CANCER ATTRACTORS & PHENOTYPE PLASTICITY IN NON-DARWINIAN SOMATIC EVOLUTION OF DRUG RESISTANCE

Translating Cancer Data and Models to Clinical Practice
IPAM, UCLA
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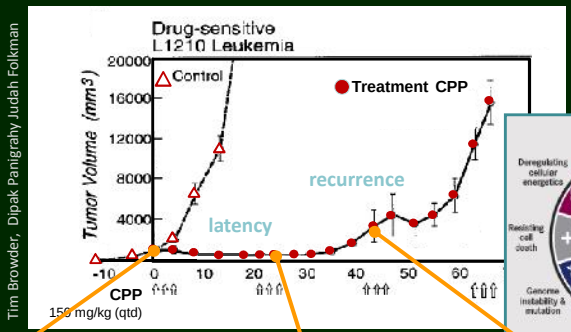
INTRODUCTION

The problem of tumor resistance / recurrence

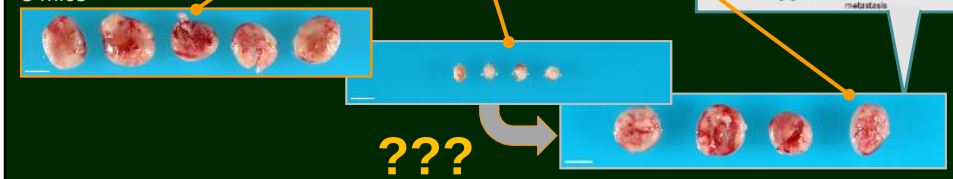


Tim Browder, Dipak Panigrahy Judah Folkman

Hallmarks of cancer

Hanahan & Weinberg, 2011

5 mice



???

➔ Why do tumors come back – often in a more malignant form?

INTRODUCTION

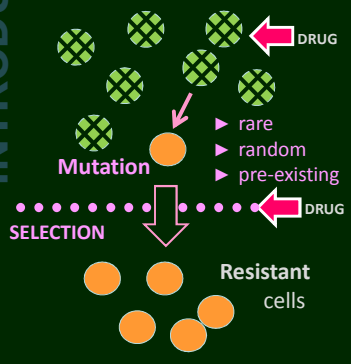
How can **non-specific perturbations**
(cytotoxic drugs, irradiation, toxins)
invariably produce
the **highly sophisticated** phenotype
(= resistant, stem cell-like)
of recurrent tumors?

INTRODUCTION

Somatic Evolution of Drug Resistance: a new model

STANDARD VIEW

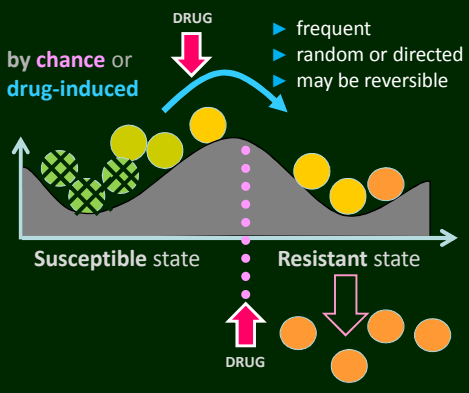
NEO-**DARWINISM**



selection

NO MUTATIONS!

We proposed a new picture:



instruction + (non-genetic) selection

➔ But what is this "**landscape**"? (➔ mechanism behind the metaphor)
➔ Whence the "**urge**" (directionality) towards the resistant, stem –cell like state?

TALK OUTLINE

Theoretical concepts

- Gene networks and the Epigenetic Landscape,
- Cancer as attractors

Experiments: *to give a feel of state transitions*

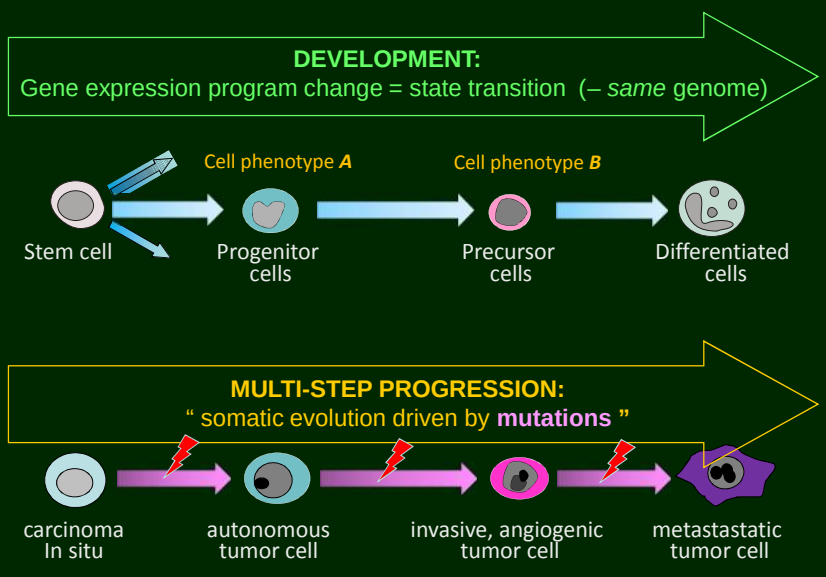
- High-dimensional attractors,
- Non-genetic heterogeneity,
- State transitions

Application to Cancer

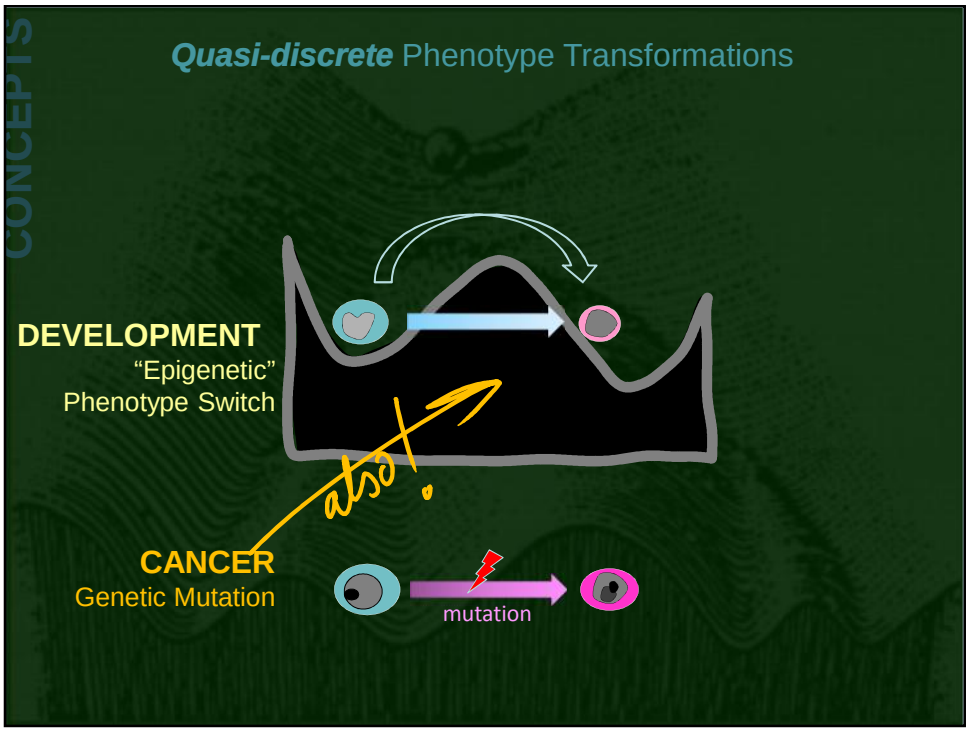
- Resistance development:
  Non-Darwinian dynamics

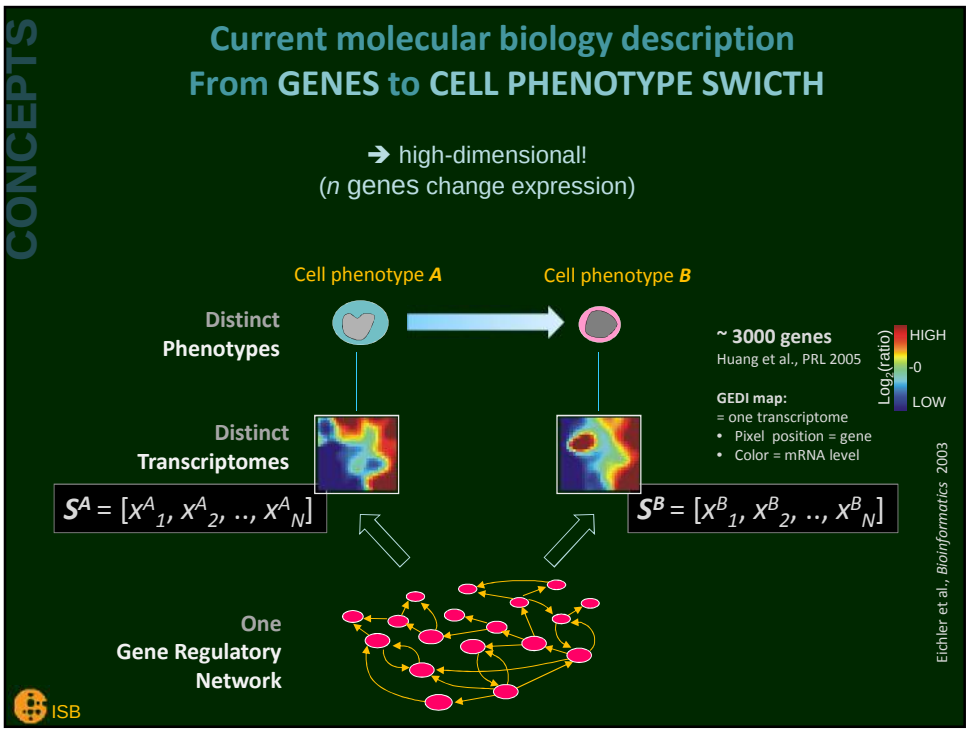
CONCEPTS

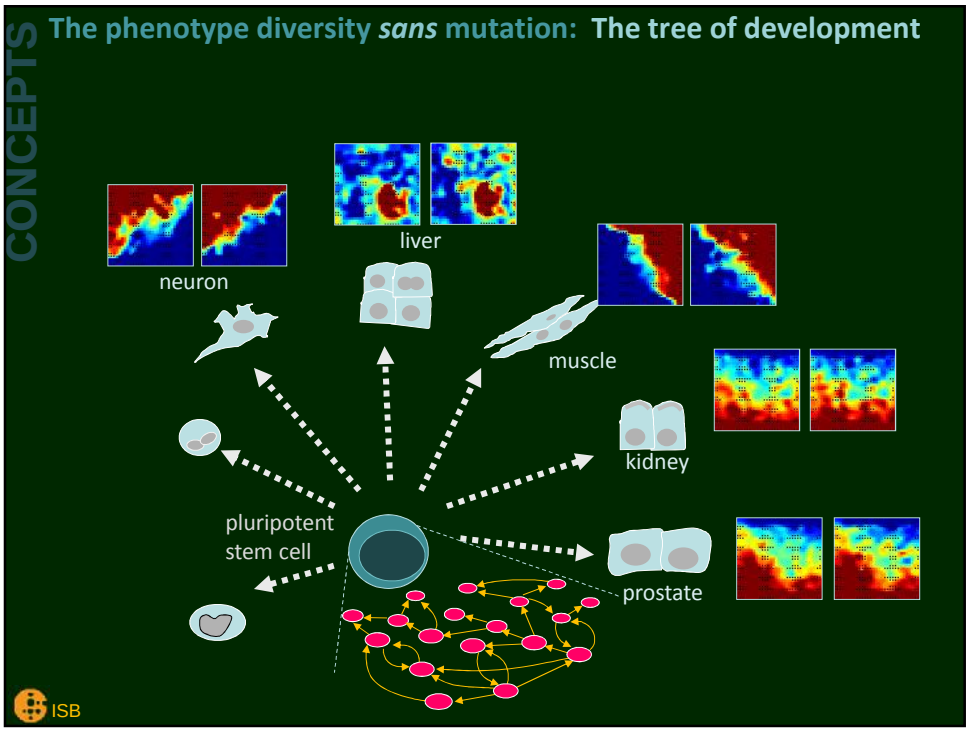
Cancer progression: an unarticulated paradox

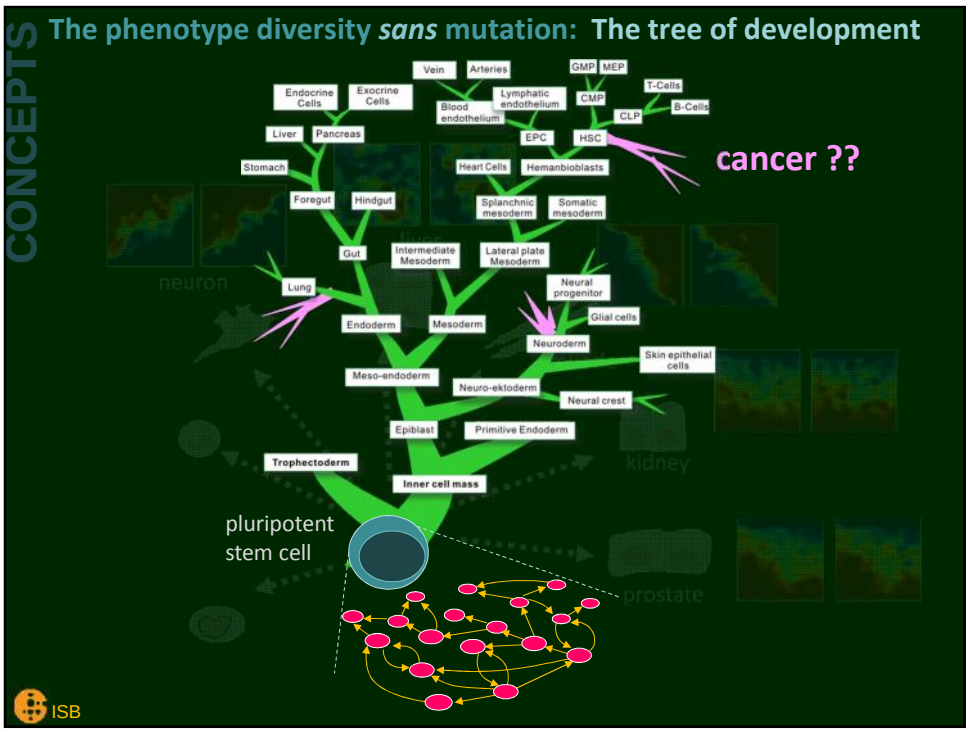


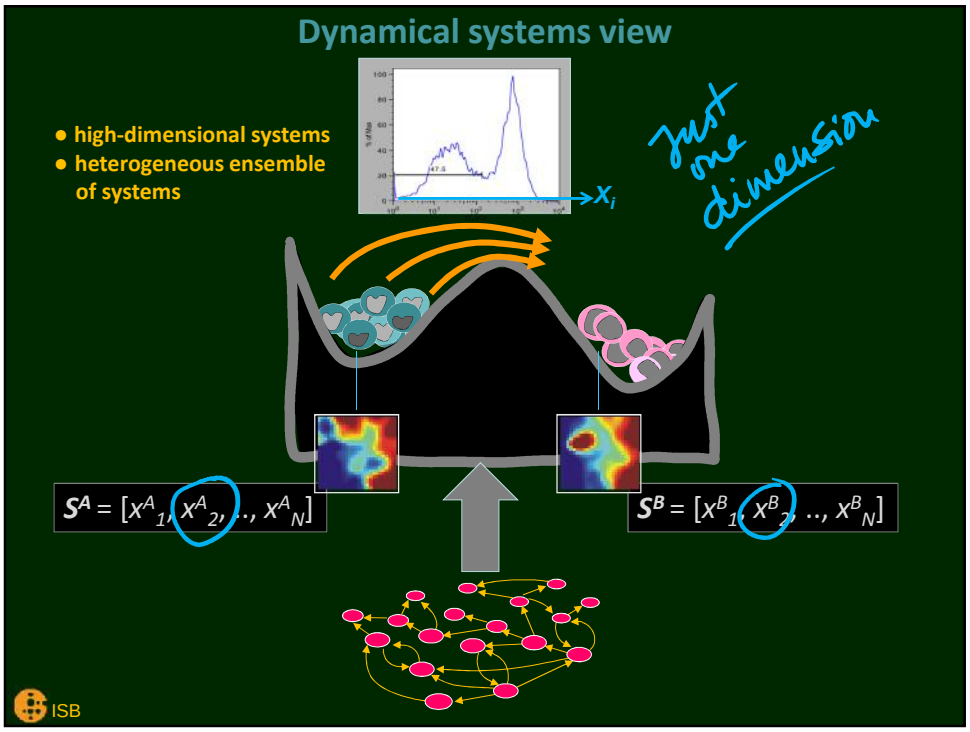
Huang S., *Progr. Biophys. & Molec. Biol.* 2012

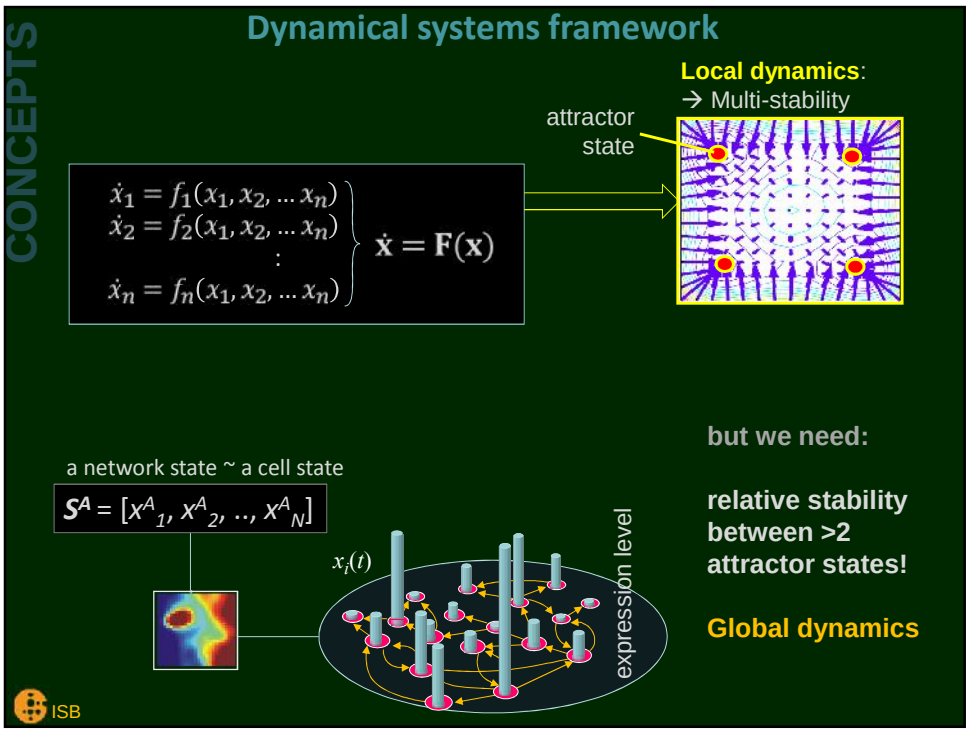

CONCEPTS
Quasi-discrete Phenotype Transformations
DEVELOPMENT
"Epigenetic"
Phenotype Switch
also !
CANCER
Genetic Mutation
mutation


CONCEPTS
Current molecular biology description
From GENES to CELL PHENOTYPE SWICTH
➔ high-dimensional!
(n genes change expression)
Cell phenotype A
Cell phenotype B
Distinct
Phenotypes
Distinct
Transcriptomes
~ 3000 genes
Huang et al., PRL 2005
GEDI map:
= one transcriptome
• Pixel position = gene
• Color = mRNA level
HIGH
0
LOW
Eichler et al., Bioinformatics 2003
One
Gene Regulatory
Network
ISB


CONCEPTS
The phenotype diversity *sans* mutation: The tree of development
neuron
liver
muscle
kidney
pluripotent
stem cell
prostate
ISB


CONCEPTS
The phenotype diversity *sans* mutation: The tree of development
Vein
Arteries
GMP
MEP
Endocrine Cells
Exocrine Cells
Lymphatic endothelium
Blood endothelium
CMP
T-Cells
B-Cells
CLP
Liver
Pancreas
EPC
HSC
cancer ??
Stomach
Heart Cells
Hemanbioblasts
Foregut
Hindgut
Splanchnic mesoderm
Somatic mesoderm
Gut
Intermediate Mesoderm
Lateral plate Mesoderm
Lung
Neural progenitor
Endoderm
Mesoderm
Glial cells
Neuroderm
Skin epithelial cells
Meso-endoderm
Neuro-ektoderm
Neural crest
Epiblast
Primitive Endoderm
Trophectoderm
Inner cell mass
pluripotent
stem cell
ISB


Dynamical systems view
high-dimensional systems
heterogeneous ensemble of systems
x_i
Just one dimension
$S^A = [x^A_1, x^A_2, .., x^A_N]$
$S^B = [x^B_1, x^B_2, .., x^B_N]$
ISB


CONCEPTS
Dynamical systems framework
Local dynamics:
→ Multi-stability
attractor state
$\dot{x}_1 = f_1(x_1, x_2, \ldots x_n)$
$\dot{x}_2 = f_2(x_1, x_2, \ldots x_n)$
$\dot{x}_n = f_n(x_1, x_2, \ldots x_n)$
$\dot{\mathbf{x}} = \mathbf{F}(\mathbf{x})$
a network state ~ a cell state
$S^A = [x^A_1, x^A_2, .., x^A_N]$
$x_i(t)$
expression level
but we need:
relative stability between >2 attractor states!
Global dynamics
ISB

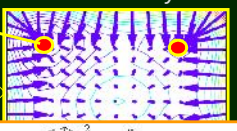
CONCEPTS

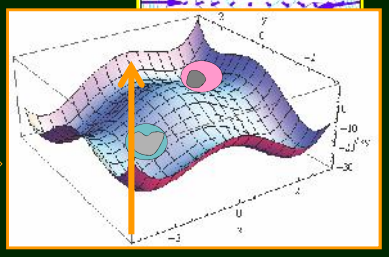
Dynamical systems framework

$$\left.\begin{aligned}\dot{x}_1 &= f_1(x_1, x_2, \ldots x_n)\\ \dot{x}_2 &= f_2(x_1, x_2, \ldots x_n)\\ &\vdots\\ \dot{x}_n &= f_n(x_1, x_2, \ldots x_n)\end{aligned}\right\} \dot{\mathbf{x}} = \mathbf{F}(\mathbf{x})$$

Local dynamics:
→ Multi-stability

attractor state

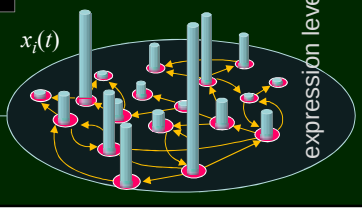




Quasi-potential

a network state ~ a cell state

$S^A = [x^A{}_1, x^A{}_2, .., x^A{}_N]$



relative stability between >2 attractor states!

Global dynamics

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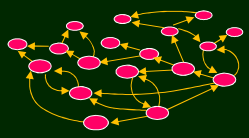
CONCEPTS

From gene regulatory network to quasi-potential landscape

A fully specified gene regulatory network

$$\dot{\mathbf{x}} = \mathbf{F}(\mathbf{x})$$



unique mapping

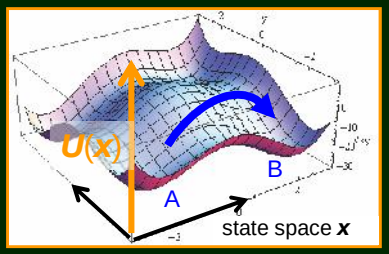
There is ***no*** function $U(x)$ that satisfies $\mathbf{F}(\mathbf{x}) = -\nabla U(\mathbf{x})$

→ Decompose vector field:
$\mathbf{F}(\mathbf{x}) = -\nabla U(\mathbf{x}) + \mathbf{G}(\mathbf{x})$

2 $\mathbf{F}(\mathbf{x}) = -\nabla U(x) + F_{curl}(x)$

$U = -\ln(P_{ss})$

steady-state probability distribution (measurable) *(J. Wang)*



1 such that $(\nabla U(x), \mathbf{G}(x)) = 0$

$P(A \to B) = e^{-2\Delta U_{AB}/\varepsilon^2}$ ε : magnitude of **noise**

"Cond. probability for A → B"

$$2\Delta U_{AB} = \Delta V_{AB} = \frac{1}{2} min\left\{\int_{t_A}^{t_{AB}} \|\dot{\mathbf{x}} - \mathbf{F}(\mathbf{x})\|^2 dt\right\}$$

Wentzell-Freidlin quasi-potential

with E. **Aurell** (Zhou J, et al., J Royal Soc Interface, 2012

CONCEPTS

Meaning of the Quasi-potential landscape

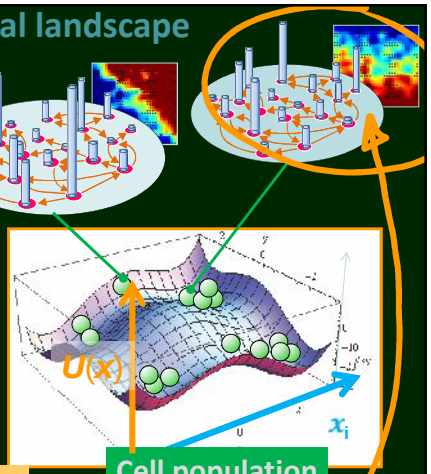
A fully specified gene regulatory network

$\dot{x} = F(x)$

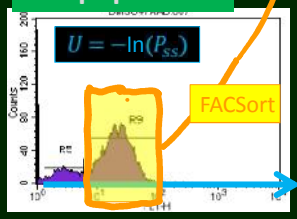
unique mapping

= network state

= cell state



Cell population



REMEMBER FOR LATER

- Change in **specification of the network** (incl. mutation, … etc) ➔ change in **shape** (=**topography**) of the landscape
- Transient ***perturbations*** of expression variables ➔ **attractor transition**

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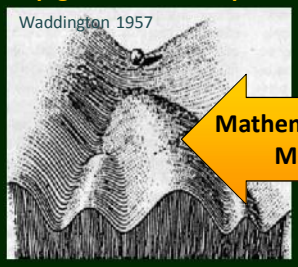
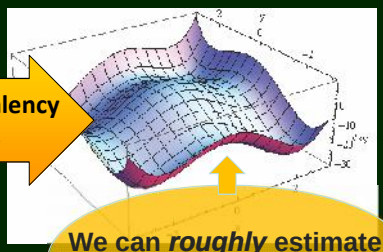
CONCEPTS

Waddington's "Epigenetic Landscape"

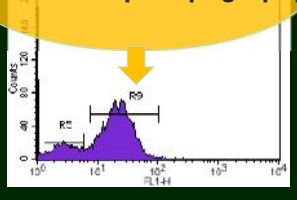


Mathematical equivalency
Molecular basis

Modern quasi-potential landscape
based on notion of network dynamics
(but: often still equally metaphoric)



We can *roughly* estimate the landscape topography



Similar ideas ("Biological cell state as attractor")

- 1940 **Waddington** ('valleys' in landscape)
- 1949 **Delbrück** (bistability)
- 1961 **Jacob & Monod** (gene circuits)
- 1969 **Kauffman** (networks)

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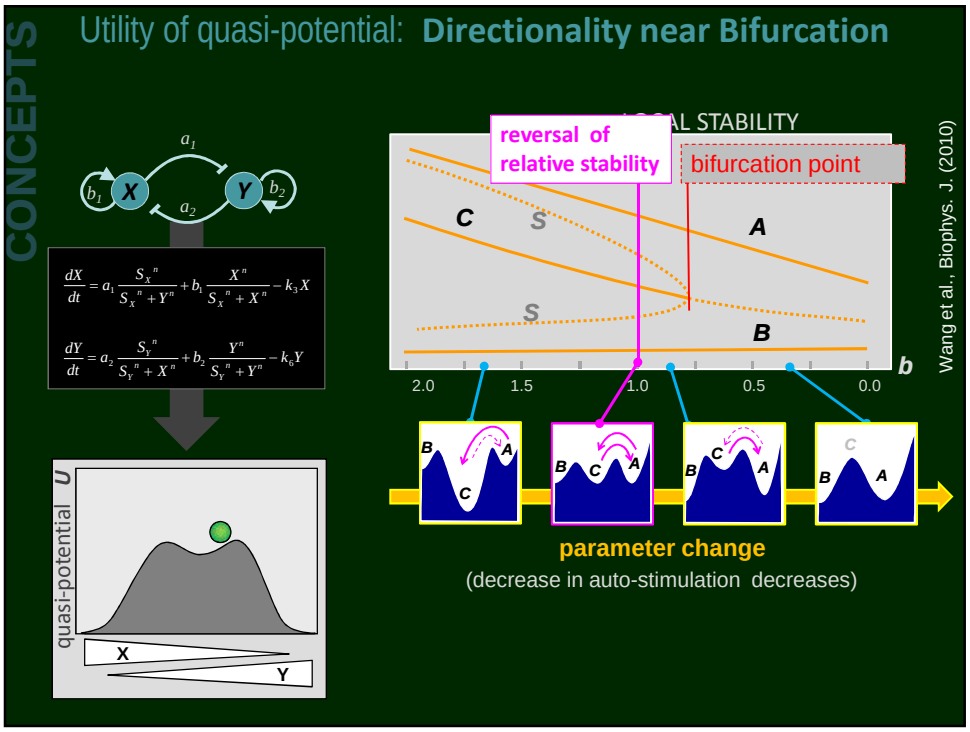
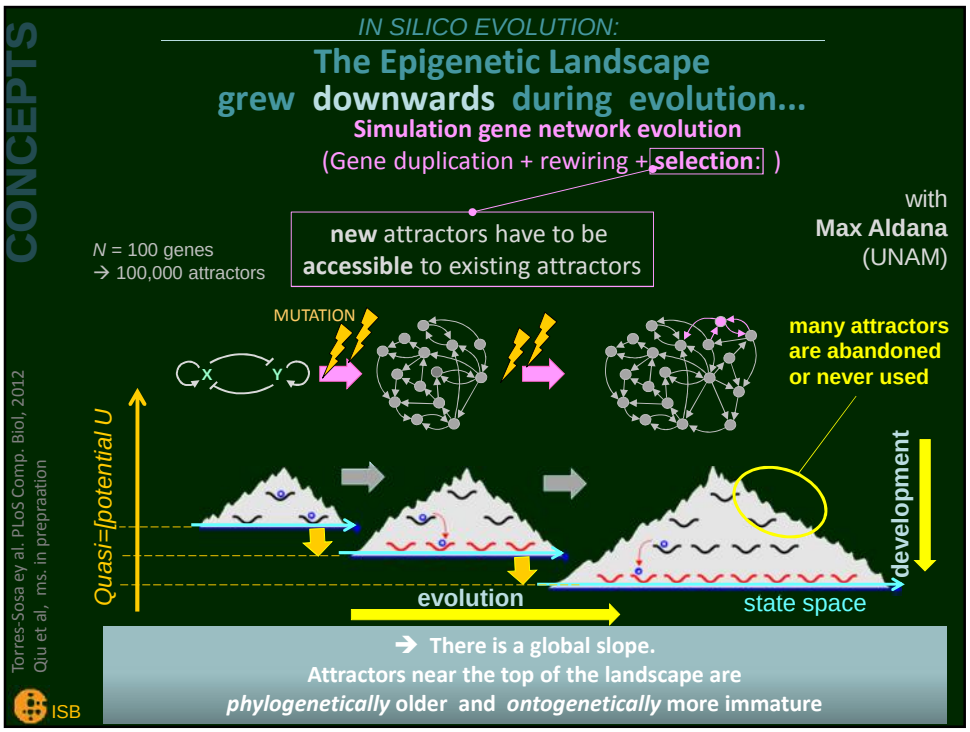

CONCEPTS
Utility of quasi-potential: Directionality near Bifurcation
reversal of relative stability
bifurcation point
C
S
A
S
B
b
2.0
1.5
1.0
0.5
0.0
Wang et al., Biophys. J. (2010)
quasi-potential U
X
Y
parameter change
(decrease in auto-stimulation decreases)


CONCEPTS
IN SILICO EVOLUTION:
The Epigenetic Landscape
grew downwards during evolution...
Simulation gene network evolution
(Gene duplication + rewiring + selection:)
with
Max Aldana
(UNAM)
N = 100 genes
→ 100,000 attractors
new attractors have to be accessible to existing attractors
MUTATION
many attractors are abandoned or never used
Quasi=[potential U
development
evolution
state space
→ There is a global slope.
Attractors near the top of the landscape are
phylogenetically older and ontogenetically more immature
Torres-Sosa ey al. PLoS Comp. Biol, 2012
Qiu et al, ms. in prepration
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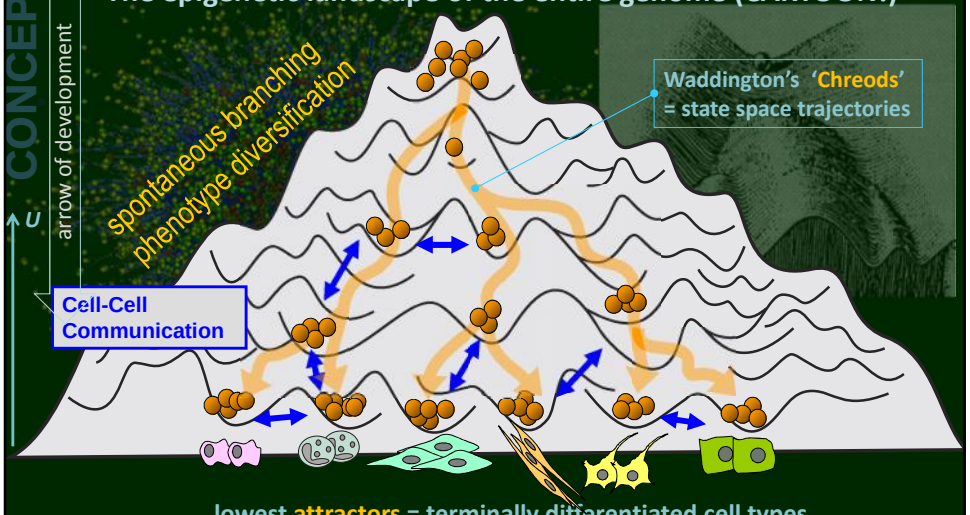
CONCEPTS

The Cancer Attractor Hypothesis

CONCEPTS

ASSEMBLING THE CONCEPTS TO DREAM MODEL:

The epigenetic landscape of the entire genome (CARTOON!)



lowest attractors = terminally differentiated cell types

● Stable ● discrete ● no fate option ("restricted")

OK, still qualitative – but: in principle inevitable.
→ allows specific qualitative predictions . . .

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CONCEPTS

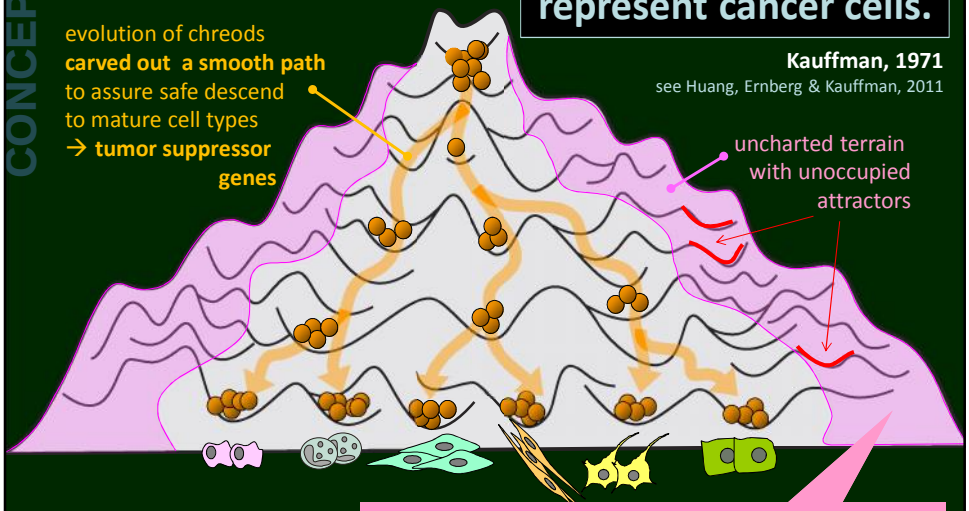
EXPANDING AN OLD HYPOTHESIS:

Unoccupied attractors represent cancer cells.

Kauffman, 1971
see Huang, Ernberg & Kauffman, 2011

evolution of chreods **carved out a smooth path** to assure safe descend to mature cell types → **tumor suppressor genes**

uncharted terrain with unoccupied attractors



Properties of **cancer attractors**

- ► **Never evolved to serve tissue function**
  → "asocial", primordial cell functions, unstable
- ► **No access to normal development**
  → when occupied: cells stuck in **immature** state

Huang, *Progr. Biophys. Mol. Bio*, 2012

TALK OUTLINE

✓ **Theoretical concepts**

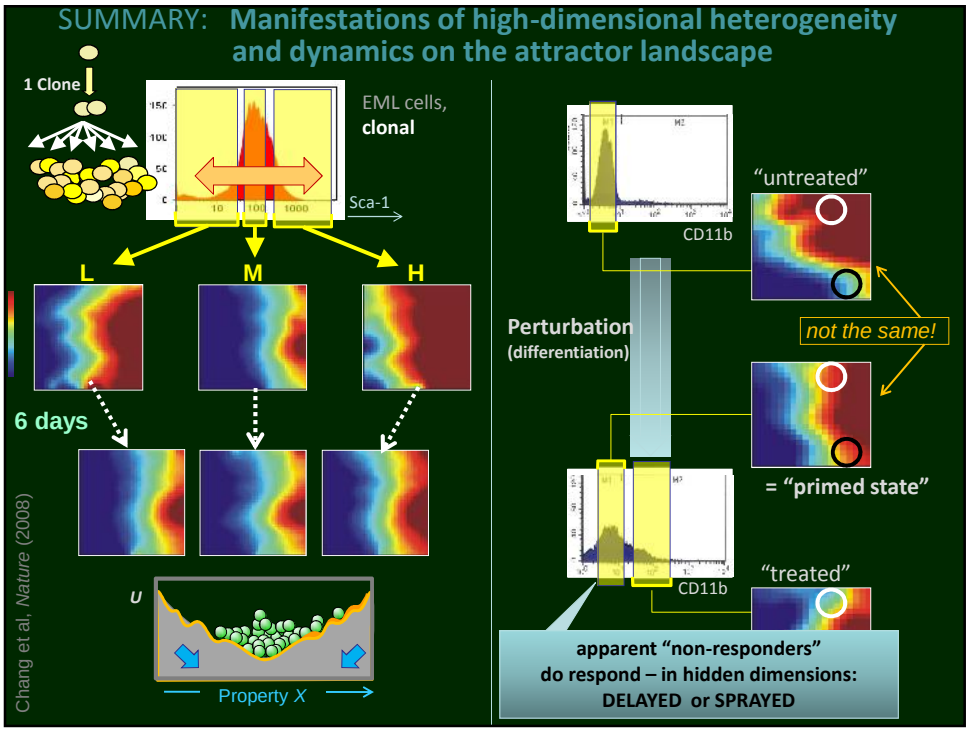
- Gene networks and Epigenetic landscape,
- Cancer as attractors

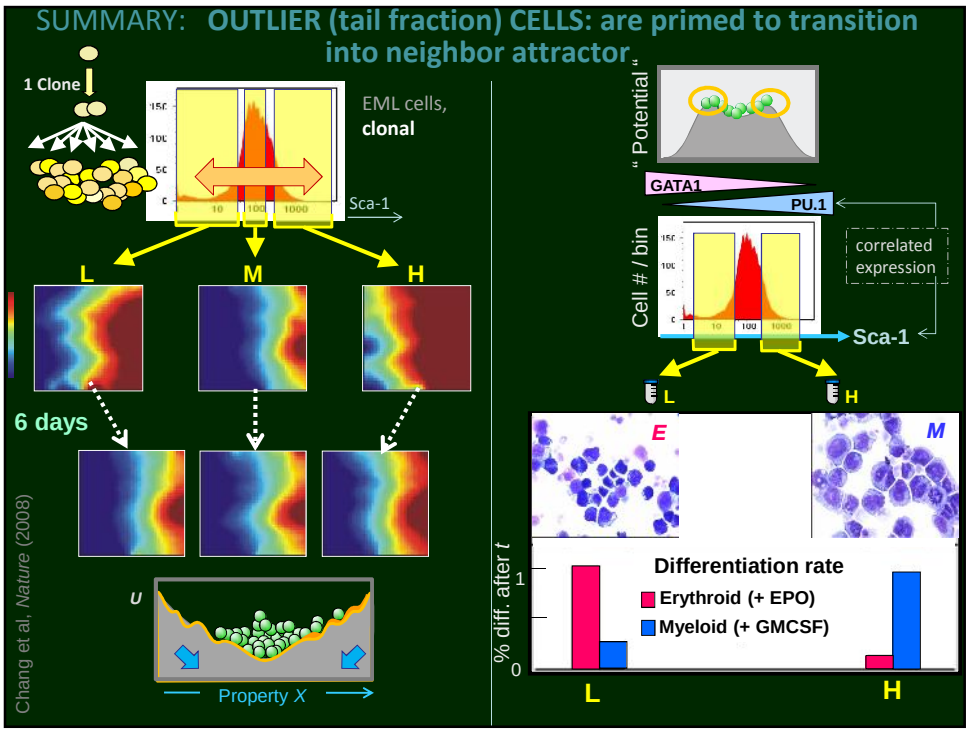
Experiments: *to give a feel of state transitions*

- High-dimensional attractors,
- Non-genetic heterogeneity,
- State transitions

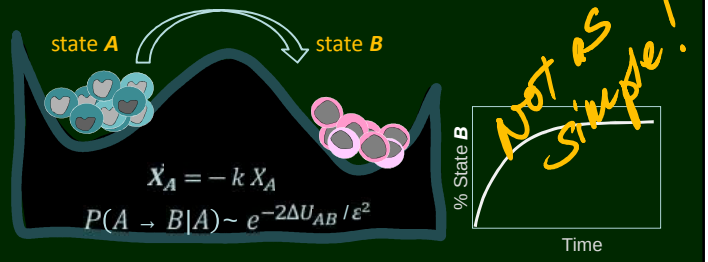
Application to Cancer

- Non-genetic dynamics
- Drug screening: The Perturbation space
- Resistance development: Non-Darwinian


SUMMARY: Manifestations of high-dimensional heterogeneity and dynamics on the attractor landscape
1 Clone
EML cells, clonal
Sca-1
L
M
H
6 days
Chang et al, Nature (2008)
U
Property X
"untreated"
CD11b
Perturbation (differentiation)
not the same!
= "primed state"
CD11b
"treated"
apparent "non-responders" do respond – in hidden dimensions: DELAYED or SPRAYED


SUMMARY: OUTLIER (tail fraction) CELLS: are primed to transition into neighbor attractor
1 Clone
EML cells, clonal
Sca-1
L
M
H
6 days
Chang et al, Nature (2008)
U
Property X
" Potential "
GATA1
PU.1
correlated expression
Cell # / bin
Sca-1
L
H
E
M
% diff. after t
Differentiation rate
Erythroid (+ EPO)
Myeloid (+ GMCSF)
L
H

Theory predicts many non-intuitive properties for state transitions



- Rugged landscape: **fractionated response to perturbation**
- Bifurcation → "**rebellious**" **cells**
- Heterogenization → Heterotypic cell-cell **interactions**
- Differential growth rates → complex population dynamics
- …

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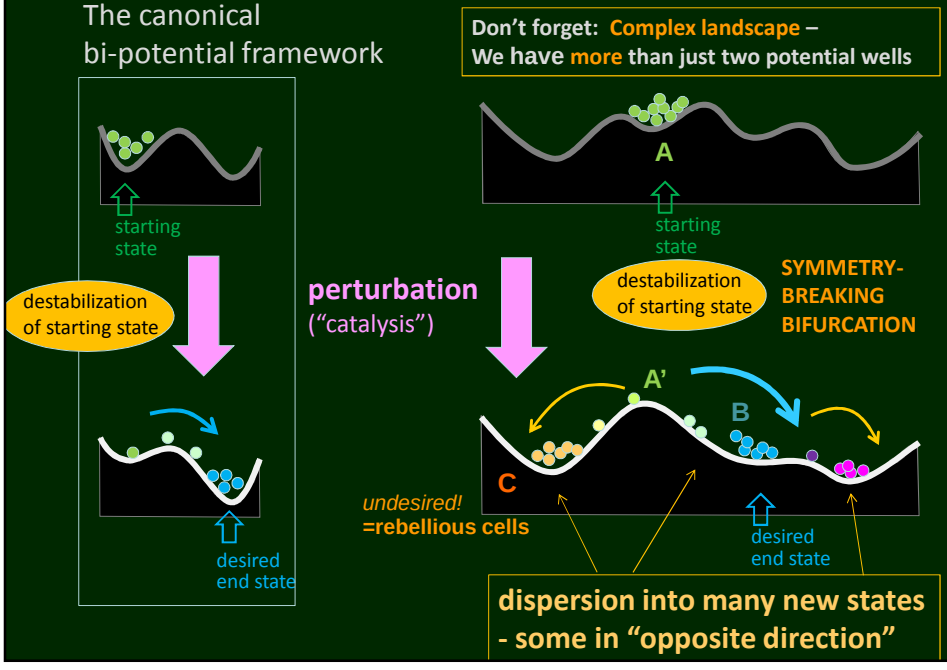
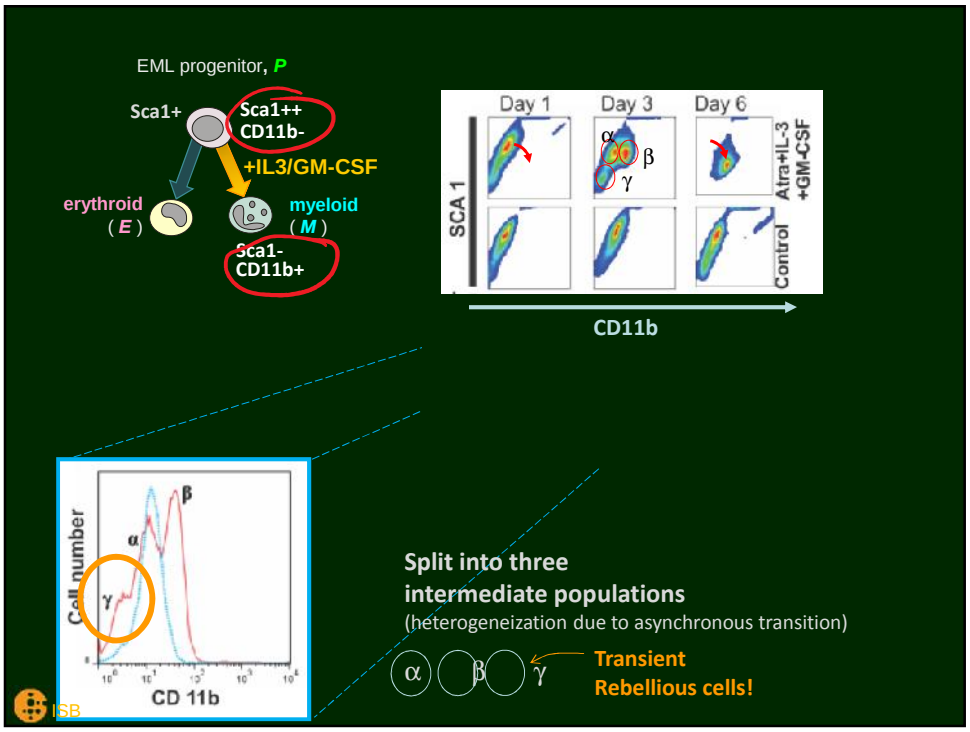
The canonical bi-potential framework




EML progenitor, P
Sca1+
Sca1++
CD11b-
+IL3/GM-CSF
erythroid
(E)
myeloid
(M)
Sca1-
CD11b+
Day 1
Day 3
Day 6
SCA 1
α
β
γ
Atra+IL-3
+GM-CSF
Control
CD11b
Cell number
CD 11b
Split into three
intermediate populations
(heterogeneization due to asynchronous transition)
Transient
Rebellious cells!
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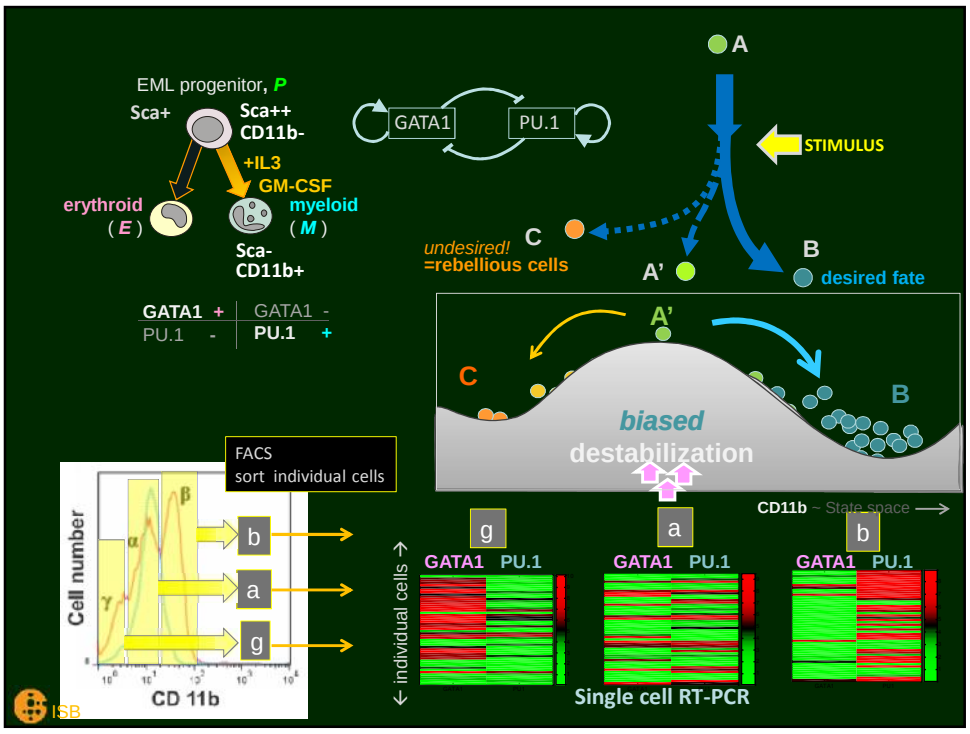
EML progenitor, P
Sca+
Sca++
CD11b-
+IL3
GM-CSF
erythroid
(E)
myeloid
(M)
Sca-
CD11b+
GATA1 +
PU.1 -
GATA1 -
PU.1 +
GATA1
PU.1
A
STIMULUS
undesired!
=rebellious cells
C
A'
B
desired fate
biased
destabilization
FACS
sort individual cells
Cell number
CD 11b
b
a
g
↓ individual cells →
GATA1 PU.1
Single cell RT-PCR
CD11b ~ State space
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TALK OUTLINE

Theoretical concepts

- Gene networks and Epigenetic landscape,
- *Cancer as attractors*

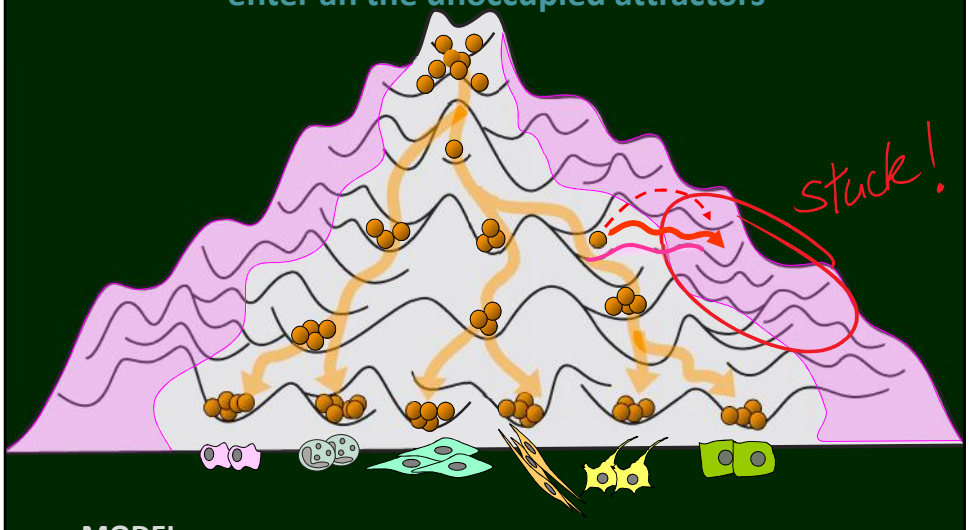
Experiments: *to give a feel of state transitions*

- High-dimensional attractors,
- Non-genetic heterogeneity,
- State transitions: *Rebellious cells*

Application to Cancer

- Non-genetic dynamics
- Drug screening: The Perturbation space
- Resistance development: Non-Darwinian

Mutation or chronic perturbation can allow cells to enter an the unoccupied attractors



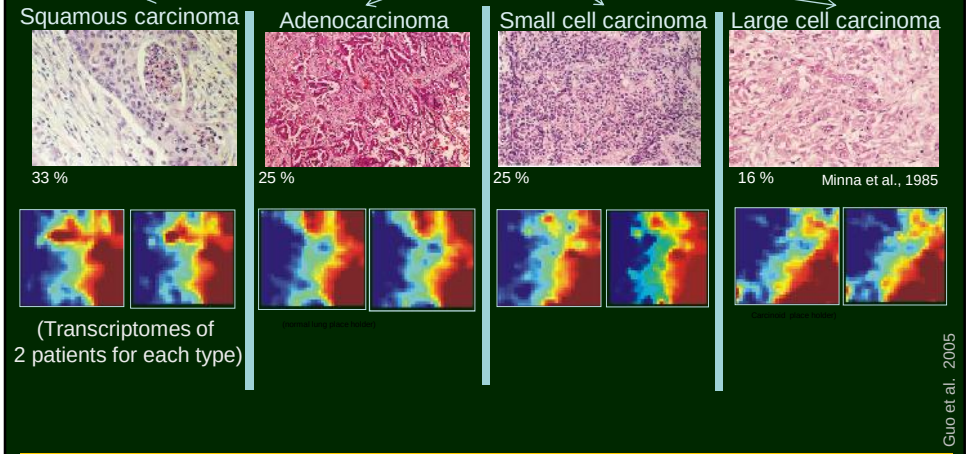
MODEL

– unites **genetic** and **non-genetic** causes of cancer

– explains **multiple discrete substates** in clonal cancer populations

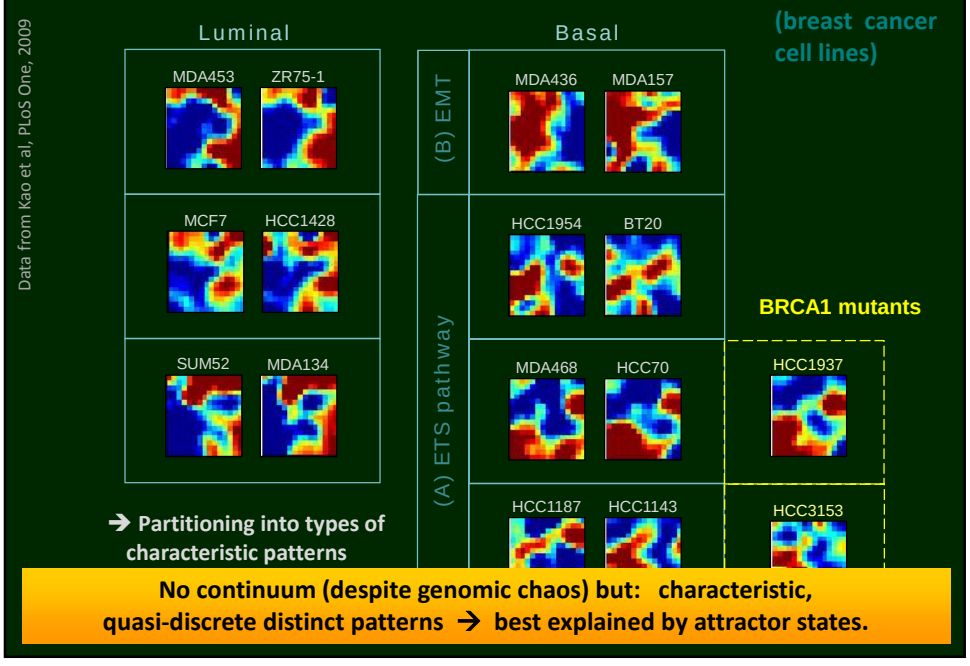
Cancer cells also partition into discrete types

Major Lung cancer histologic types
(> 90% of all cases)



Tumors partition into discrete, stable types:
Highly specific patterns that do not exist in healthy organsims

Breast cancer: natural types

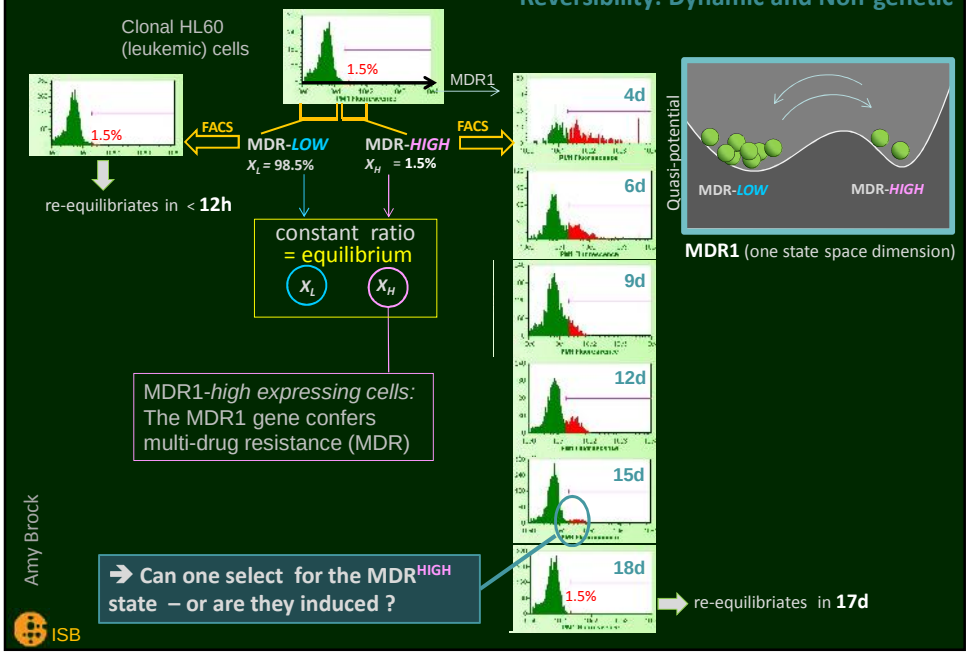


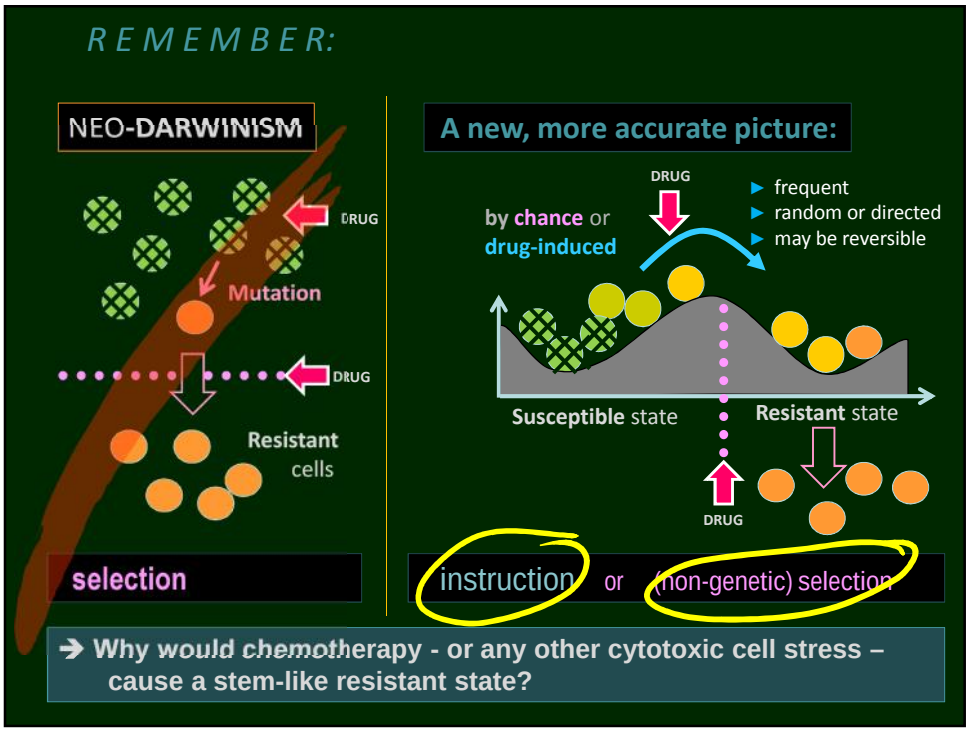
No continuum (despite genomic chaos) but: characteristic, quasi-discrete distinct patterns → best explained by attractor states.

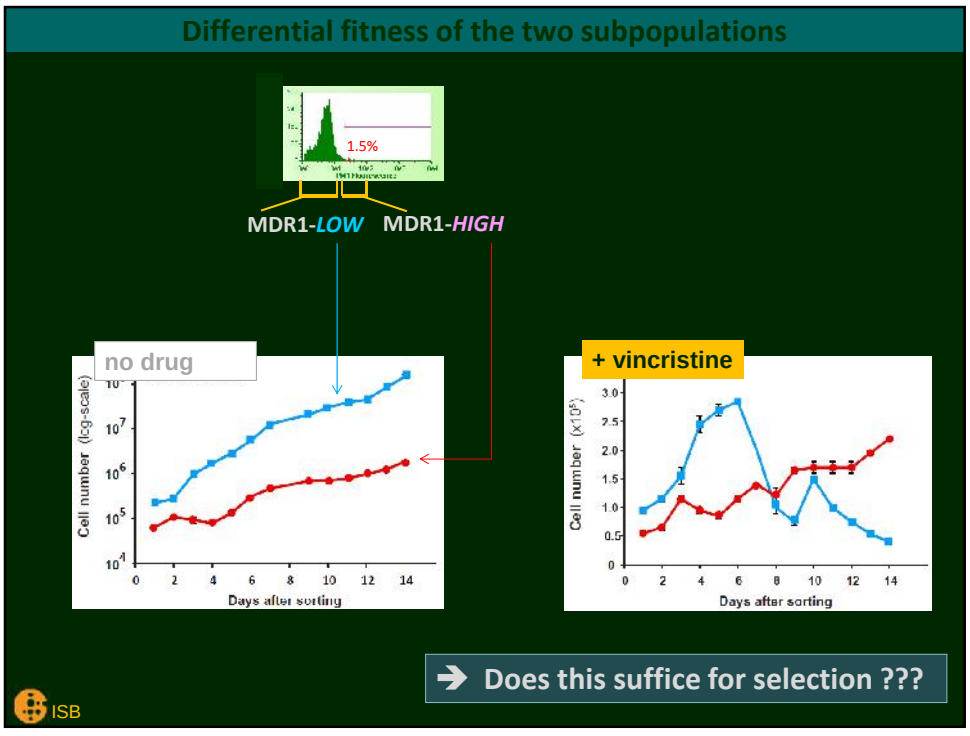
DRUG RESISTANCE DEVELOPMENT

Dynamics of MDR1-*low* and MDR1-*high* subpopulations

Reversibility: Dynamic and Non-genetic




REMEMBER:
NEO-DARWINISM
DRUG
Mutation
DRUG
Resistant cells
selection
A new, more accurate picture:
DRUG
by chance or drug-induced
frequent
random or directed
may be reversible
Susceptible state
Resistant state
DRUG
instruction
or
(non-genetic) selection
➔ Why would chemotherapy - or any other cytotoxic cell stress – cause a stem-like resistant state?


Differential fitness of the two subpopulations
1.5%
MDR1-LOW
MDR1-HIGH
no drug
Cell number (log-scale)
Days after sorting
+ vincristine
Cell number (x10⁵)
Days after sorting
➔ Does this suffice for selection ???
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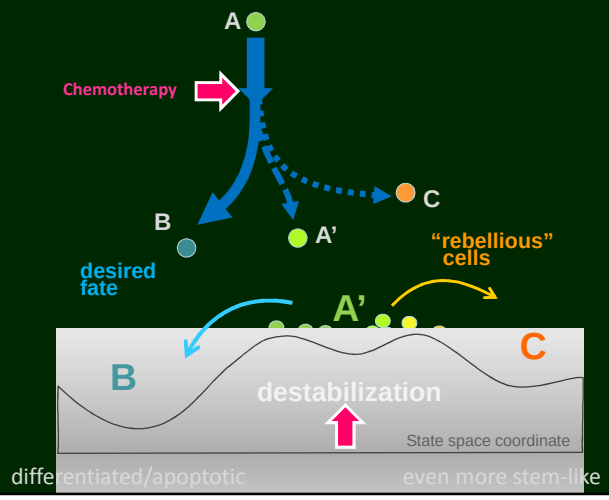
SPECULATION - MORE COMPLEX MODEL

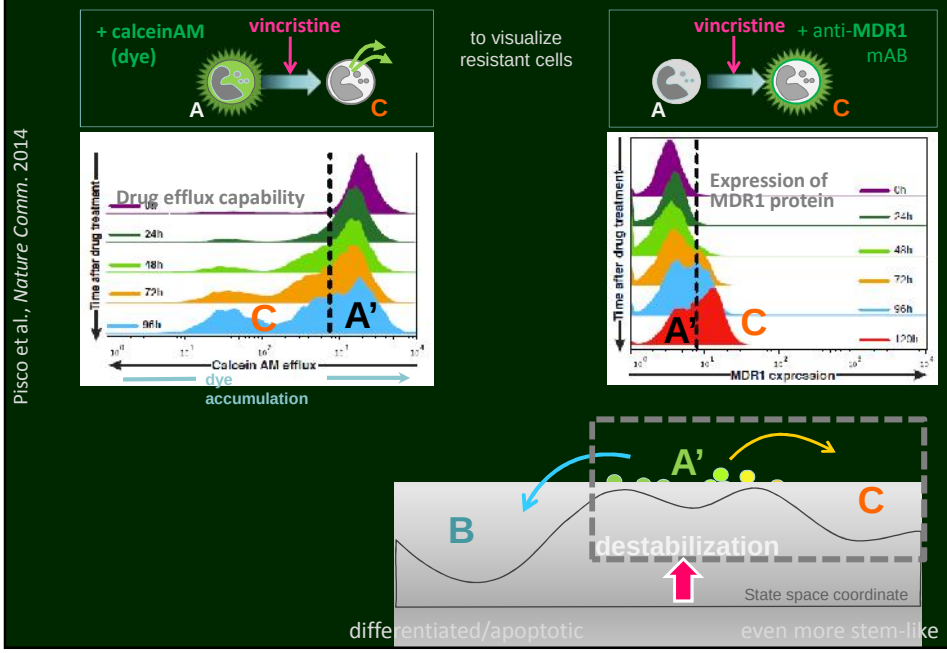
Can chemotherapy also trigger "rebellious cells"? → even more malignant

Inevitable consequence of change in parameters that promote transition into the benign attractor:

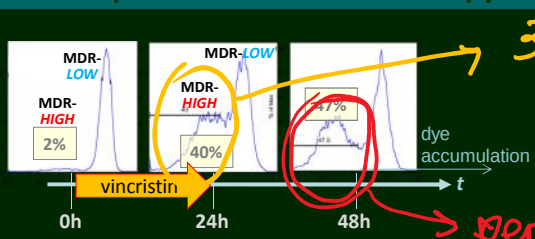
→ also access to more malignant (stem-cell like) state



Rapid appearance of Multi-drug-Resistance (MDR) + cells after vincristine



MDR1 expression after chemotherapy: instruction vs. selection?



30-40% MDR+ cells in 24h

persist...

Brock et al. (2009) *Nat Rev Genet*
see also: Charlebois et al (2011) *Phys. Rev. Lett*

Darwinian Selection
(fast selection of a non-genetic stable alternative state)

or

Lamarckian Instruction
(induction of "rebel" cells by therapy stress)

?

or both

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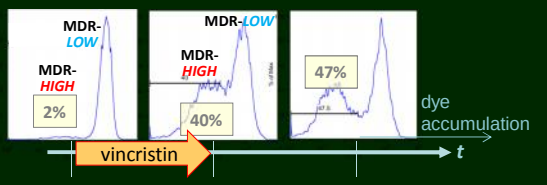
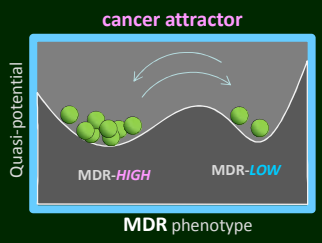
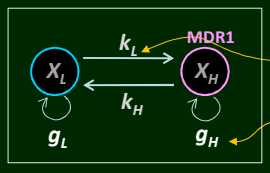
A simple quantitative model







The drug modulates :

- transition rate constant *k* ⇒ **INSTRUCTION** (*"LAMARCKIAN"* mechanism)
- effective growth rate *g* ⇒ **SELECTION** (Non-genetic *"DARWINIAN"* mechanism)

Pisco et al., *Nature Comm.* 2014

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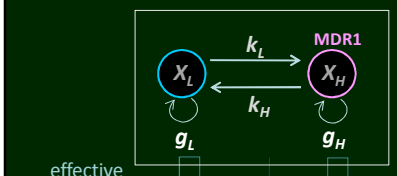
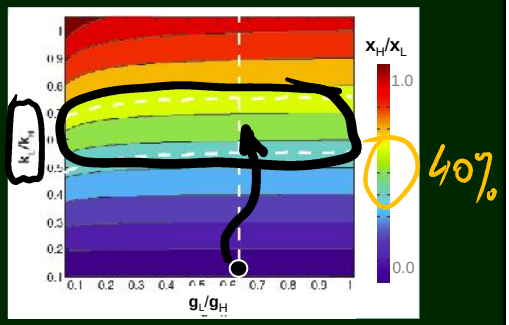
Modeling: Growth and phenotype switch kinetics

$$\dot{X}_L = X_L (g_L - k_L) + k_H X_H$$
$$\dot{X}_H = X_H (g_H - k_H) + k_L X_L$$

- Unlimited exponential growth of both subpopulations
- $(X_L / X_H)_{\text{steady state}} \approx 50$ is invariant & stable
- Presence of MDR+ cells after **24h:** x_H=~ **40%**





effective growth rate:		
control	0.50 /d	~ 0.10 /d
vincristin	0.25 /d*	0.37 /d*

*Initial growth rate first day

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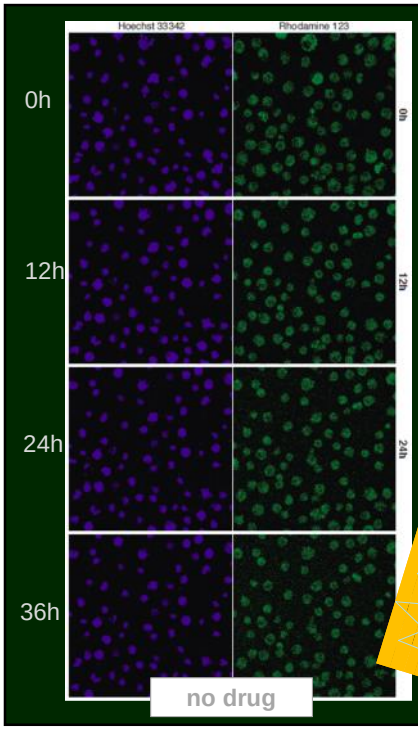
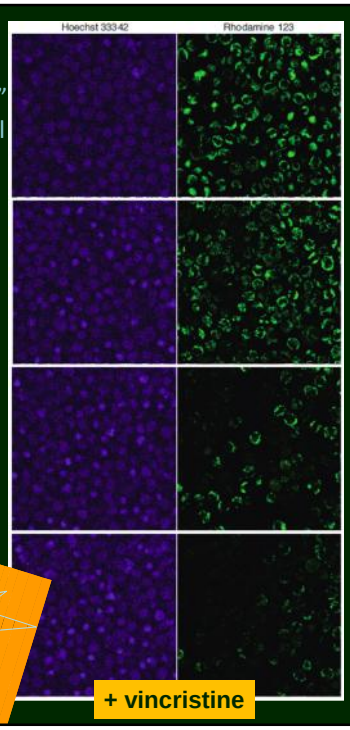


The only "proof" of cell-individual adaption (instruction):

→ **Single**-cell **longitudinal** monitoring of phenotype change

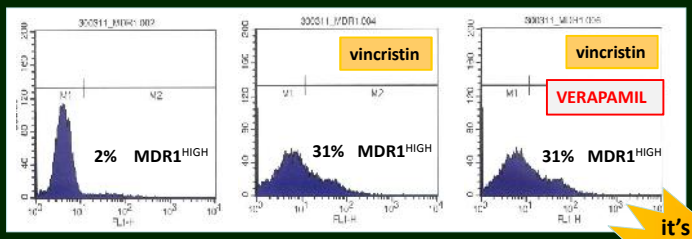


→ **instruction not selection**

EXPERIMENT: blocking fitness advantage …

To really exclude "*selection* of the fittest " by vincristin → **decouple:**

- fitness phenotype = **MDR1 pump function** — from — **MDR1 expression induction**
- **MDR1 pump function** ⊣ VERAPAMIL
- **MDR1 expression induction** ← vincristin



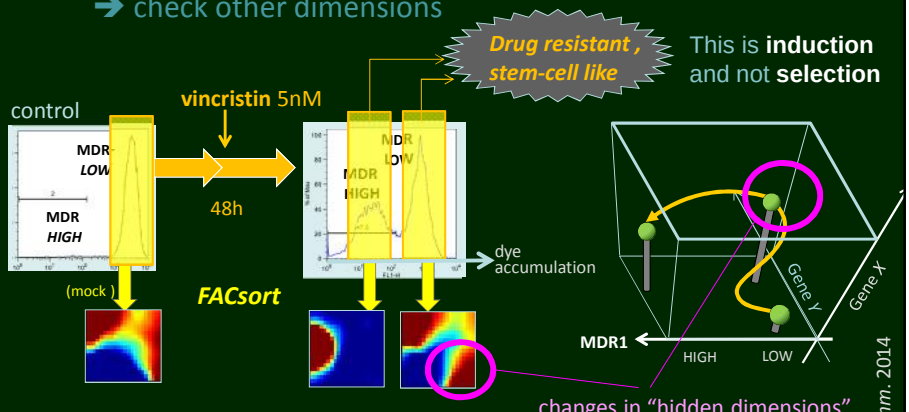
MDR1 expression →

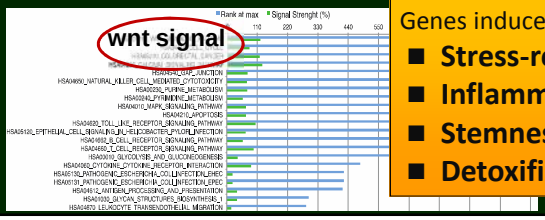
it's not selection

ISB

Transcriptome analysis of vincristine-*induced* genes

➔ check other dimensions

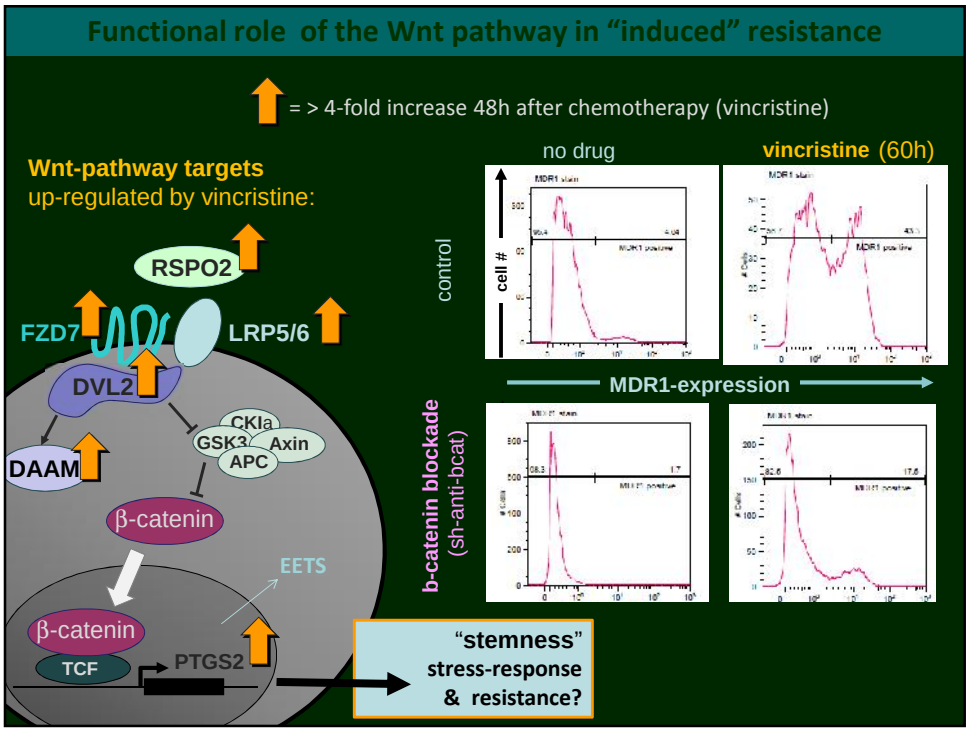


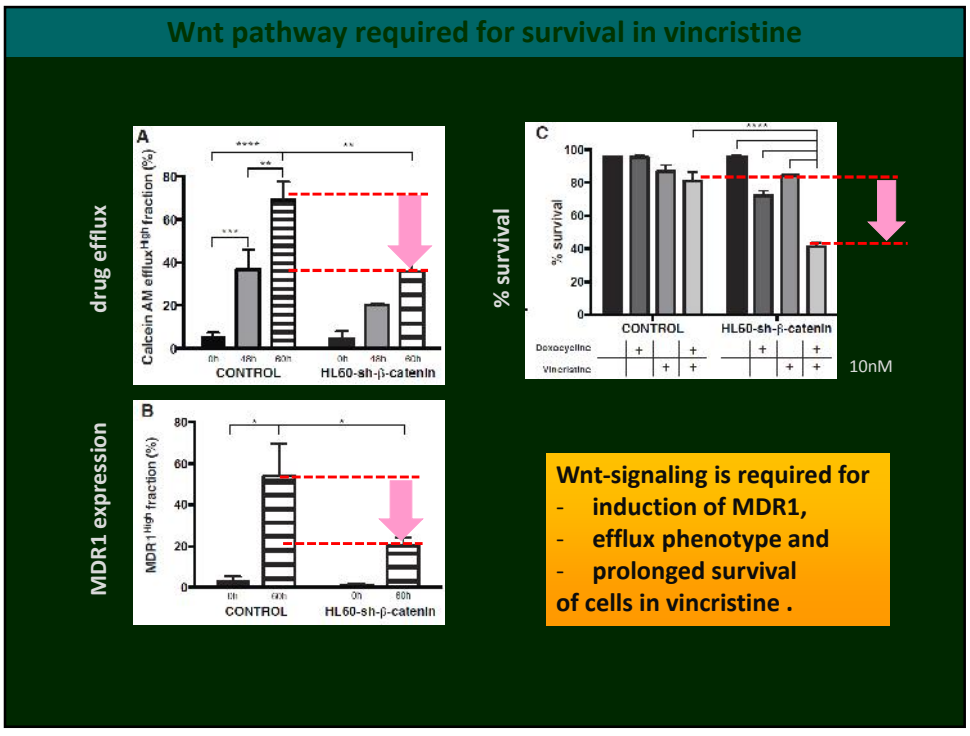


Genes induced:

- **Stress-response** genes
- **Inflammatory** genes
- **Stemness** genes
- **Detoxification**

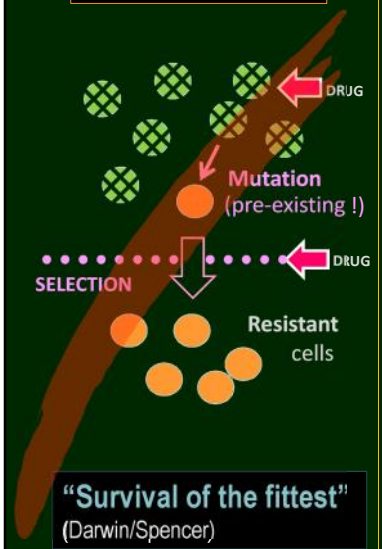
Pisco et al., *Nature Comm.* 2014


Functional role of the Wnt pathway in "induced" resistance
= > 4-fold increase 48h after chemotherapy (vincristine)
Wnt-pathway targets
up-regulated by vincristine:
RSPO2
FZD7
LRP5/6
DVL2
CKIa
GSK3
Axin
APC
DAAM
β-catenin
EETS
β-catenin
TCF
PTGS2
"stemness"
stress-response
& resistance?
no drug
vincristine (60h)
control
cell #
MDR1-expression
b-catenin blockade
(sh-anti-bcat)


Wnt pathway required for survival in vincristine
drug efflux
A
Calcein AM efflux^High fraction (%)
CONTROL
HL60-sh-β-catenin
MDR1 expression
B
MDR1^High fraction (%)
CONTROL
HL60-sh-β-catenin
% survival
C
% survival
CONTROL
HL60-sh-β-catenin
Doxocycline
Vincristine
10nM
Wnt-signaling is required for
- induction of MDR1,
- efflux phenotype and
- prolonged survival
of cells in vincristine .

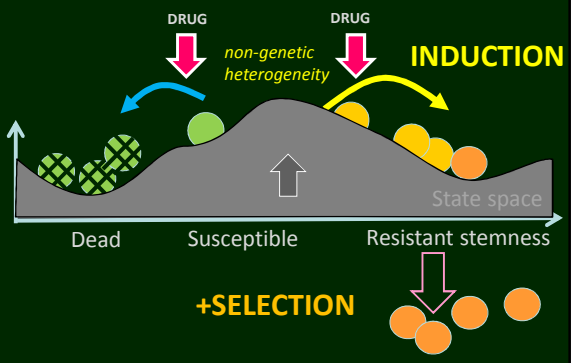
NON-DARWINIAN evolution of resistance

NEO-DARWINISM



"Survival of the fittest"
(Darwin/Spencer)

a kind of LAMARCKISM



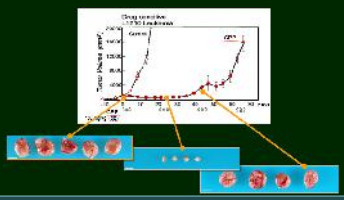
+SELECTION

+ many new stem-like "atavistic" survival functions

"What doesn't kill me makes me stronger "
(Nietzsche)

see: Huang S., *Progr. Biophys. Mol. Biol.* (2012)

BROAD LESSONS



➔ **Why do tumors come back – often in a more malignant form?**

- **Cancer is not** (just) **a "genetic disease" !**
- **There is an inherent limitation to killing cancer cells.**
  – partial destruction is not partial success …
  but can be worse in the long term.
- **It is not all "mutation + selection" !**
  – there is enormous non-genetic plasticity of phenotype

thank you

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Further Reading

- Pisco, A. O. *et al.* Non-Darwinian dynamics in therapy-induced cancer drug resistance. *Nature communications* **4**, 2467, (2013). PMID: **24045430**.
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