutionary origin for the emergence of cancer cell populations
- Disrupted expression of genes in cancer hits genes of multicellularity (Domazet-Lošo & Tautz 2008, 2010, Trigos et al. 2017, 2018, 2019)
- What is coherence/cohesion within/between cells and tissues made of in a multicellular organism? Why and how is it disrupted in cancer?

Modelling cell plasticity and 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*, is a relevant setting to represent continuous evolution towards drug resistance in tumours.
- *Plasticity* in cancer cells, i.e., propension of epigenetic nature to reversal to a de-differentiated status, and resulting adaptability of cancer cell populations, makes them able to *reversibly* resist abrupt drug insult as sharp stress response.
- Such *reversible* plasticity is captured by mathematical models (PDEs) that incorporate between-cell population heterogeneity by making use of structuring *continuous phenotypic variables*.
- These models are compatible with *optimal control* methods for the design of therapeutic strategies involving combinations of cytotoxic and cytostatic drugs.

Can resistance be assessed by biological experiments?

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

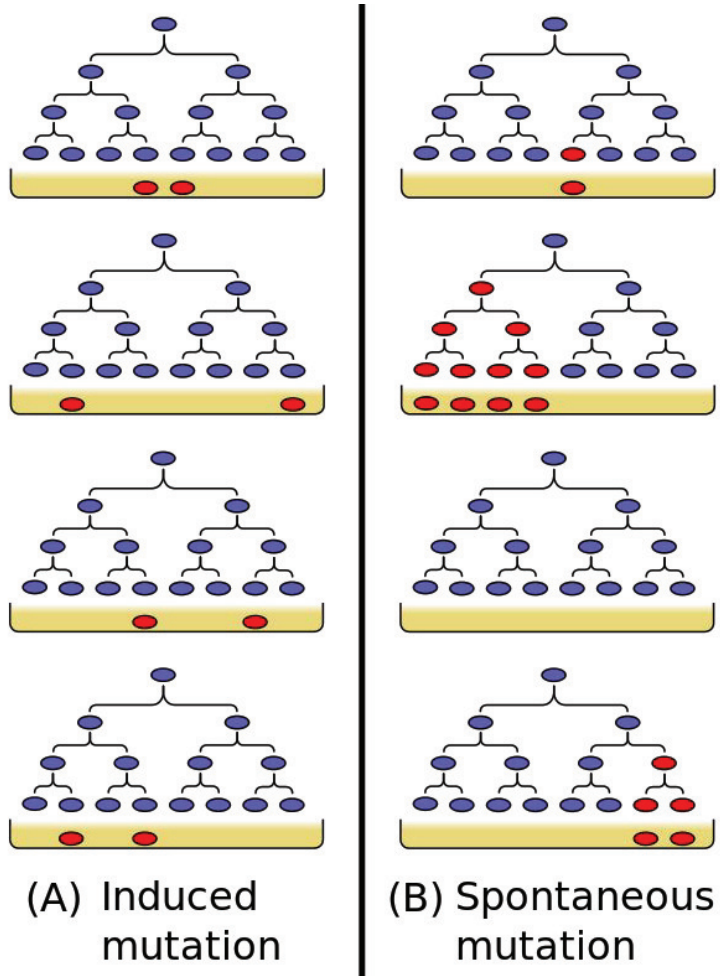
Different Petri dishes, same experimental settings

Bacterial populations firstly proliferating freely, then exposed to a phage environment: some will show resistance to the phages

Question: Is resistance induced by the phage environment, scenario (A)? Or was it preexistent in some subclones, due to random mutations at each generation, and selection by the phages, scenario (B)?

Experiment: the answer is always (B): preexistent mutations before selection (they are not phage-induced)

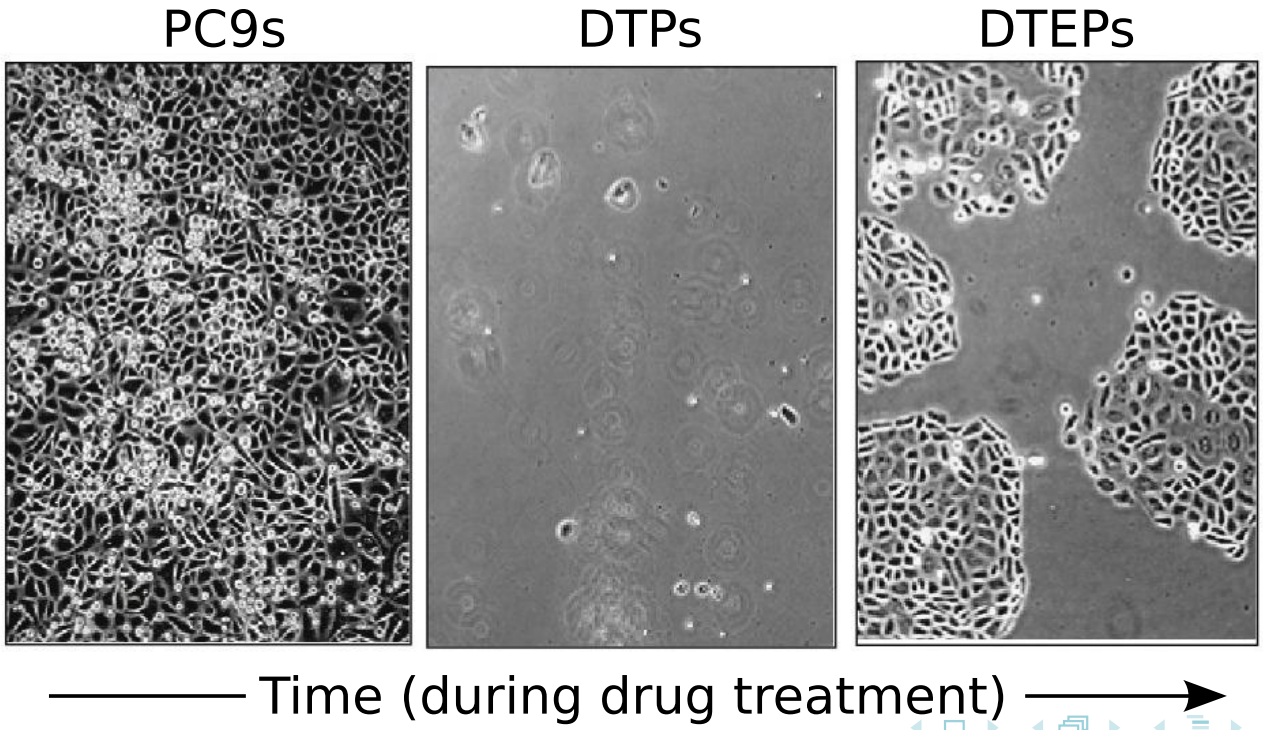
However, bacteria are not cancer cells! In particular, they are far from being able of the same plasticity (no - or poor? - differentiation leading to division of work is available for them). Otherwise said, bacterial resistance is not drug-induced *persistence* (next slide)



(*Luria & Delbrück, Genetics, 1943*)

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, modelled in Chisholm et al. Cancer Research 2015)

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...

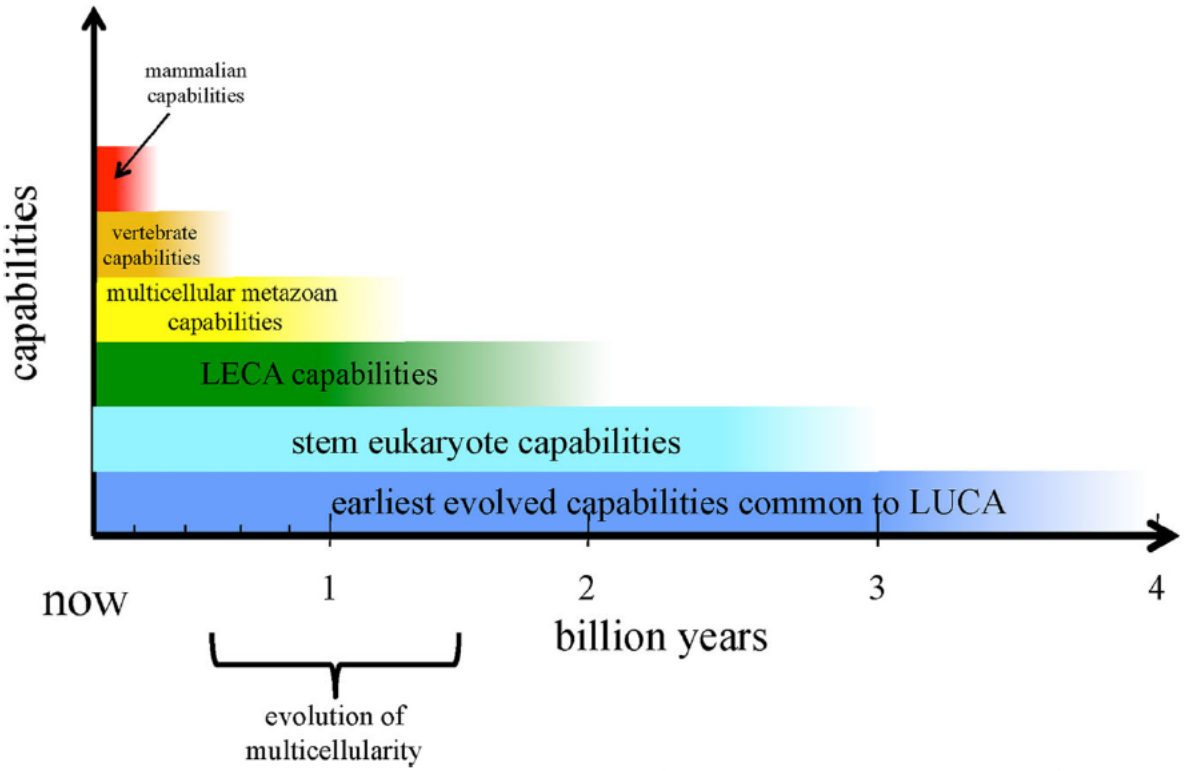
- ... Whereas the expression *drug resistance* today most frequently assumes established, *irreversible*, genetic mutations.

- 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)*.

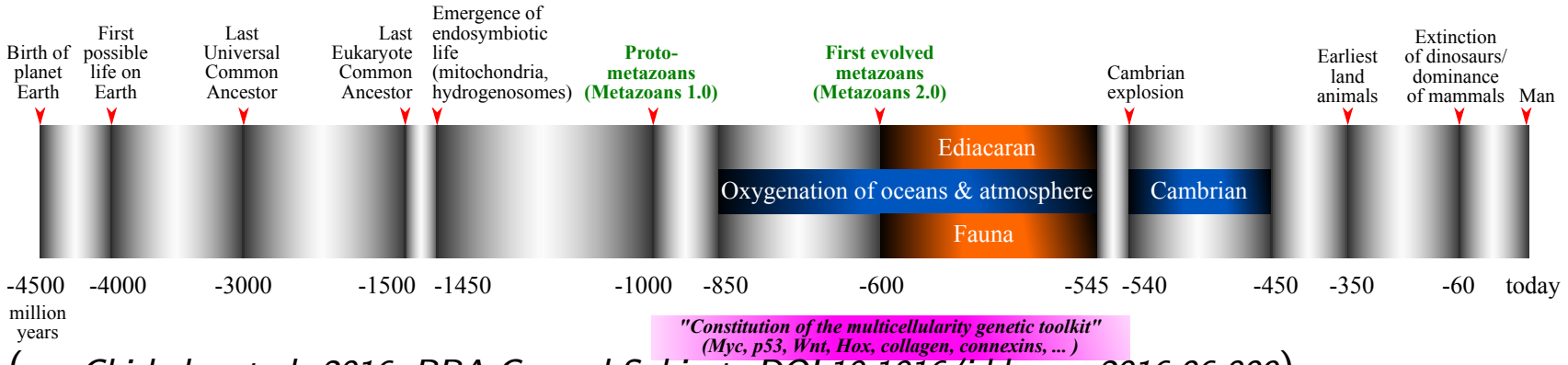
A *reverse* evolutionary framework (*billion year-term view* for multicellular organisms): the atavistic theory of cancer (1)

"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, Chen et al. Nature Comm 2015, 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*

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

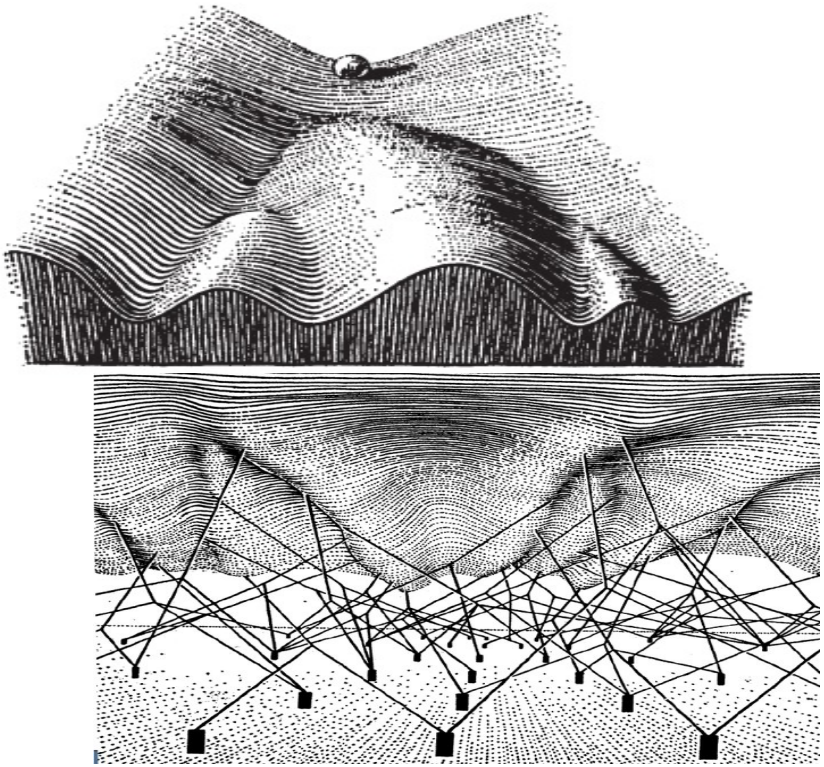


(*see Chisholm et al. 2016, BBA General Subjects DOI:10.1016/j.bbagen.2016.06.009*)

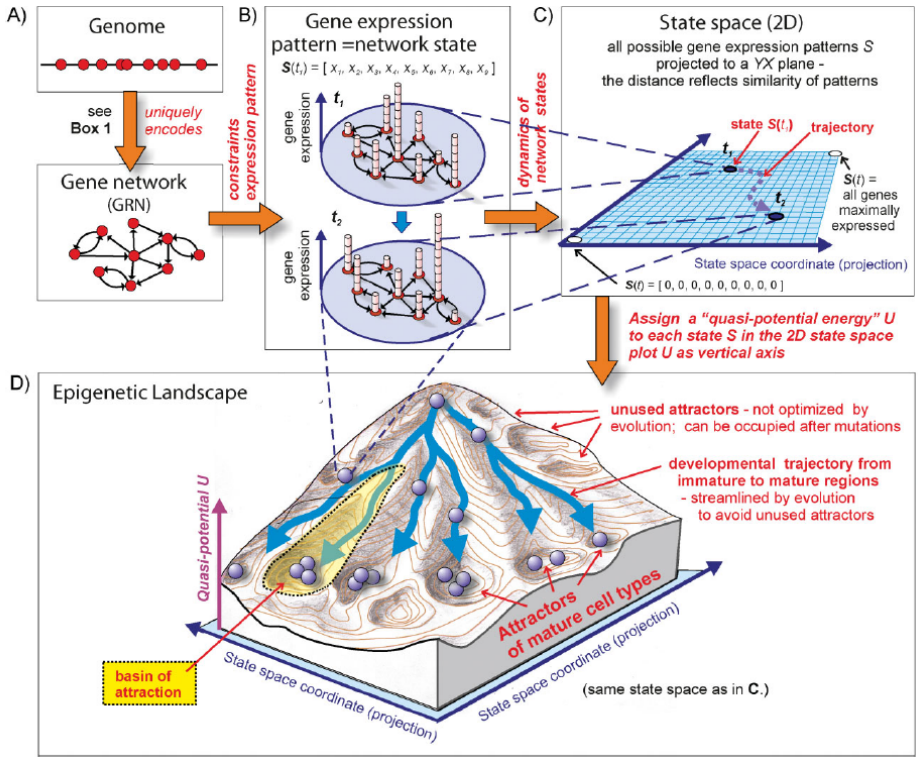
- The genes that have appeared in the development of multicellularity are those that are altered in cancer (as shown in phylostratigraphic analyses by Domazet-Lošo & Tautz 2010; investigated by Trigos *et al.* 2017, 2018, 2019)
- In order, in evolution, from 1) proliferation+apoptosis to 2) cell differentiation + 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 genetic 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)

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

The classic Waddington landscape ("The strategy of genes", 1957): differentiation of cells *within a given organism*



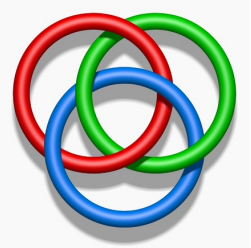
Waddington landscape revisited by S. Huang (2011, 2012, 2013)



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

Differentiation control to make a multicellular organism: yet another metaphor, the wickerwork basket

A fibre bundle (base, the body plan; fibres, the cell differentiation trees; at the rim of tips, terminally differentiated cells). Intertwining the trees that stem from the body plan are between-fibre connections that *control the coherence of differentiations* (part of a proposed extended vision of the immune system, that makes the unity of the organism), the *cohesion watch*, disrupted in cancer. These 3 elements: (1) body plan, (2) differentiation trees and (3) *cohesion watch* together make a Borromean knot.



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Structured equation models for heterogeneous 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 individuals bearing trait x at time t
- (1) Evolution in numbers of individuals constituting the population

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

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

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

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

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

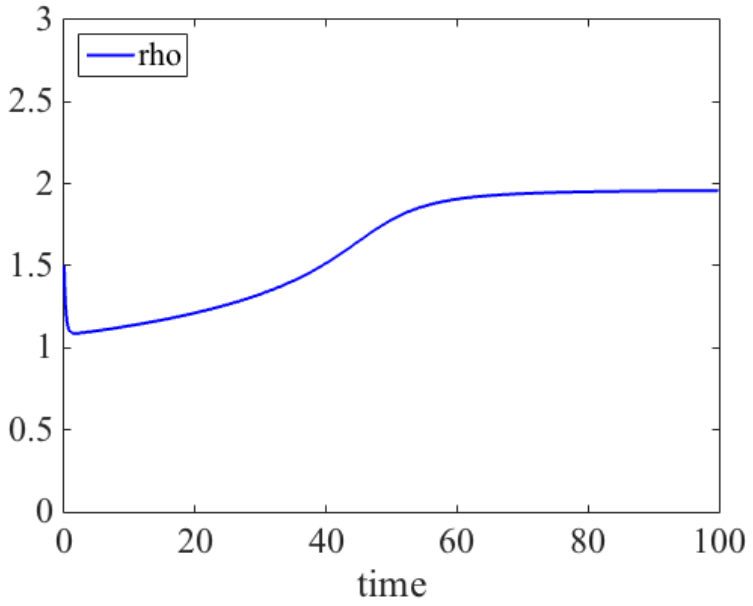
with

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

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

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

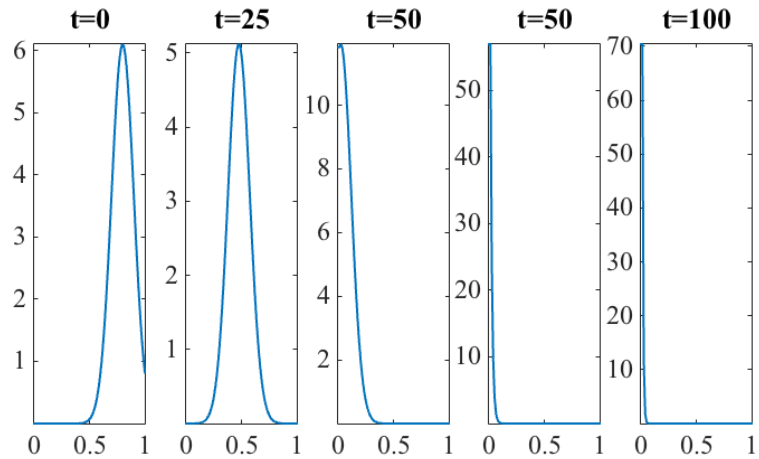
Convergence (one-population case): plot of $t \mapsto \rho(t) := \int_0^1 n(t,x)\,dx$



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 $[0,1]$.

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*

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

The same result (convergence in time t and concentration in trait x) can be shown with two or more variables, see for two *Pouchol et al. J Maths Pures Appl 2018*, and for more *Pouchol & Trélat J Biol Dynamics 2018*

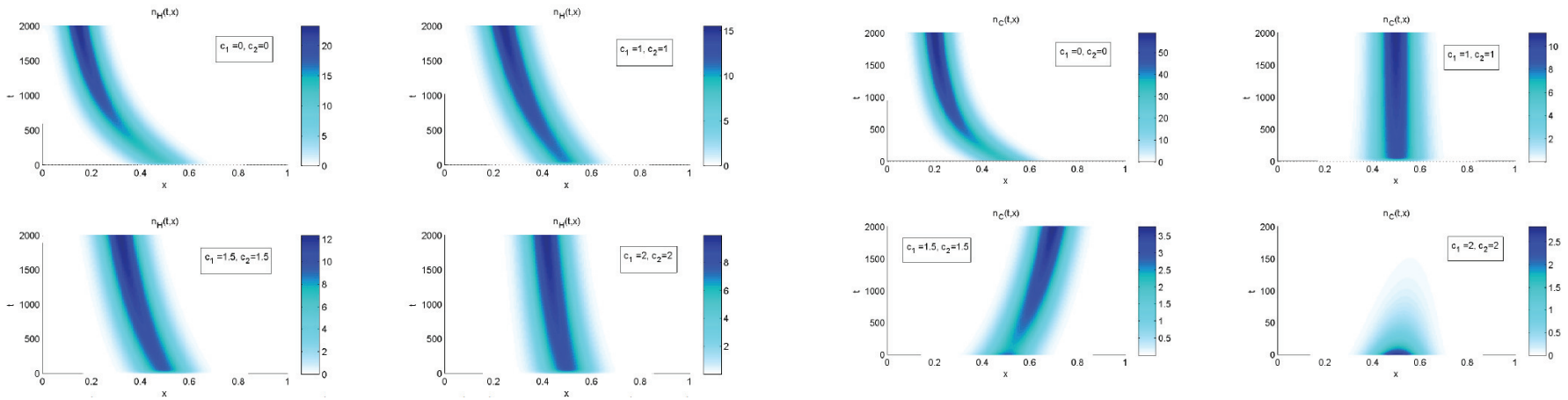
Non-local Lotka-Volterra 2-population integrodifferential model: two different drugs and 1D resistance phenotype x

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

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

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

[A kernel integral has been added for small mutations]

Cancer cells: eventually extinct

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

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 $u_1(t)$

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

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

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

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

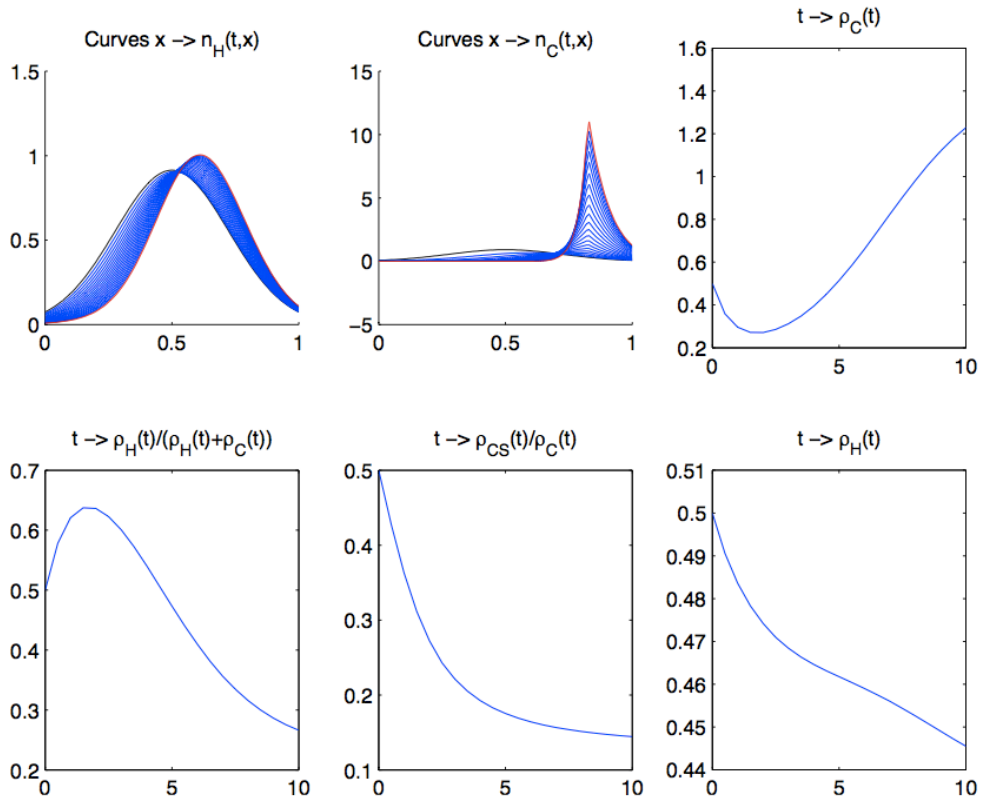
$$\frac{\rho_H(t)}{\rho_H(t) + \rho_C(t)} \ge \theta_{HC}, \qquad \rho_H(t) \ge \theta_H.\rho_H(0)$$

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

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}.

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 $u_2 = u_2^{\max}$.

Simulations illustrating this theorem

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

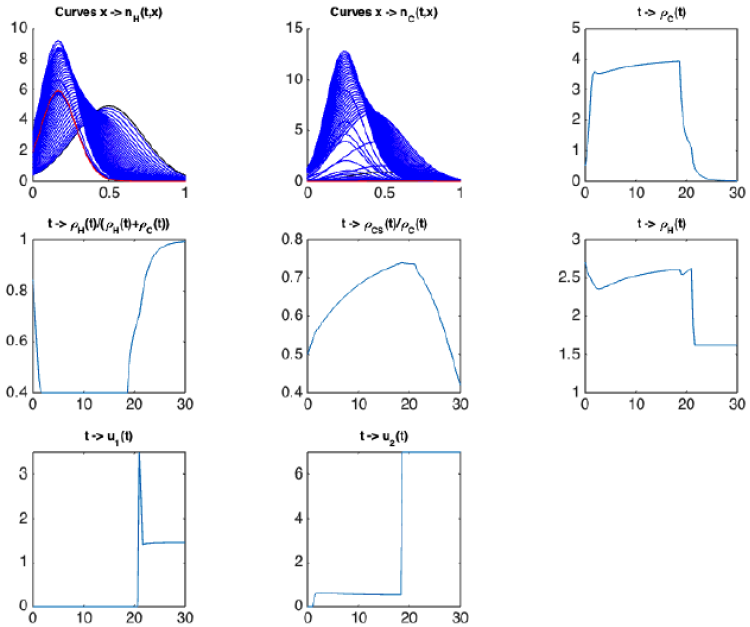


Figure 4: Simulation of (**OCP**) for $T = 30$.

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

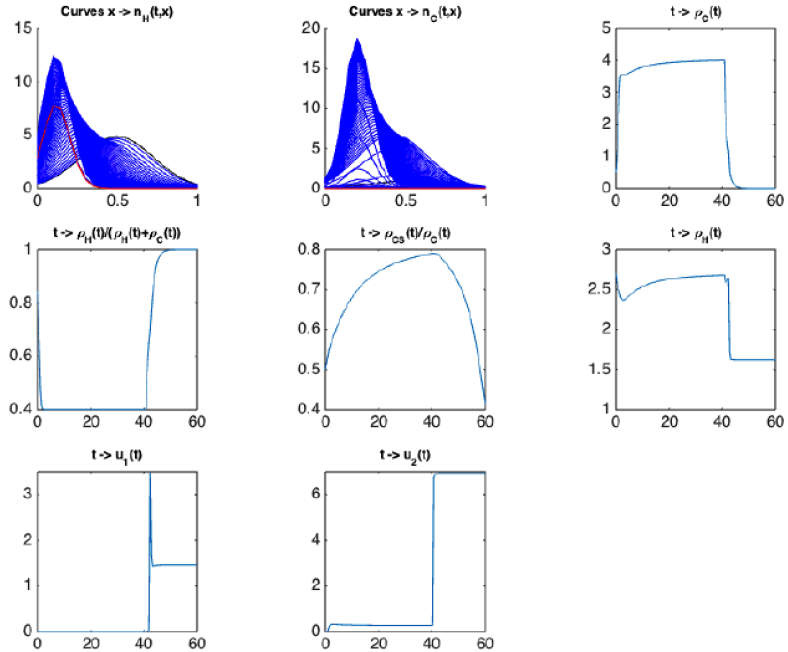


Figure 5: Simulation of (**OCP**) for $T = 60$.

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.

Comparison with "almost periodic" therapeutic strategies

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

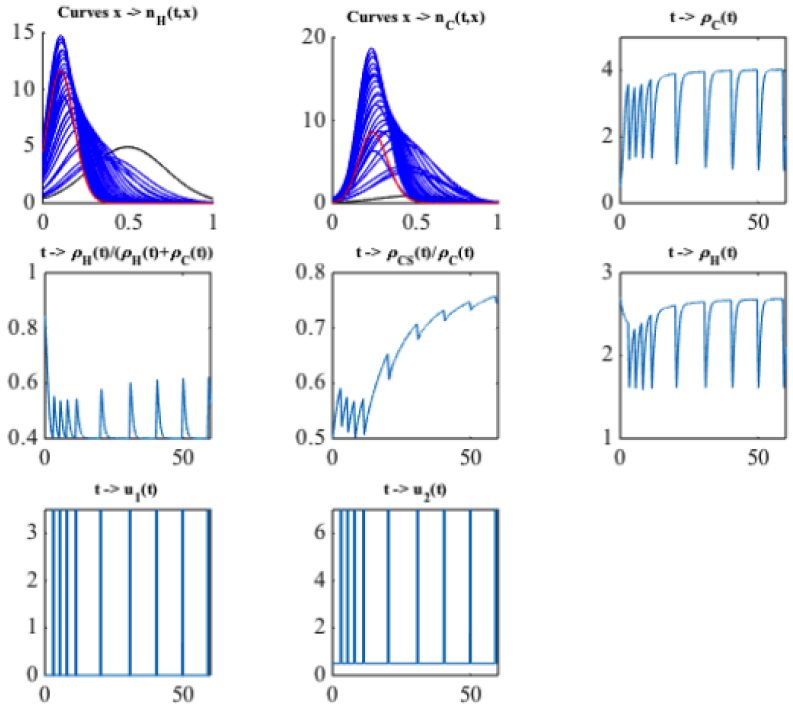


Figure 6: Quasi-periodic strategy, for $T = 60$.

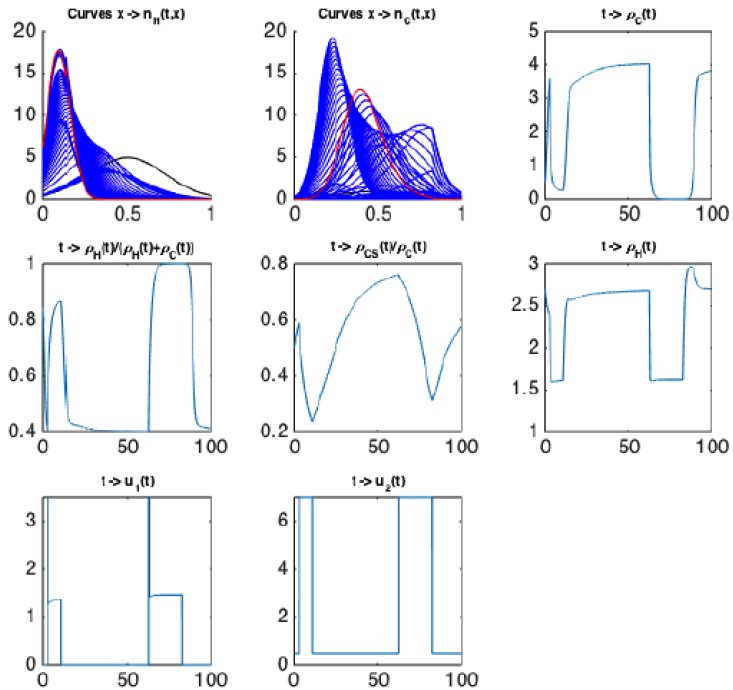


Figure 7: Second quasi-periodic strategy, for $T = 100$.

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?).

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

- 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 *plastic* cells expressing 'cold genes' (*Wu et al. PNAS 2015*), able to launch different resistance mechanisms in different cells?

- *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...)

- Bet hedging setting for $n(x, y, \theta, t)$, with x=fecundity, y=viability, θ=plasticity:

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

- 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, germen/soma) yielding either a homogeneous population of hybrid cells (sort of Pareto optimum?) or a heterogeneous cell population of two coexisting subpopulations separately maximising each phenotype... and nevertheless somehow sticking together. *Is not the latter choice at the origin of multicellularity in eucaryote cell populations, admitting that tumours constantly reinvent multicellularity?* (work underway)

Immune checkpoint inhibitor (ICI) 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 immune cell police.
- Immune checkpoint inhibitors, i.e., 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.
- CAR T-cells have also achieved remarkable cures (LLC, lymphomas), however with the same limitations: boosting the power of the immune police may have unexpected and unpredictable counter-productive effects (e.g., cytokine release syndrome).
- (ICI therapy modelling with optimal control: also work underway, again using structured population models for immune and cancer cells)

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

- If we admit 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 program launched by fecundation, then material as a set of cohesive intercellular connections within the constituted organism, it remains for 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. 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 these connections, 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.

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 by designing mathematical models of heterogeneous cell populations with built-in therapeutic targets and optimal control for the therapeutic means of action.

- 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 so. 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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... plus a recent, easy-reading conference paper

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