Combination therapies and drug resistance in heterogeneous tumoral population.

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Mathematical Perspectives in the Biology and Therapeutics of Cancer,
9-13 July 2018, CIRM, Marseille, France

Guidelines of the research

- Mathematical **models** for complex systems. Data and phenomena driven
- **Frameworks**: structured populations and population dynamics
- **General aim**: study patterns of evolution and adaptation through mathematical modelling, asymptotic analysis and numerical simulations
- **Virtual laboratories**: test hypothetical scenarios giving new insights into behaviors emerging from the complex interactions

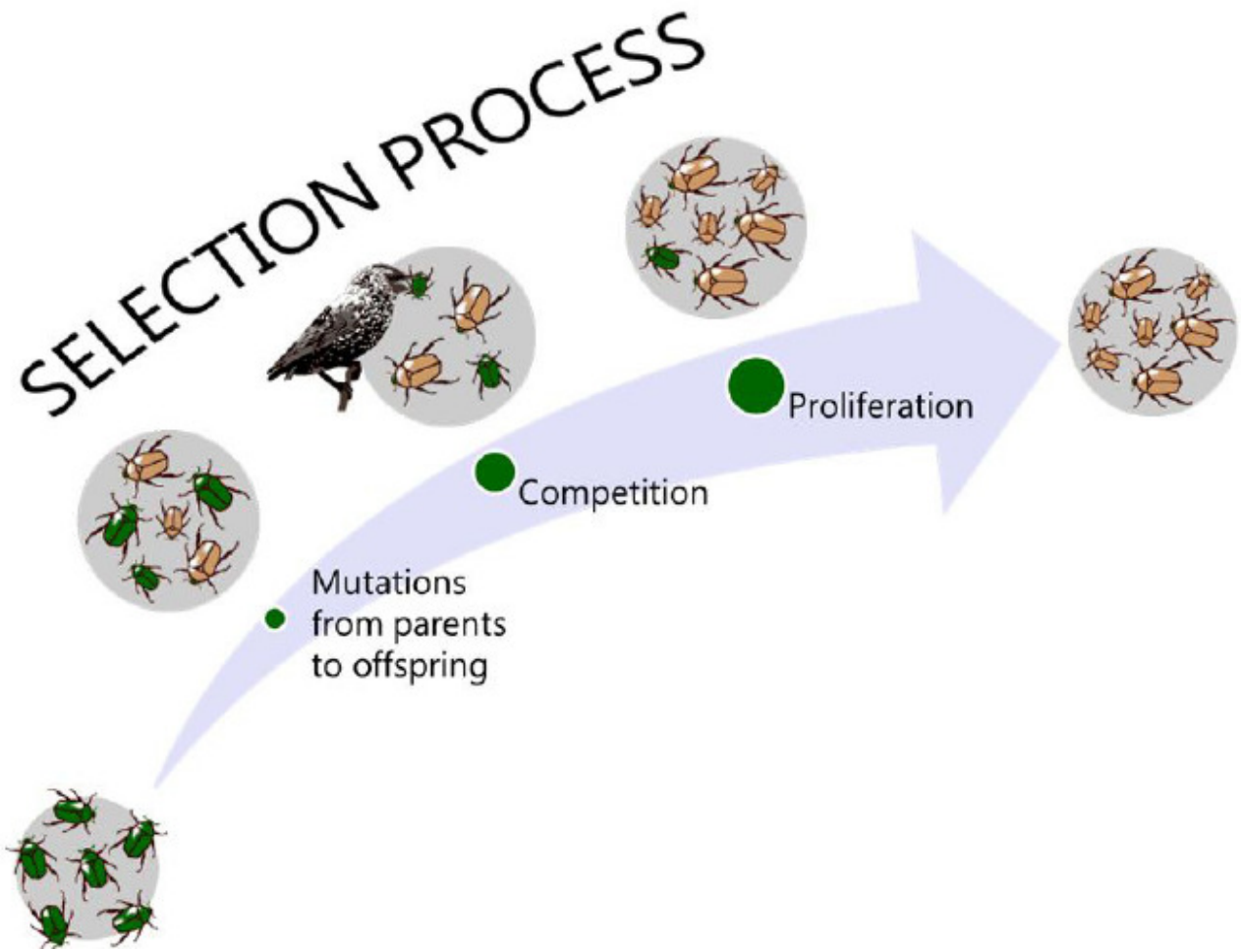
Outline of the talk

- Ecology of cancer and evolutionary dynamics
- Mathematical model and asymptotic behaviour
- Experimental data and parameter estimation
- In silico laboratory. Monotherapy and combination therapy: different does and temporal order of administration (concomitant and sequantial)
- NSCLC lung cancer

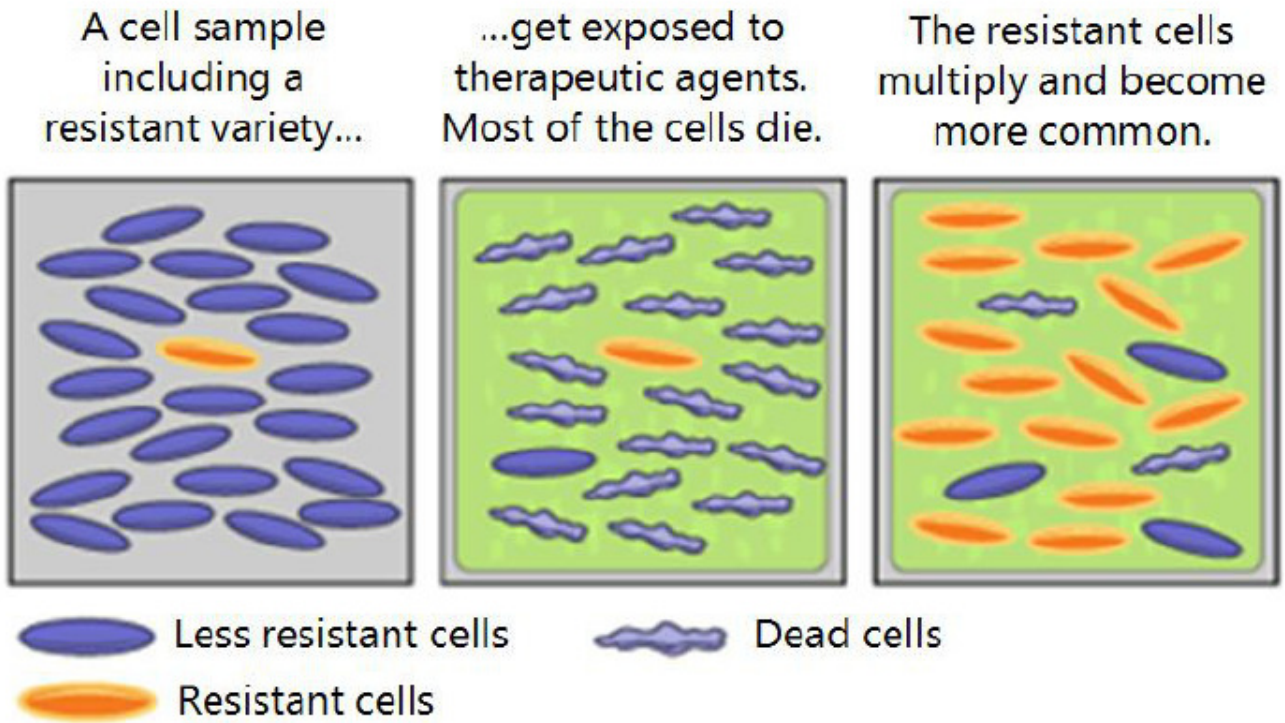
Darwinian evolution

- **Phenotypes**: organism's observable characteristics
- **Fitness** of a phenotype: measure of ability to survive and reproduce
- Theory of evolution by means of **natural selection**. Traits **more fitting**, i.e. better **adapted to survive** and reproduce in the environment, are preserved
- Give rise to **diversity** at every level of biological organization

Selection


SELECTION PROCESS
Proliferation
Competition
Mutations
from parents
to offspring

Emergence of resistance in cancer



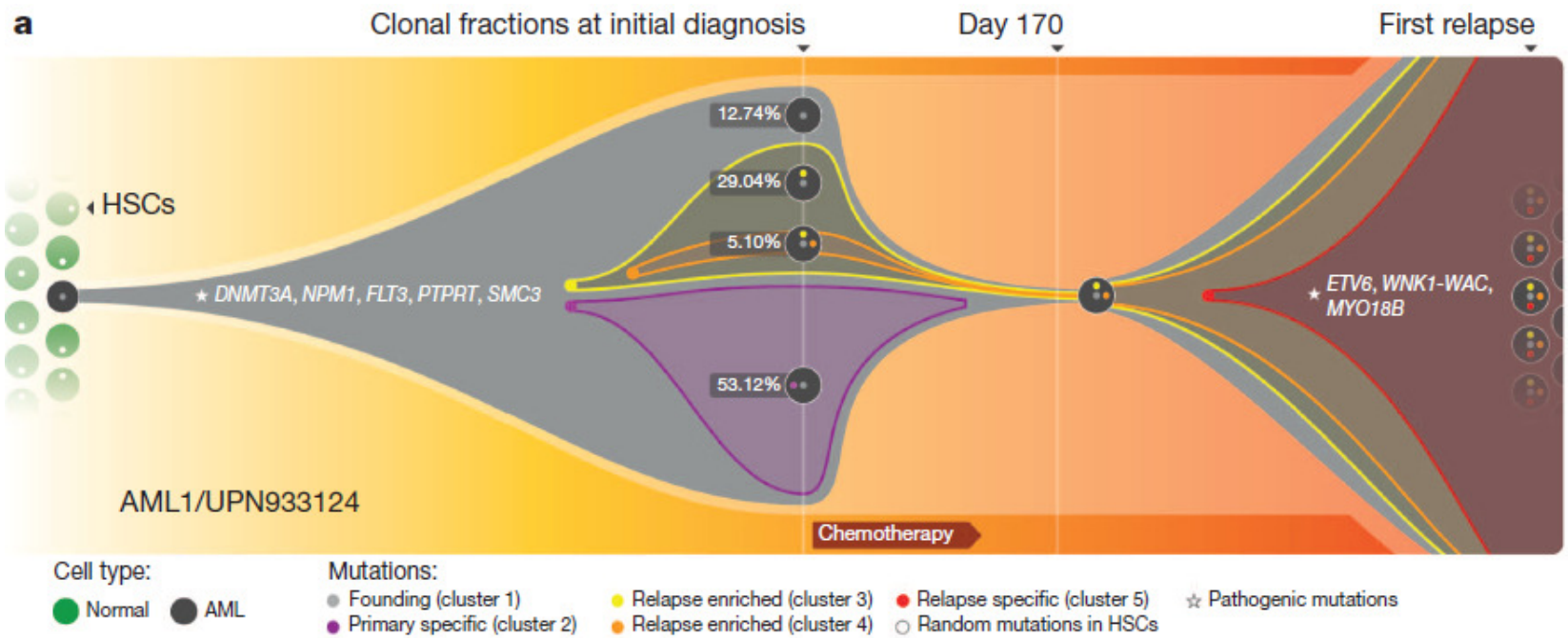
Heterogeneity and clonal selection in AML

LETTER

doi:10.1038/nature10738

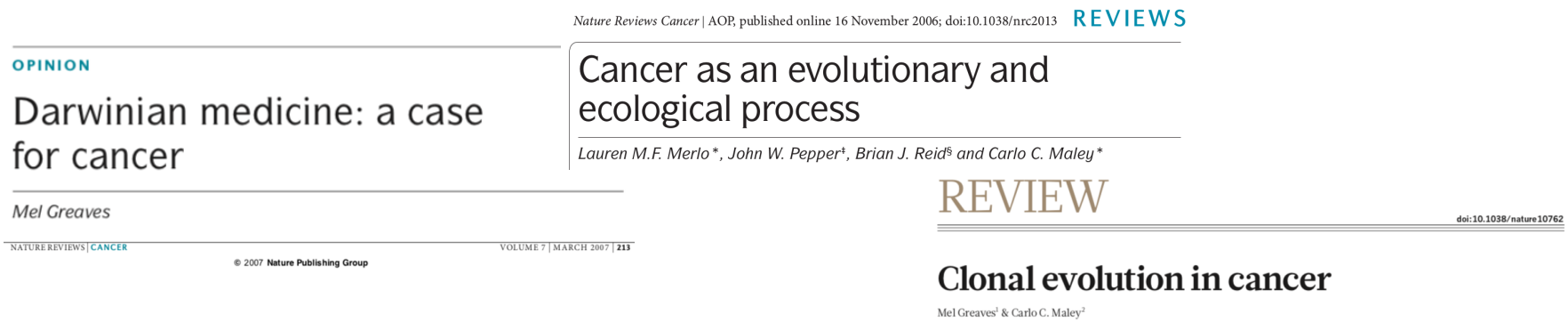
Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing

Li Ding[1,2]*, Timothy J. Ley[1,3,4]*, David E. Larson[1], Christopher A. Miller[1], Daniel C. Koboldt[1], John S. Welch[3], Julie K. Ritchey[3], Margaret A. Young[3], Tamara Lamprecht[3], Michael D. McLellan[1], Joshua F. McMichael[1], John W. Wallis[1,2], Charles Lu[1], Dong Shen[1], Christopher C. Harris[1], David J. Dooling[1,2], Robert S. Fulton[1,2], Lucinda L. Fulton[1,2], Ken Chen[1,2], Heather Schmidt[1], Joelle Kalicki-Veizer[1], Vincent J. Magrini[1,2], Lisa Cook[1], Sean D. McGrath[1], Tammi L. Vickery[1], Michael C. Wendl[1,2], Sharon Heath[3], Mark A. Watson[5], Daniel C. Link[3,4], Michael H. Tomasson[3,4], William D. Shannon[6], Jacqueline E. Payton[5], Shashikant Kulkarni[2,4,5], Peter Westervelt[3,4], Matthew J. Walter[3,4], Timothy A. Graubert[3,4], Elaine R. Mardis[1,2,4], Richard K. Wilson[1,2,4] & John F. DiPersio[3,4]



From primary tumour to relapse with selection of resistant clones

Cancer progression from an evolutionary perspective



OPINION

Darwinian medicine: a case for cancer

Mel Greaves

NATURE REVIEWS | CANCER VOLUME 7 | MARCH 2007 | 213

Nature Reviews Cancer | AOP, published online 16 November 2006; doi:10.1038/nrc2013 REVIEWS

Cancer as an evolutionary and ecological process

Lauren M.F. Merlo, John W. Pepper‡, Brian J. Reid§ and Carlo C. Maley**

REVIEW

doi:10.1038/nature10762

Clonal evolution in cancer

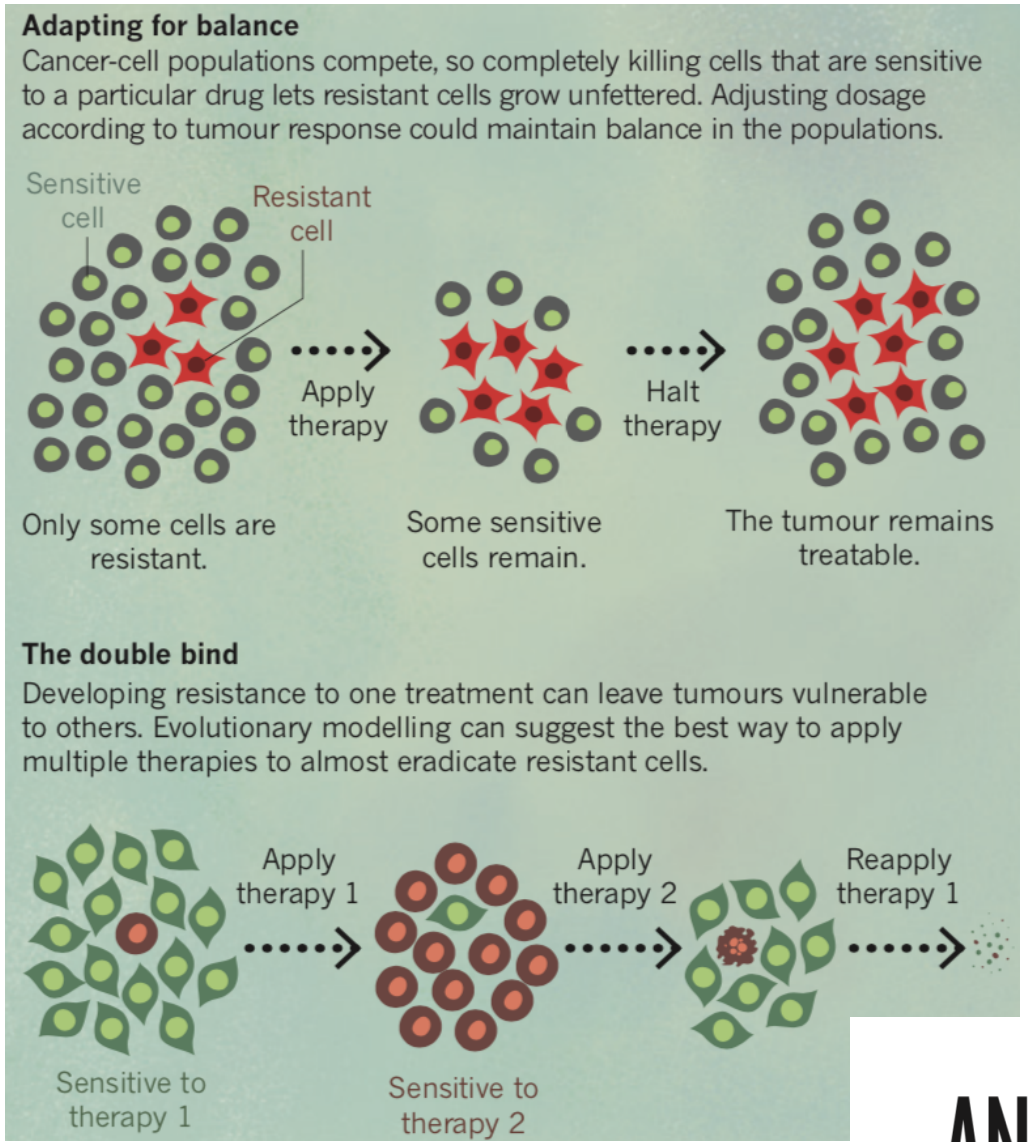
Mel Greaves[1] & Carlo C. Maley[2]

- **Competition for space and resources** (e.g. oxygen and glucose) among healthy and cancer cells
- **Evolutionary bottleneck** due to different selective pressures, e.g. the immune system and anti-cancer therapies
- **Darwinian micro-evolution** selecting for increased proliferation and survival, and might lead to invasion, metastasis and therapeutic resistance

Adaptive therapy

- **Integrate the principles of evolution** into quantitative and experimental methods, and into new strategies for cancer therapy
- **Avoid extinction of sensitive clones** to therapies with low doses of drugs
- Limit (in certain cases) the emergence of the new clone of resistant cells, and ultimately **allow to control the total number of tumor cells**.

and double blind strategies



CANCER:
AN EVOLVING
THREAT

Combination therapies and drug resistance in heterogeneous tumoral population.

166 | NATURE | VOL 532 | 14 APRIL 2016

Ecology of cancer

Translational Oncology
www.transonc.com
Volume 4 Number 5 October 2011 pp. 266–270 266

CANCER ORIGINS

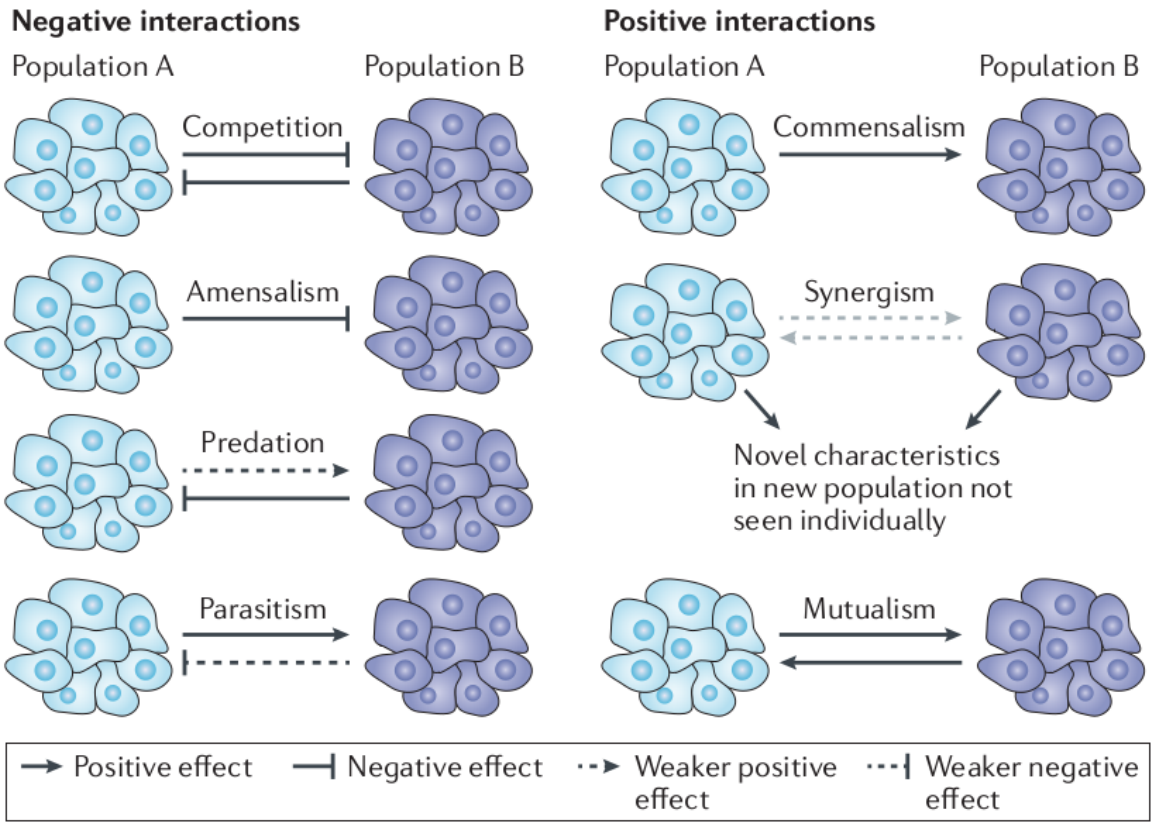
Tumorigenesis: it takes a village

Doris P. Tabassum[1,2] and Kornelia Polyak[1–4]

What Can Ecology Teach Us About Cancer?[1]

Irina Kareva*,†

*School of Human Evolution and Social Change, Arizona State University, Tempe, AZ, USA; †Mathematical, Computational and Modeling Sciences Center, Arizona State University, Tempe, AZ, USA



Marcello Delitala Combination therapies and drug resistance in heterogeneous tumoral population.

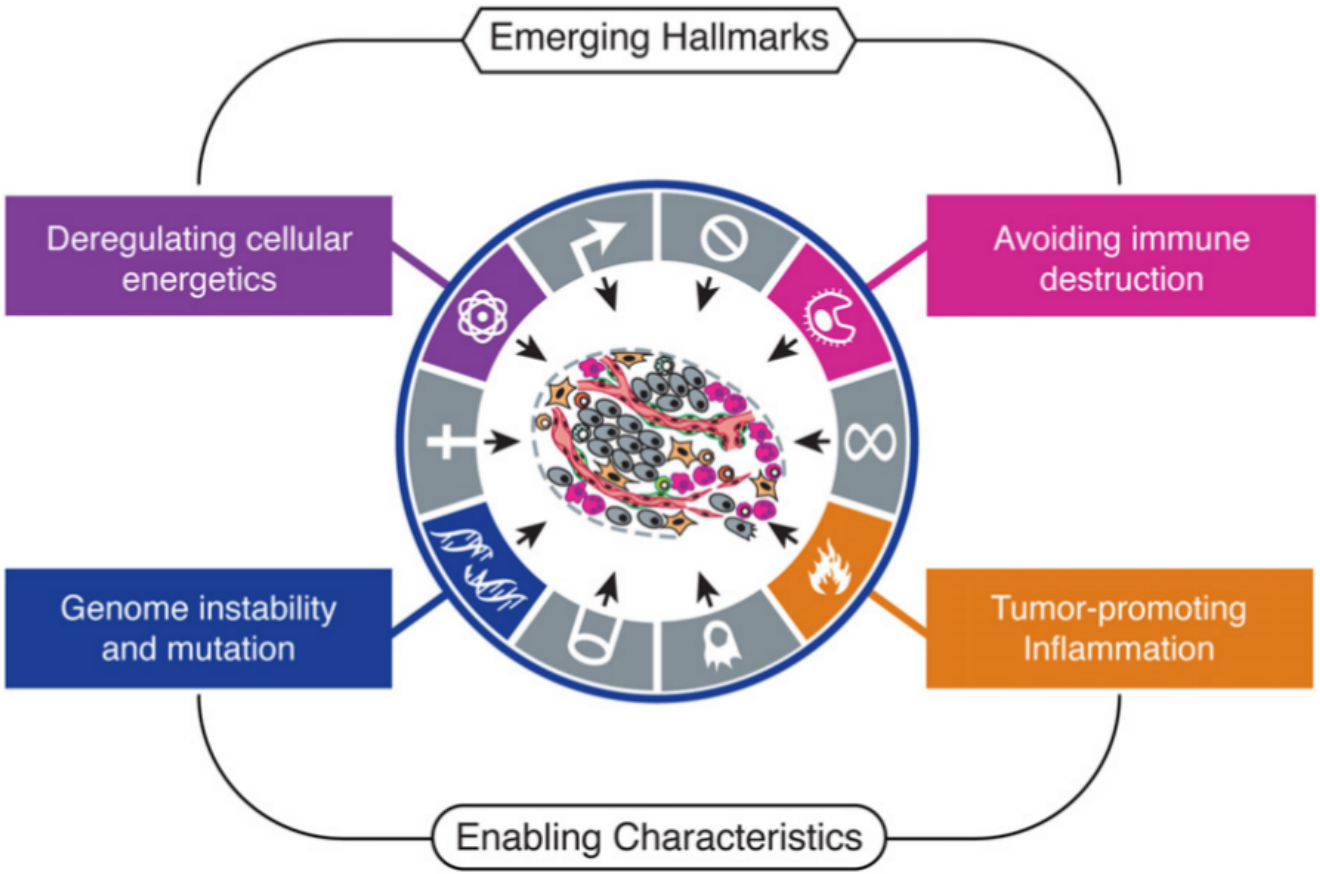
Role of the immune system

Cell

Leading Edge
Review

Hallmarks of Cancer: The Next Generation

Douglas Hanahan[1,2,*] and Robert A. Weinberg[3,*]
[1]The Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, EPFL, Lausanne CH-1015, Switzerland
[2]The Department of Biochemistry & Biophysics, UCSF, San Francisco, CA 94158, USA
[3]Whitehead Institute for Biomedical Research, Ludwig/MIT Center for Molecular Oncology, and MIT Department of Biology, Cambridge, MA 02142, USA
*Correspondence: dh@epfl.ch (D.H.), weinberg@wi.mit.edu (R.A.W.)
DOI 10.1016/j.cell.2011.02.013



Immunotherapies

- **Forefront** of cancer treatment


Immunity
Review

Cell PRESS

Oncology Meets Immunology: The Cancer-Immunity Cycle

Daniel S. Chen[1,3] and Ira Mellman[2,3,*]

- Immunotherapy and antigen-specific protocols to **boost** or restore the **ability of the immune system** to fight cancer, see e.g. *U. Ledzewicz, M.S. Faraji Mosalman, and H. Schhattler, DCDS-B 2013*.

- - **Adoptive cell transfer** identification of immune cells in a tumor, *in lab* expansion and re-infusion into the patients
  - Dendritic cell **(DC)-based vaccines** to induce a long-lasting tumor specific CTL response (sustaining memory T cells)

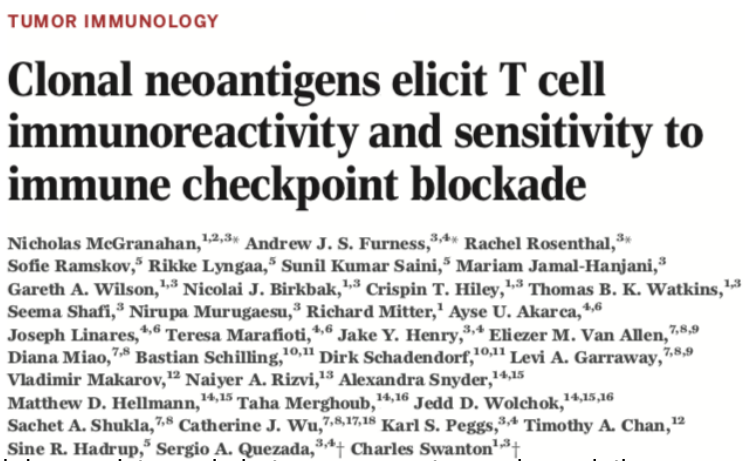

Published OnlineFirst March 28, 2013; DOI: 10.1158/0008-5472.CAN-12-2449

Microenvironment and Immunology

Cancer Research

Booster Vaccinations against Cancer Are Critical in Prophylactic but Detrimental in Therapeutic Settings

Alessia Ricupito[1,2,4], Matteo Grioni[1,2], Arianna Calcinotto[1,2,4], Rodrigo Hess Michelini[1,2,3], Renato Longhi[5], Anna Mondino[2,3], and Matteo Bellone[1,2]


TUMOR IMMUNOLOGY

Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade

Nicholas McGranahan,[1,2,3*] Andrew J. S. Furness,[3,4*] Rachel Rosenthal,[3*] Sofie Ramskov,[5] Rikke Lyngaa,[5] Sunil Kumar Saini,[5] Mariam Jamal-Hanjani,[3] Gareth A. Wilson,[1,3] Nicolai J. Birkbak,[1,3] Crispin T. Hiley,[1,3] Thomas B. K. Watkins,[1,3] Seema Shafi,[3] Nirupa Murugaesu,[3] Richard Mitter,[1] Ayse U. Akarca,[4,6] Joseph Linares,[4,6] Teresa Marafioti,[4,6] Jake Y. Henry,[3,4] Eliezer M. Van Allen,[7,8,9] Diana Miao,[7,8] Bastian Schilling,[10,11] Dirk Schadendorf,[10,11] Levi A. Garraway,[7,8,9] Vladimir Makarov,[12] Naiyer A. Rizvi,[13] Alexandra Snyder,[14,15] Matthew D. Hellmann,[14,15] Taha Merghoub,[14,16] Jedd D. Wolchok,[14,15,16] Sachet A. Shukla,[7,8] Catherine J. Wu,[7,8,17,18] Karl S. Peggs,[3,4] Timothy A. Chan,[12] Sine R. Hadrup,[5] Sergio A. Quezada,[3,4,†] Charles Swanton[1,3,†]

Aims of the virtual laboratory

- Better **comprehension** of mechanisms underlying cancer cell evolution
- Support the design of **optimized anti-cancer protocols**
- Suggestion of **hypothesis-driven experiments**

Reference papers

Journal of Theoretical Biology 446 (2018) 149–159

Contents lists available at ScienceDirect

Journal of Theoretical Biology

journal homepage: www.elsevier.com/locate/jtbi

Combination therapies and intra-tumoral competition: Insights from mathematical modeling

Elena Piretto[a,b], Marcello Delitala[b,*], Mario Ferraro[c]

LETTERS IN BIOMATHEMATICS, 2018
https://doi.org/10.1080/23737867.2018.1465862

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How combination therapies shape drug resistance in heterogeneous tumoral populations

E. Piretto[a,b], M. Delitala[a] and M. Ferraro[c]

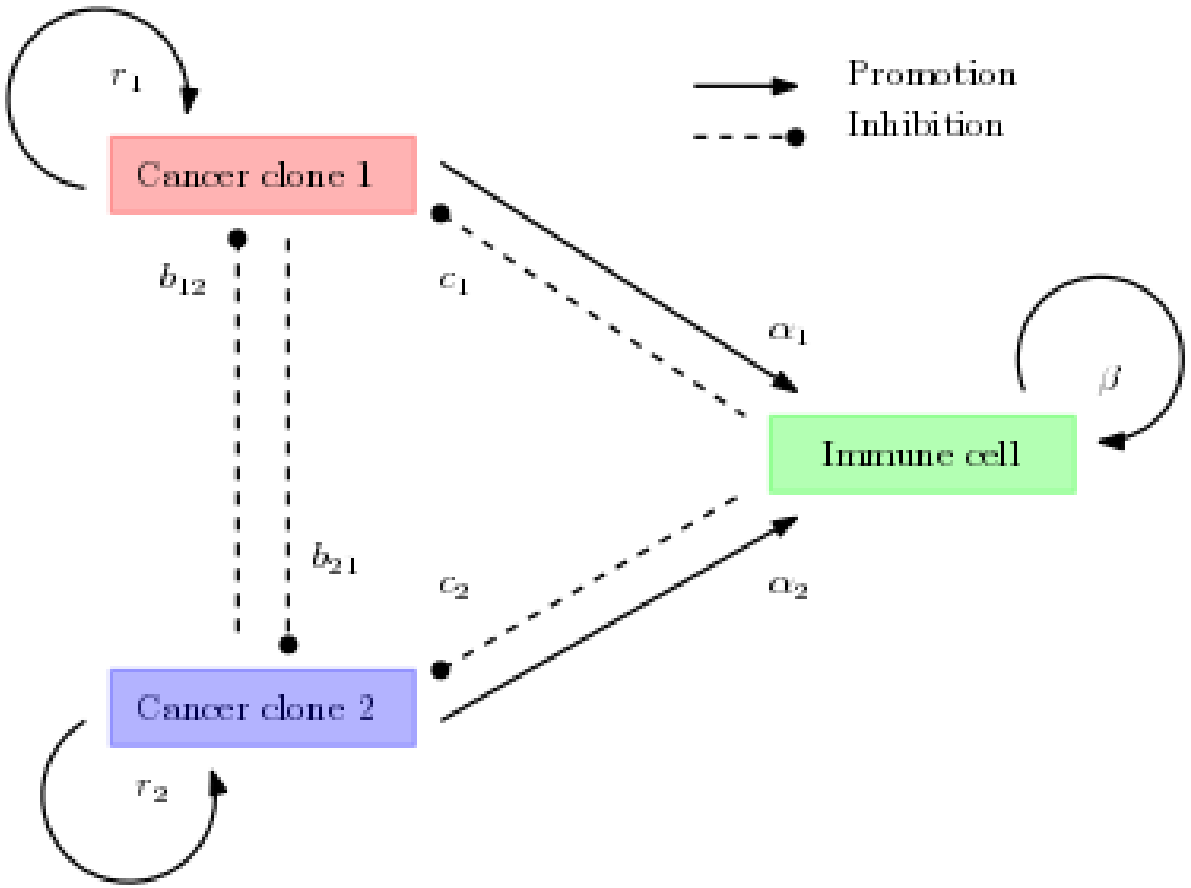
Biological questions

- Which are the **evolutionary mechanisms** that modify the efficacy of the medical treatments?
- **Monotherapy** or **combination therapy**. What to choose?
- In combination therapy, how do different therapy doses and the temporal order of administration affect the outcome?
- Focusing on **Lung Cancer**, how it is possible to reduce the insurgence of resistance?

Modeling approach and populations

- **Population dynamics** approach
- **Cancer cells**: Two clones x_i sensitive and resistant to specific therapies
- **Immune system**: activated T-cells z
- Cells are **homogeneously mixed** *i.e.* no space effects
- Two types of therapies:
  - $g_i(t)$ therapeutic drug such as **chemotherapy**
  - $h(t)$ **immunotherapy** boosting the immune system

A mathematical model



Evolution equations

$$
\begin{aligned}
\frac{dx_1}{dt} &= \underbrace{r_1 x_1 - \frac{r_1}{K_1} x_1^2}_{\textit{proliferation}} - \underbrace{\frac{b_{12}}{K_1} x_1 x_2}_{\textit{competition}} - \underbrace{\frac{c_1}{K_1} x_1 z}_{\textit{predation}} - g_1(t) x_1 - \frac{h(t)}{K_1} x_1 z, \\
\frac{dx_2}{dt} &= r_2 x_2 - \frac{r_2}{K_2} x_2^2 - \frac{b_{21}}{K_2} x_1 x_2 - \frac{c_2}{K_2} x_2 z - g_2(t) x_2 - \frac{h(t)}{K_2} x_2 z, \\
\frac{dz}{dt} &= \underbrace{\beta z \left(1 - \frac{z}{H}\right)}_{\textit{proliferation}} + \underbrace{\frac{\alpha_1}{H} x_1 z + \frac{\alpha_2}{H} x_2 z}_{\textit{recognition}}.
\end{aligned}
$$

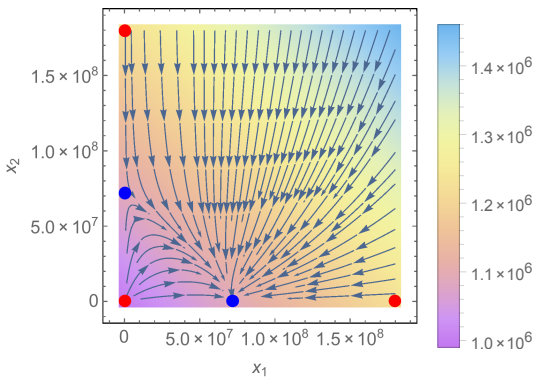
Stability analysis

8 stationary points:

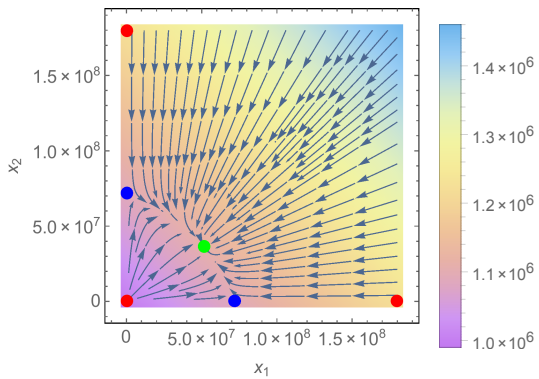
- Four unstable points, of no importance from a biological perspective;
- One point representing **cancer eradication** by the immune system;
- Two points representing the **survival of one cancer clone**: ($EC1$, $EC2$)
- One point of **coexistence** (COE).

Stability analysis

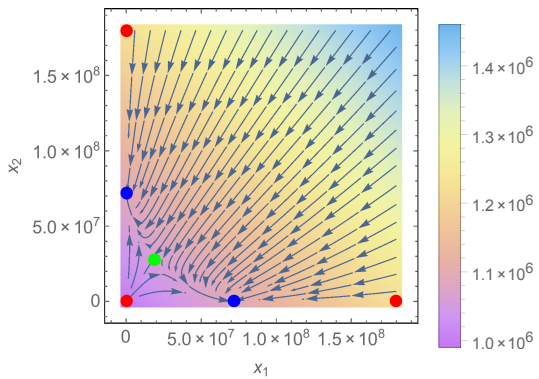
"Survival of the fittest"



Coexistence



"Survival of the first"



Correspondence between different trends and parameters, i.e. composition of the cancer population.

Stability analysis

- Cancer eradication is guaranteed if both $r_1 K_1$ and $r_2 K_2$ are smaller than cH.
- If $r_1 K_1 > cH$ and $r_2 K_2 < cH$, just the species x_1 survives and vice versa.
- If both $r_1 k_1$ and $r_2 K_2$ are larger than cH, the competitive exclusion applies. Survival of the species depends on parameters $A_1 = \frac{c\alpha/\beta + r_1}{K_1 r_1 - cH}$, $A_2 = \frac{c\alpha/\beta + b_{12}}{K_1 r_1 - cH}$, $B_1 = \frac{c\alpha/\beta + b_{21}}{K_2 r_2 - cH}$ and $B_2 = \frac{c\alpha/\beta + r_2}{K_2 r_2 - cH}$.

- If $A_1 < B_1$ and $A_2 < B_2$, the species x_1 dominates (and vice versa). "Survival of the fittest".
- If $A_1 < B_1$ and $A_2 > B_2$, only one of the two cancer clones survives depending on the initial conditions. "Survival of the first".
- If $A_1 > B_1$ and $A_2 < B_2$, coexistence point is stable.

Stability analysis

- **Coexistence** (*COE*). Before therapy, x_1, the susceptible clone, is also the fittest, as it has the larger rate of growth and also a competitive advantage ($b_{12} = 0.02$ and $b_{21} = 0.07$) even though x_2 is not totally eliminated.

- **Competitive exclusion** (*EC*1). x_1 is the dominant clone. In this configuration x_1 increases its competitive advantage ($b_{12} = 0.1$ and $b_{21} = 0.5$) so that, asymptotically, x_2 is wiped out.

- **Competitive exclusion** (*EC*2). The resistant clone x_2 dominates x_1 ($b_{12} = 0.5$ and $b_{21} = 0.09$), so that, asymptotically, x_1 disappears.

Modelling of therapies

r-th Therapies decreasing the rate of growth r_i to f_i:
Chemotherapy such as *paclitaxel* (TAX) or **Molecular Target-therapy**: such as the anti-EGFR, *Cetuximab*, and the anti-MEK, *Pimasertib*.

c-th Therapeutic agents increasing the efficiency of the immune system, from c_i to κ_i:
Immunotherapy such as adenovirus containing full-length mouse wild-type p53 *Adp53 DC*.

We have investigated the action of monotherapies alone and in combination, concomitant and sequential administration.

Therapies Dose

Administered doses:

$$\mathrm{Ch} = \int_{T_i}^{T_f} g(t)\mathrm{d}t = \int_{T_i}^{T_f} [r_i - f_i(t)] \ \ \mathrm{d}t,$$

$$\mathrm{I} = \int_{T_i}^{T_f} h(t)\mathrm{d}t = \int_{T_i}^{T_f} [\kappa_i(t) - c_i] \ \ \mathrm{d}t,$$

where $[T_i, T_f]$ is the time interval of administration of the treatments.

Efficiency of a treatment

Total cancer loads and resistant clones before and after therapy:

$$\epsilon = \frac{x_{pre} - x_{post}}{x_{pre}} \qquad \delta = \frac{x_{2,pre} - x_{2,post}}{x_{pre}}$$

Normalize by means of a sigmoid function

$$g(a) = \frac{1}{1 + \exp(-a)}.$$

and define an efficiency index that depends on the decrease of both x and x_2

$$\gamma = \frac{g(\epsilon)}{g(1)} \frac{g(\delta)}{g(1)}.$$

Threshold for the effectiveness of therapies: $\widehat{\gamma} = 0.5$, i.e. $\epsilon = \delta = 0$.

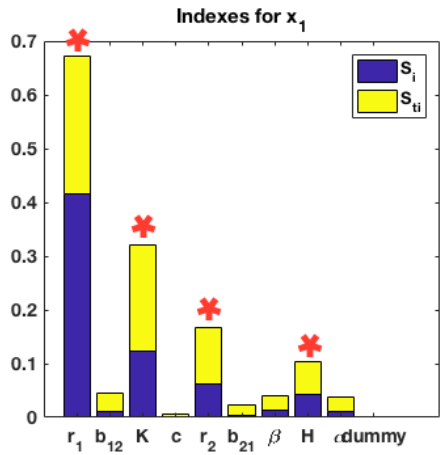
Parameters

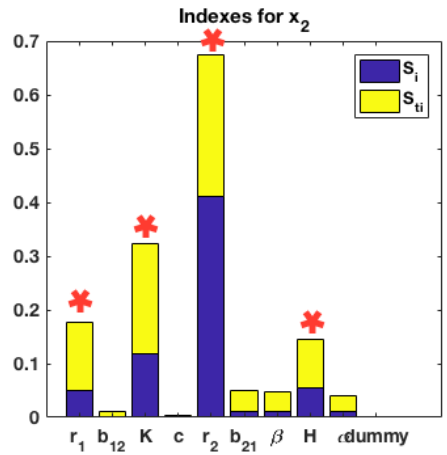
Parameter	Unit	Range or Value	Interpretation	Source
r_i	$days^{-1}$	$0.02 - 02$	Tumour growth rate	De Pillis[1]
b_{ij}	$days^{-1}$	$0.01 - 0.02$	Competition	Estimated
c/K	$days^{-1}$	$1.1 \cdot 10^{-7}$	Killing rate	Kuznetsov[2]
K	cells	$10^7 - 5 \cdot 10^9$	Tumour carrying capacity	De Pillis[1]
H	cells	$5 \cdot 10^4 - 5 \cdot 10^5$	Immune system carrying capacity	Kuznetsov[2]
α/H	$days^{-1}$	$2.4 \cdot 10^{-10} - 6 \cdot 10^{-9}$	Immune recruitment	Kuznetsov[2]
β	$days^{-1}$	0.8	Immune system growth rate	De Pillis[1]
$f(t)$	$days^{-1}$	0.22	Death rate due to chemotherapic drug	data
$g(t)$	$days^{-1}$	$4.9 \cdot 10^{-7}$	killing rate due to vaccination	data

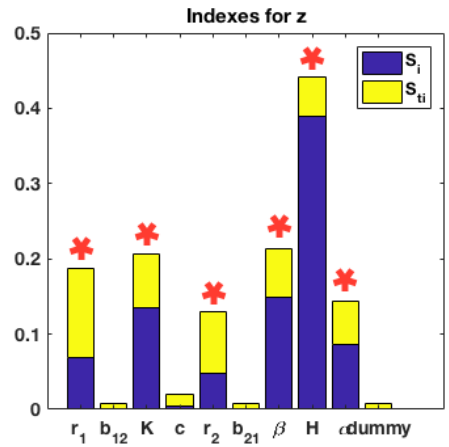
[1] AE Radunskaya, LG de Pillis, W Gu, JTB, 2006

[2] VA Kuznetsov, IA Makalkin, MA Taylor, AS Perelson, Bulletin of Mathematical Biology,1994.

Global sensitivity analysis

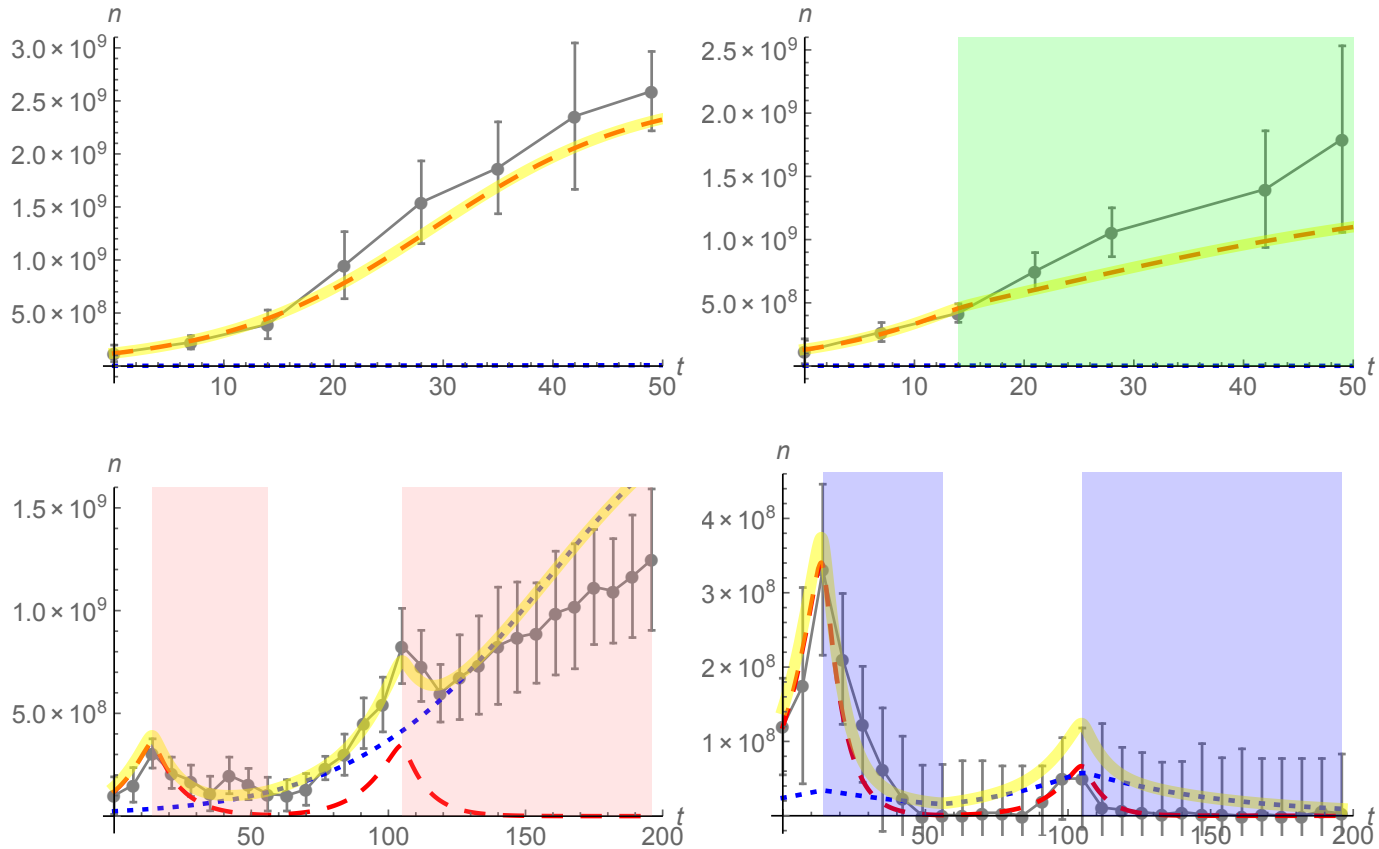






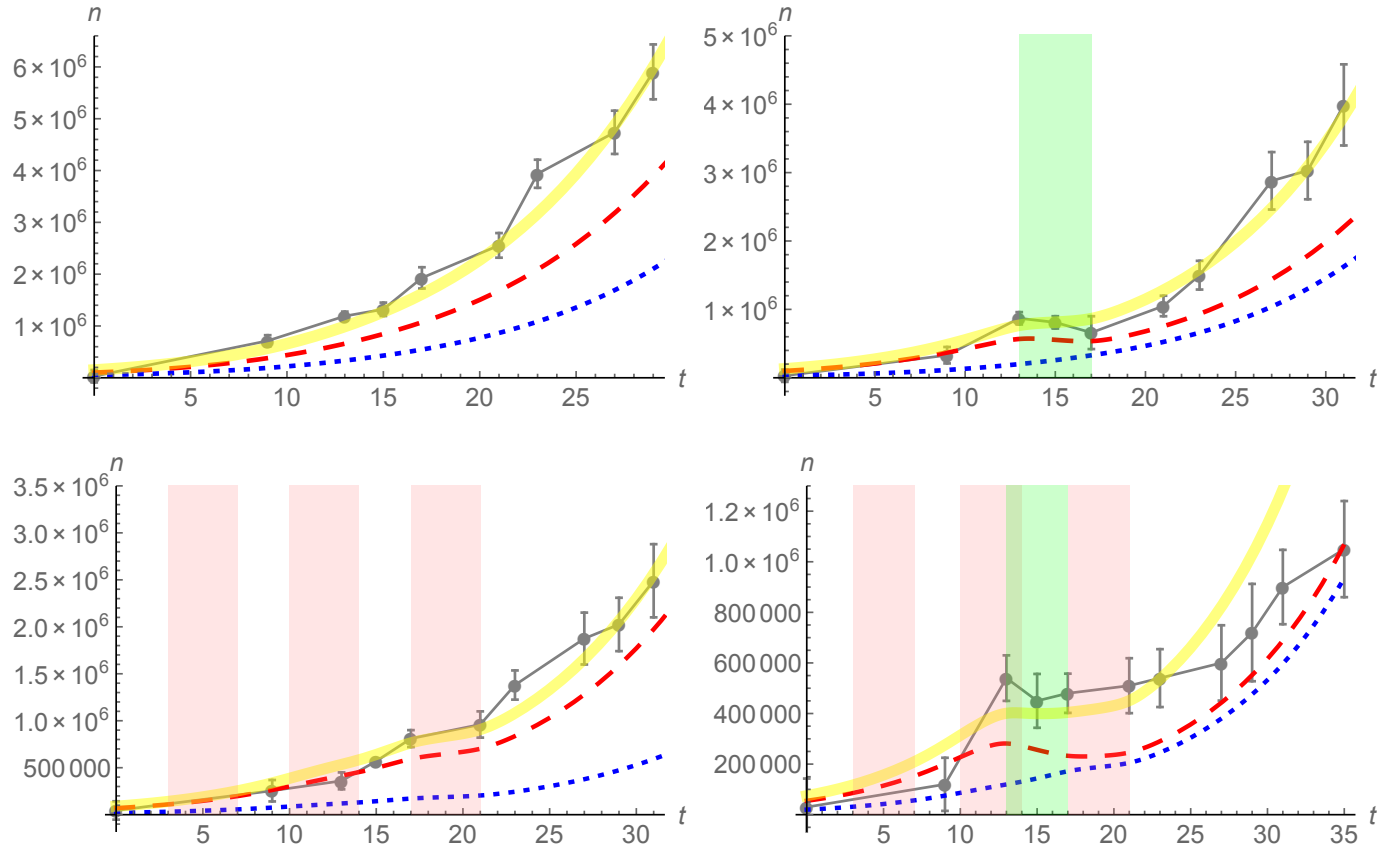
Evaluation of the effects on an error in the parameter estimation: parameters with greater impact on the output of the model are fit to experimental data

Parameters estimation from CRC data



Comparison of the simulations with data from *(Bardelli et alii., Nat. Commun., 2015)* under two different types of molecular target drugs
Top left panel, control case. Top right, the effect of Pimasertib drug. Bottom left the action of Cetuximab drug. Bottom right, the **combination therapy**.

Only x_1 is sensitive to Cetuximab and correspondingly $f_1 = -0.1\ days^{-1}$, $f_2 = r_2$, whereas Pimasertib affects both clones: $f_1 = 0.06\ days^{-1}$ and $f_2 = -0.015\ days^{-1}$;

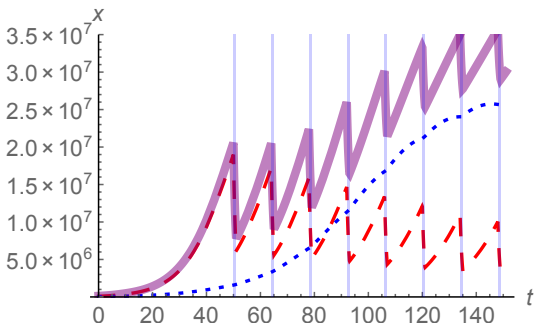


Comparison of the simulations with data from *(Ramakrishnan et alii. J. Clin. Invest. 2010)* under combination of immunotherapy and chemotherapy
Upper panels: on the left the control case, on the right the TAX therapy (chemotherapy);
Lower panels: on the left the Ad-p53 therapy (immunotherapy), on the right results of combination therapy.

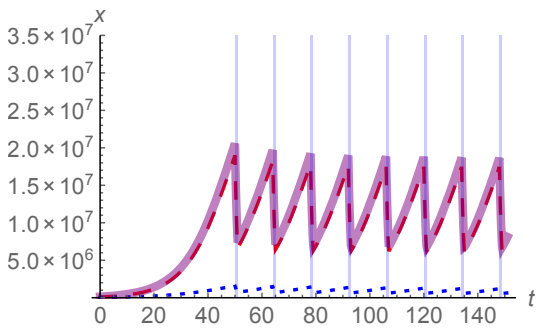
Only x_1 is sensitive to chemotherapy and r_1 is lowered to $f_1 = -0.072\ days^{-1}$, whereas r_2 is kept unchanged. The effect of immunotherapy is to increase c_i to $\kappa_i = 21.5\ days^{-1}$.

Clonal composition matters. The *COE* case

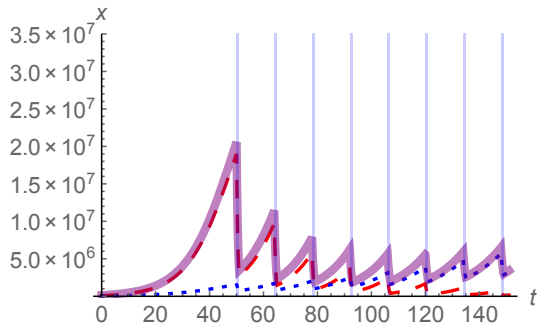
Ch_+



$\mathrm{Ch}_-\mathrm{I}$



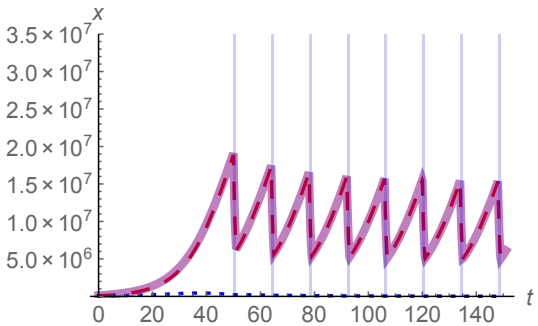
$\mathrm{Ch}_+\mathrm{I}$



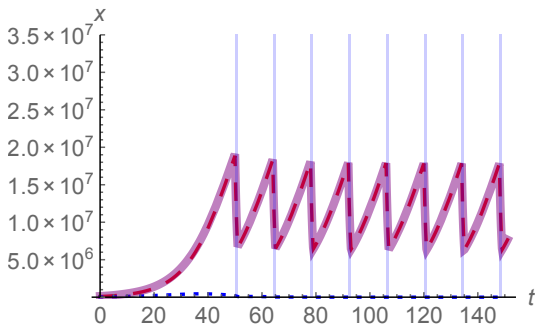
- Standard chemotherapy dose (Ch_+) is inefficient, $\gamma = 0.15$
- Combination of low chemotherapy dose and immunotherapy ($\mathrm{Ch}_-\mathrm{I}$) is efficient, $\gamma = 0.61$
- Combination of standard chemotherapy dose and immunotherapy ($\mathrm{Ch}_+\mathrm{I}$) is more efficent, $\gamma = 0.62$
- Trade off between decrease the total load and the control of resistant clones. ($\mathrm{Ch}_-\mathrm{I}$) has the advantage of less dangerous side effects on the patient

Competitive exclusion *EC*1: clone x_1 dominates

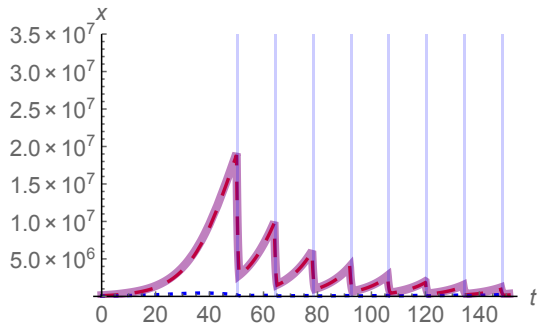
Ch_+



$\mathrm{Ch}_-\mathrm{I}$



$\mathrm{Ch}_+\mathrm{I}$

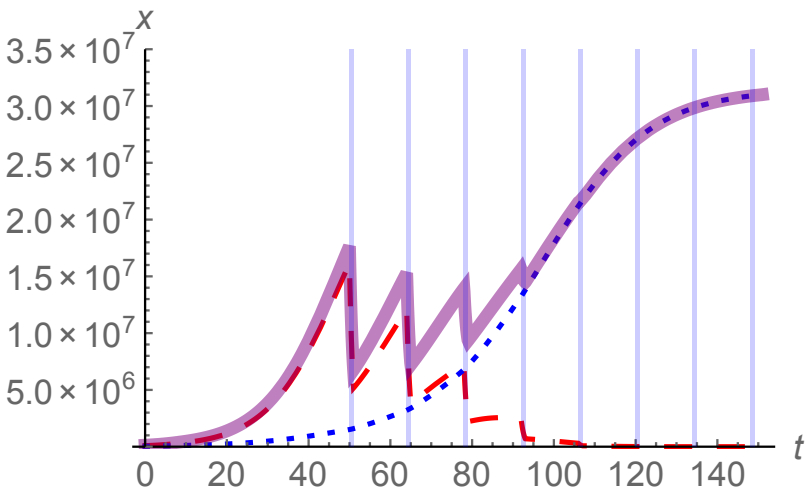


- Standard chemotherapy (Ch_+) is effective, $\gamma = 0.62$
- Combination of low dose chemotherapy and immunotherapy ($\mathrm{Ch}_-\mathrm{I}$) is less efficient than in *COE* configuration, $\gamma = 0.60$
- Combination of standard dose chemotherapy and immunotherapy ($\mathrm{Ch}_+\mathrm{I}$) is an effective treatment, $\gamma = 0.68$

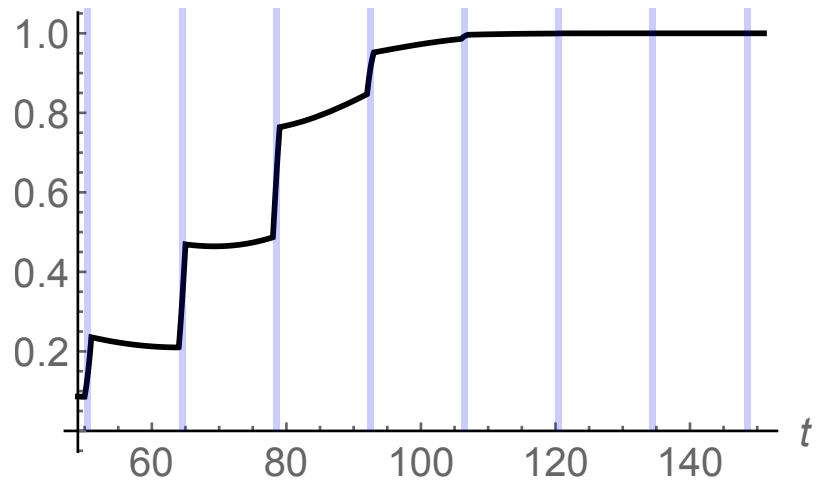
Competitive exclusion: clone x_2 dominates, EC2

Therapy outcomes similar to *COE*, but with the effect of clonal inversion somehow intensified by the fact that x_2 is now dominant.

Ch_+



Clonal inversion

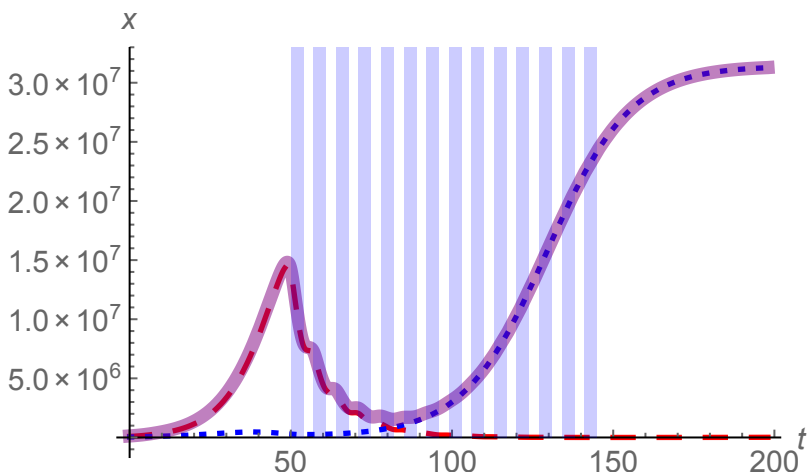


Pulsed chemotherapy Ch_+. On the left cancer load and on the right fraction of the resistant clone.

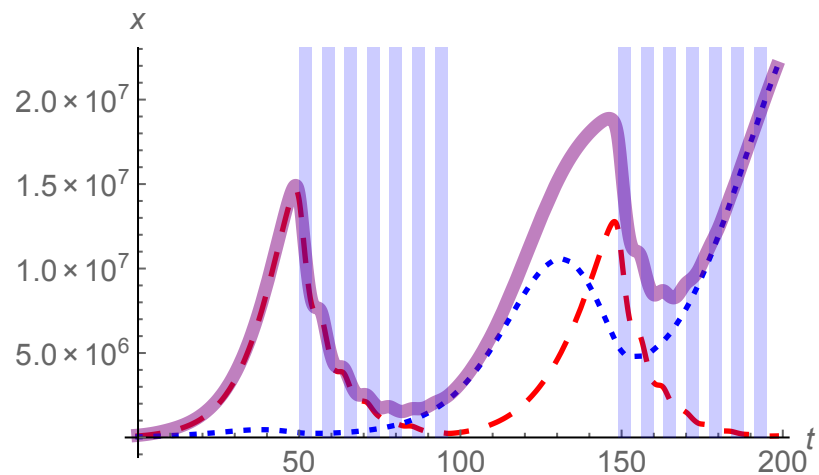
Drug holiday

- Drug resistance is a dynamical process *(Sharma et alii 2010)*
- Drug holiday enables a regrowth of sensitive cells reestablishing the effectiveness of a therapy, moreover it allows patients to recover from the therapy side effects, e.g. *(Pouchol, Clairambault, Lortz, Trelat, 2017)*

Ch_+ without drug-holiday



Ch_+ with drug-holiday

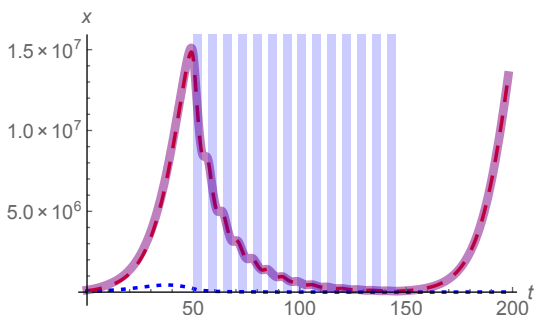


Effect of a drug holiday in the *EC*1 case and the decrease of the resistant clone besides, obviously, confirming that chemotherapy alone is inefficient.

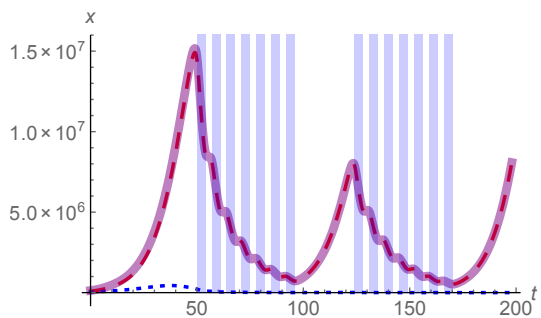
Timing and duration of the holiday

When a combination therapy is adopted, Ch_I, the action of x_1 over x_2 is less relevant since immunotherapy affects both cancer types.

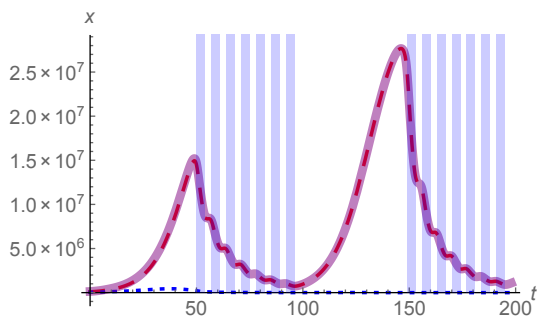
without drug-holiday



25 days of drug-holiday



50 days of drug-holiday



Values of γ are, respectively, $\gamma_a = 0.48$, $\gamma_b = 0.57$, $\gamma_c = 0.67$.

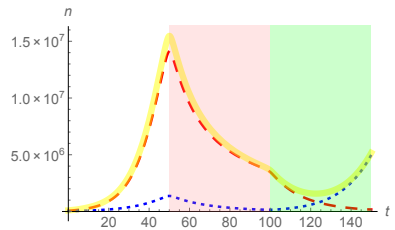
Influence of temporal order on combination therapies

Fitness of the i-th cancer population:

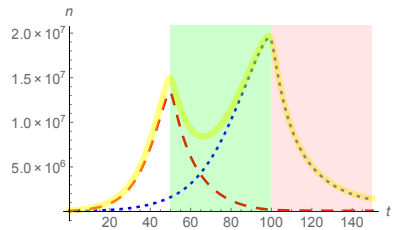
$$w_i = r_i - \frac{b_{ij}}{k} x_j - cz;$$

Mean fitness: $\frac{w_1(T)x_1(T)+w_2(T)x_2(T)}{x_1(T)+x_2(T)}$.

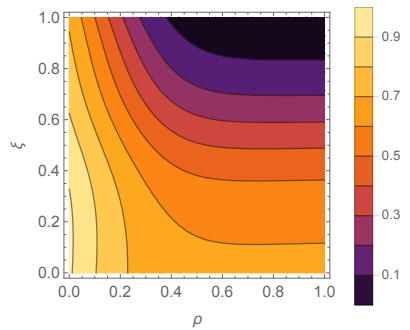
Immuno first

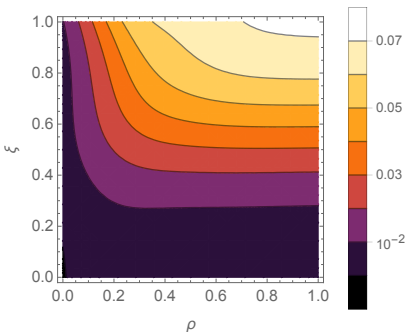


Chemo first

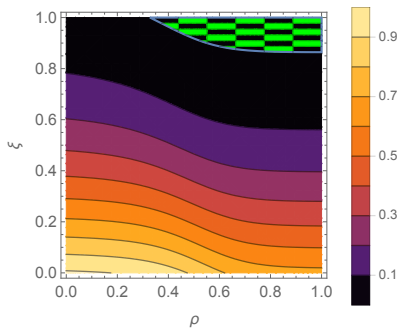


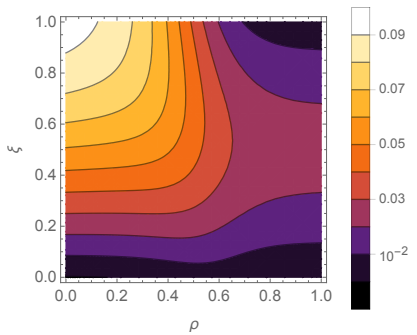
Immunotherapy first





Chemotherapy first





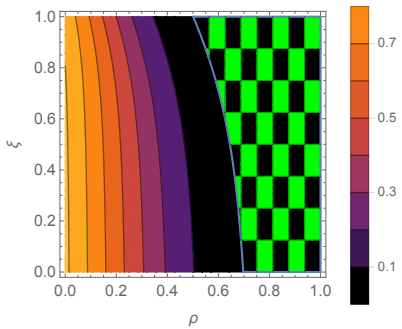
Different immunotherapy doses (vertical axes) and chemotherapy doses (horizontal axes) in the ***COE* case**

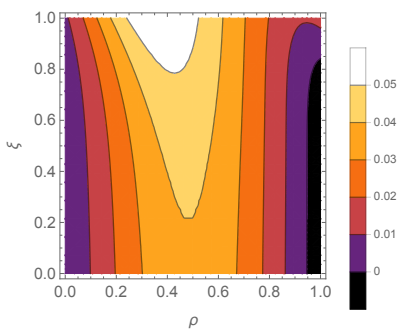
Above Heat map of the fraction of total cancer cells surviving at the end of treatment

Below Heat map of the mean fitness of total cancer cells

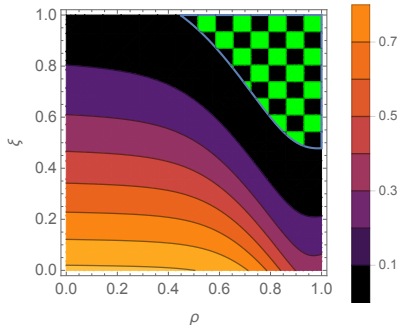
Dominating clone sensitive to chemotherapy, *EC*1.

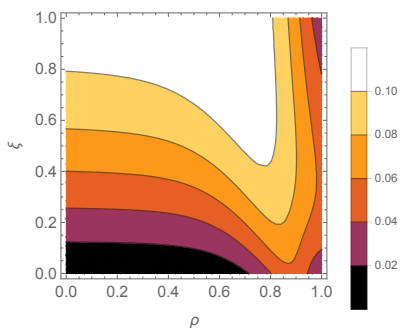
Immunotherapy first





Chemotherapy first





Above Heat map of the fraction of total cancer cells surviving at the end of treatment

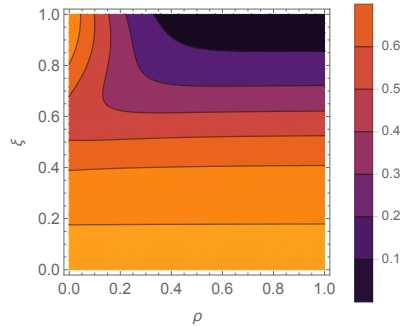
Below Heat map of the mean fitness of total cancer cells

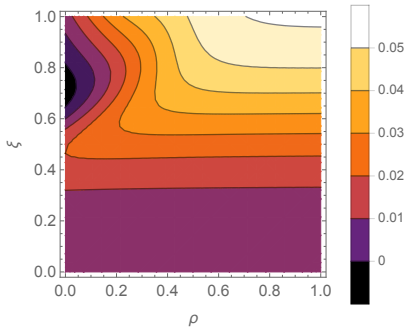
Different immunotherapy doses (vertical axes) and chemotherapy doses (horizontal axes) in the *EC*1 case

If the dominating clone is susceptible to chemotherapy (*EC*1), an immuno-first therapy yields better results,

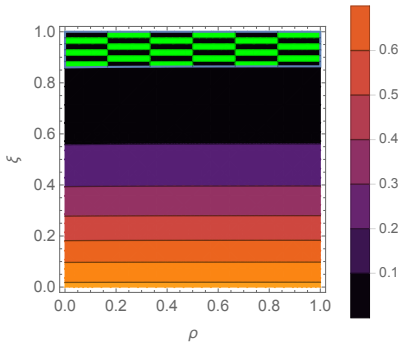
Dominating clone resistant to chemotherapy, *EC2*

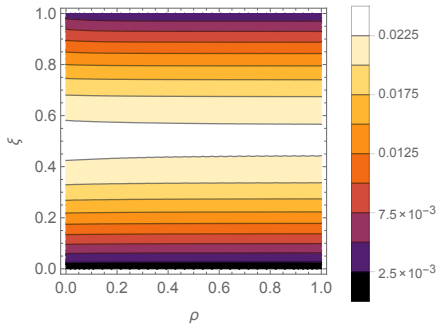
Immunotherapy first





Chemotherapy first





Above Heat map of the fraction of total cancer cells surviving at the end of treatment

Below Heat map of the mean fitness of total cancer cells

Different immunotherapy doses (vertical axes) and chemotherapy doses (horizontal axes) in the *EC2* case

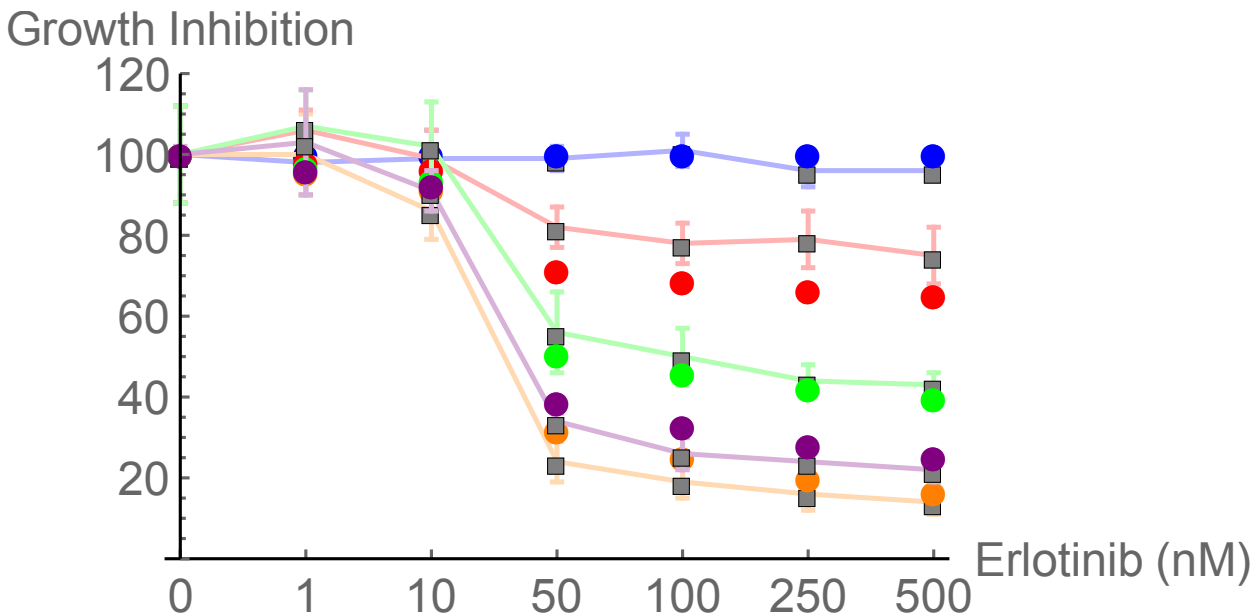
A chemo-first treatment is better if the dominant clone is resistant to chemical treatment (*EC2*)

Summarizing

- Relevance of the interactions among cancer species, i.e. cancer types, in designing effective therapies
- Weak competition between cancer clones, *COE* case, mild environment, the sequence with chemotherapy first appears to be more efficient
- When the competition is strong, harsh environment, the situation is less clear-cut and different strategies yields better results in the *EC*1 and *EC*2 case
- These findings may explain why there exist apparently contradictory experimental findings about optimal order of cures administration, e.g. chemo first *(Antonia et al. Cancer Res., 2006)* and immuno first *(Hodi et al. PNAS, 2008)*

Application to non-small cell lung cancer (NSCLC)

For NSCLC the problem of resistance seems to be crucial: **controversial results** in the efficacy of the Erlotinib drug (a tyrosin kinase inhibitors).



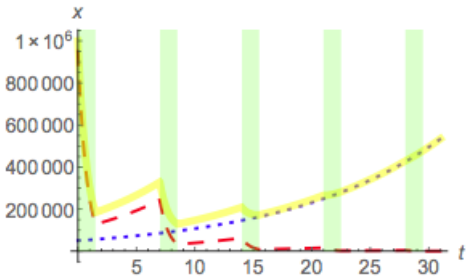
A comparison with some "in vitro" data[1] from literature for NSCLCs.

[1] Chmielecki et al. 2011, Science translational medicine.

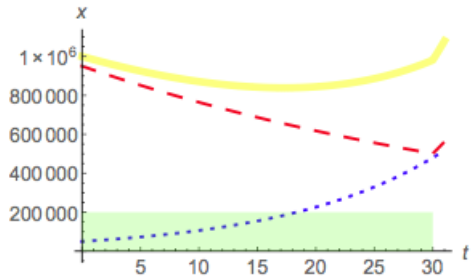
Clinical protocols

Test of some clinical protocols used in the treatment of NSCLC[1] where a second mutation resulting in a cancer clone resistant to Erlotinib:

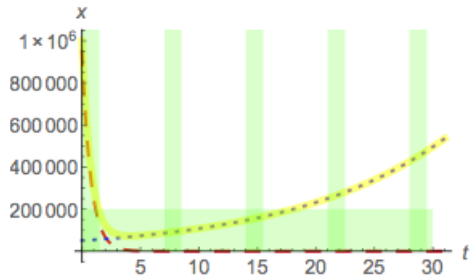
- A **pulsed** protocol
- Low-dose concentrations in a **metronomic** schedule
- Mixture schedules of **switch to pulse** protocol



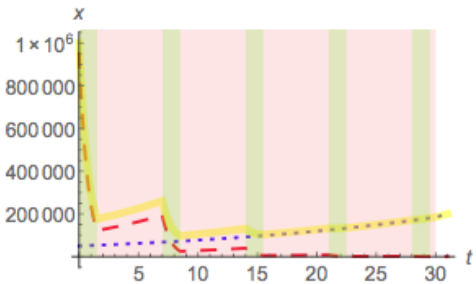
(a) Pulsed high dose TKI



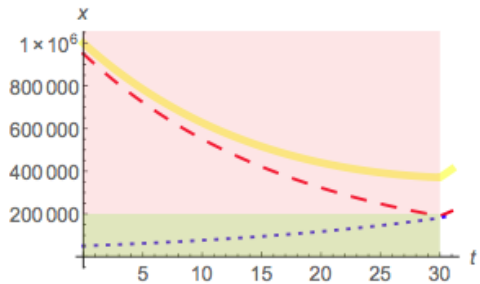
(b) Continuous low dose TKI



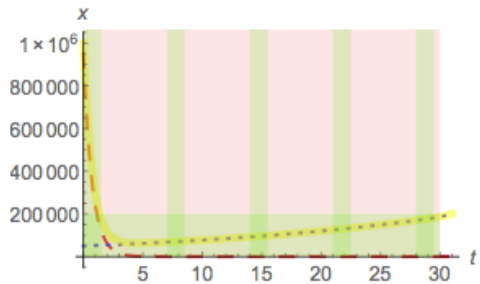
(c) Switch to pulse TKI



(a) Combination of pulsed high dose TKI and low dose continous immunotherapy



(b) Combination of continuous low dose TKI and low dose continous immunotherapy

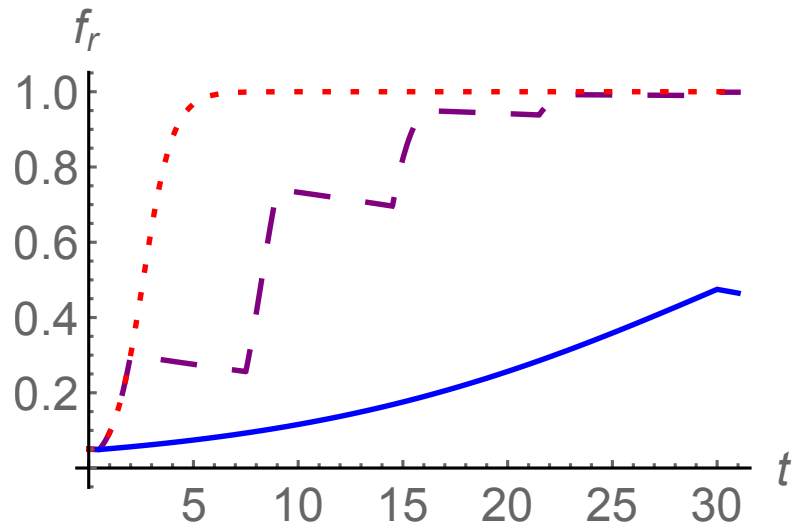


(c) Combination of switch to pulse TKI and low dose continous immunotherapy

[1] Foo, Jasmine, Juliann Chmielecki, William Pao, and Franziska Michor. 2012, Journal of Thoracic Oncology

Resistance

Relative frequencies of resistant cells in mono and in combination schedules. Fraction is similar while the total cancer load is reduced in the combination protocol:



Red Switch to pulse protocol: 1600 mg/wk + 150 mg/day;
Purple Pulsed protocol: 1600 mg/wk;
Blue Metronomic protocols[1]: 150 mg/day 50mg/day, 25mg/day.

[1] André, Carré, Pasquier, Nat. Rev. clin. Oncol. 2014.

Conclusions

Influence of temporal order:

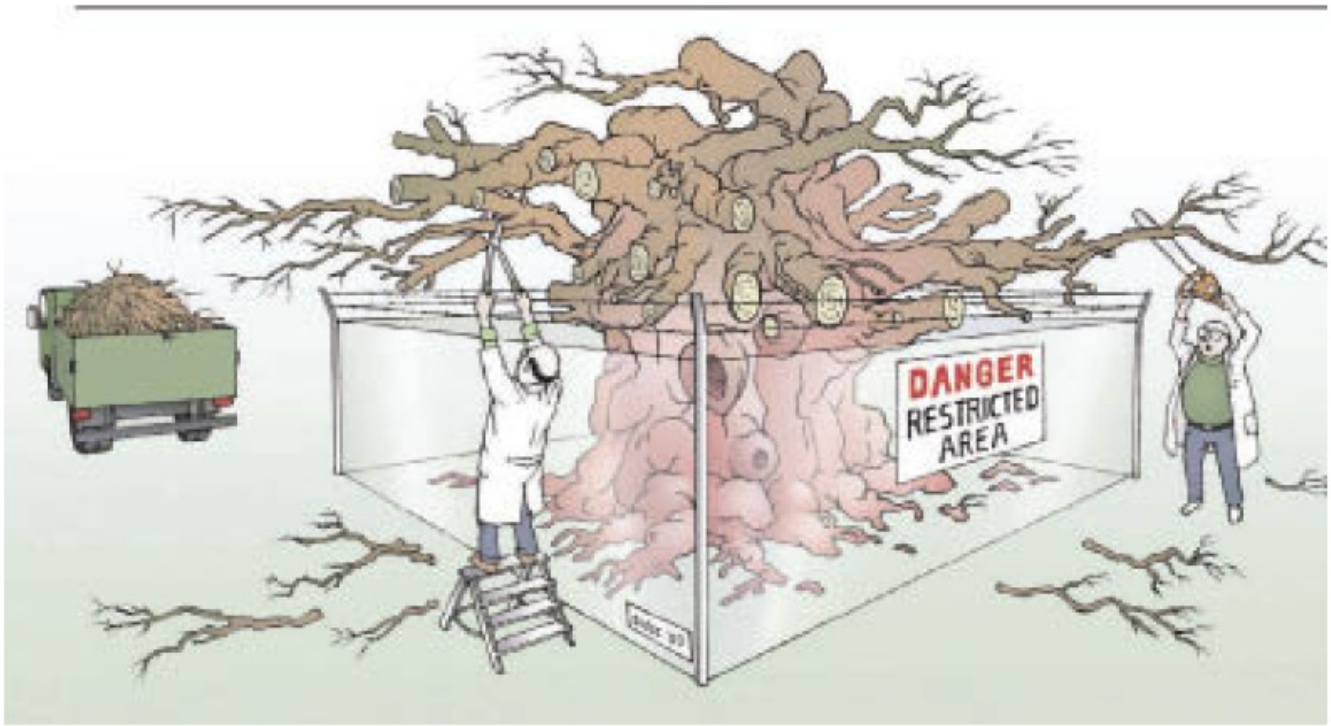
- Clonal composition is crucial to the efficacy of treatments
- Inoculation of the 'Maximum Tolerable Dose' (MTD) is not always the best choice

Application to Lung Cancer:

- Pulsed protocol (or Switch to Pulse protocol) has stronger results in term of reduction of tumor expansion
- Resistance is accelerated with stronger protocols
- The combination of treatments makes low dose continuous protocols (metronomic) more efficient and reduces considerably the resistance
- Ongoing project with P. Bironzo, San Luigi Hospital, Orbassano, Torino

Change of strategy

Integrate the principles of evolution to design new strategies for cancer therapy.



R.A. Gatenby, A change of strategy in the war on cancer, Nature, 459 (2009) 508–509.

Thank you for your attention!