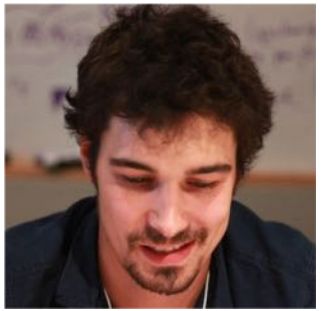
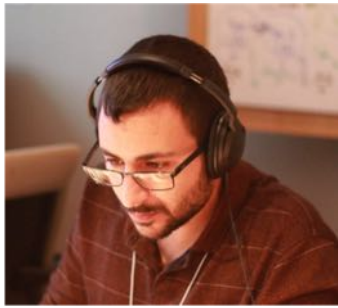
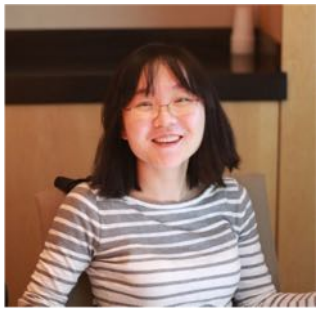
Dark selection for JAK/STAT-inhibitor resistance in CMML

The Orange team, the future is bright...



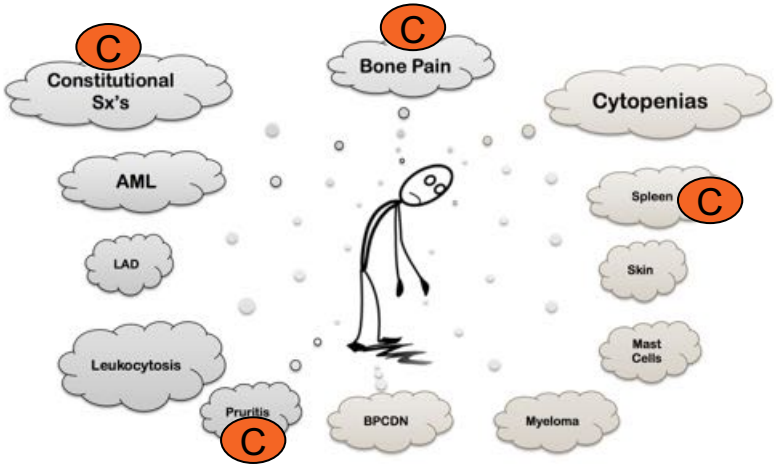
ORANGE

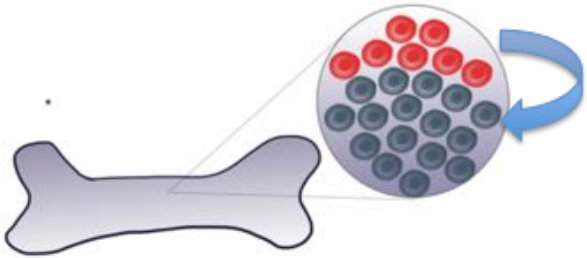




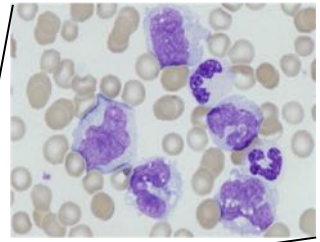


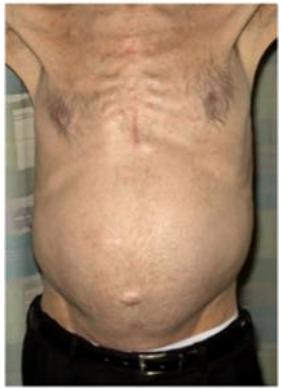


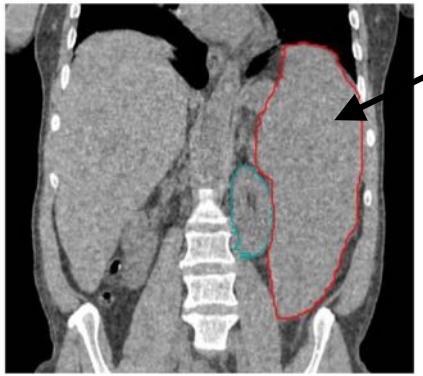
Chronic Myelomonocytic Leukemia (CMML)



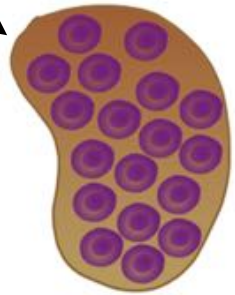


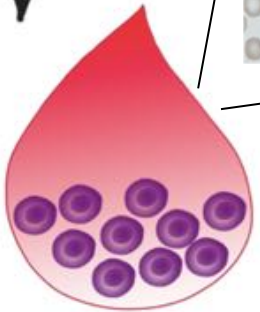




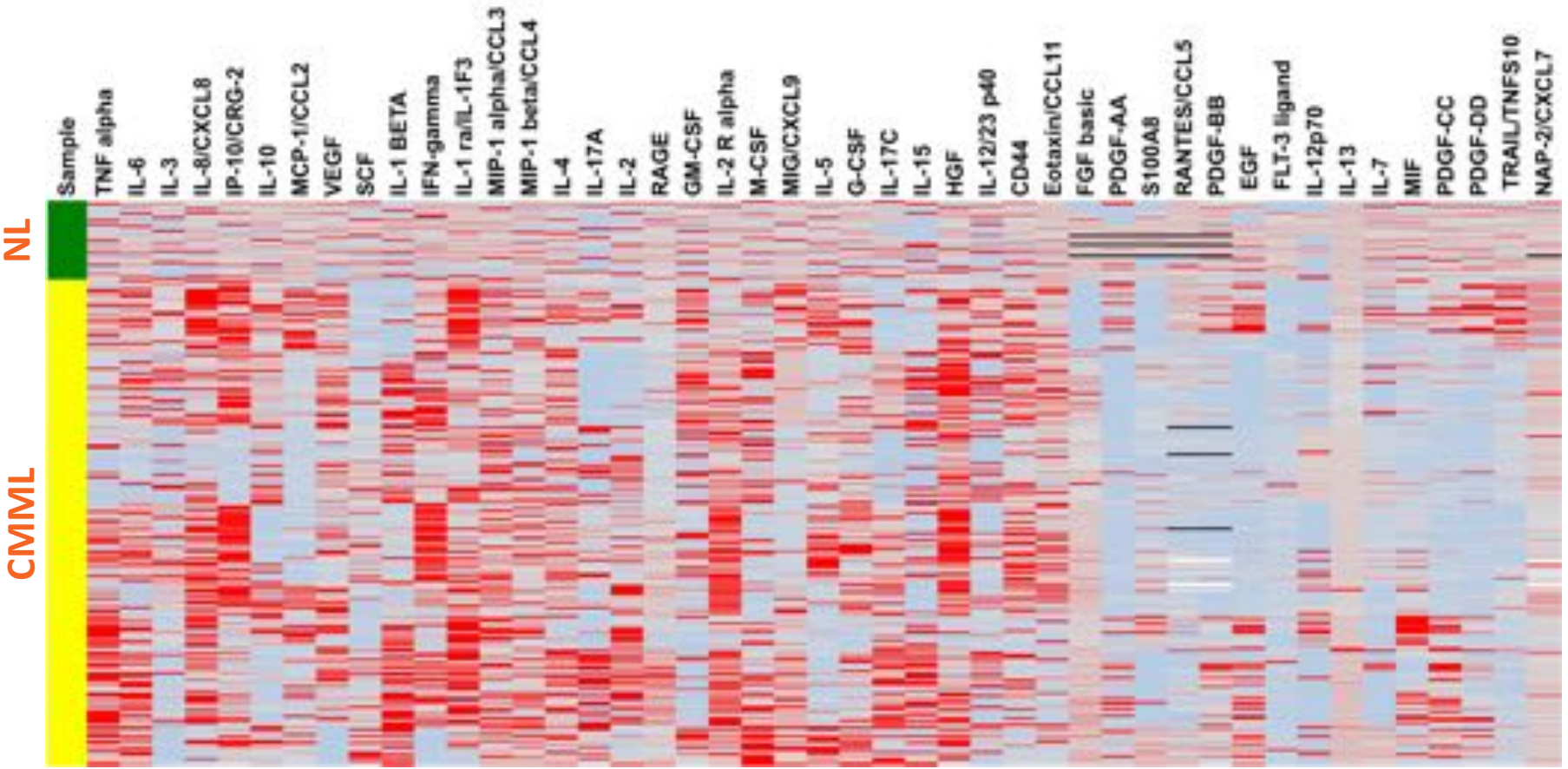


splenomegaly





Inflammatory cytokines are elevated in CMML



N=219 CMML n=35 age-matched controls

Blue (10%) percentile Red (90%) percentile

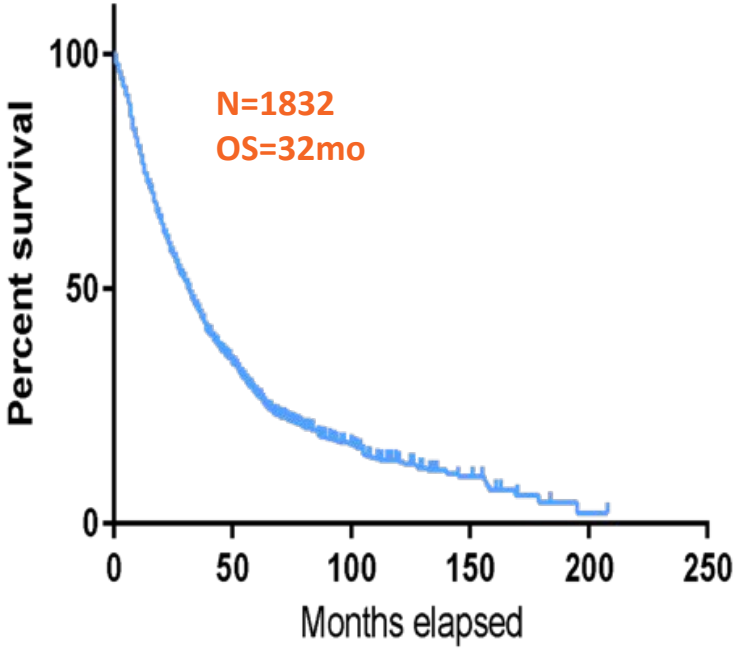
Our analysis revealed prognostic links

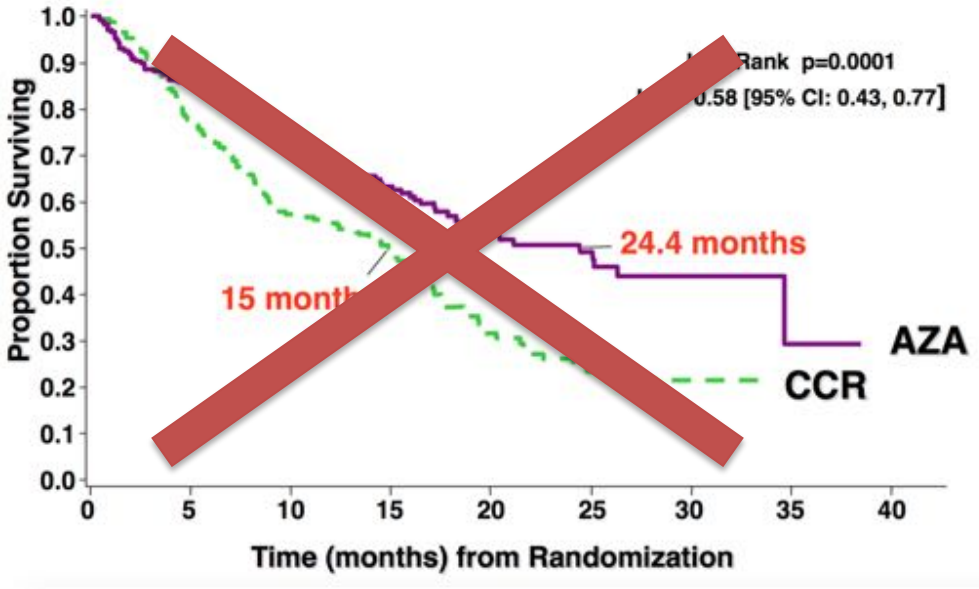
What are the important cytokines related to:

	How patients feel?	Spleen size?	Survival?
Poorer Outcome	MCP1 **PDGFAA** EGF **RANTES** **SCF**	IL5 **SCF** TRAIL MIG IP10	IL13 **PDGFAA** RAGE **SCF** **RANTES**
Better Outcome	**FLT3** **CD44** VEGF **IL12p70** **FGFBasic**	**FLT3** **FGFBasic** RAGE **IL12p70** **CD44**	IL10 **FGFBasic** IL12.23p40 IL2Ra Exotaxin

Penalised regression from 48 plasma cytokine values of 161 CMML patients, prior to treatment

We lack good treatment options this lethal leukemia

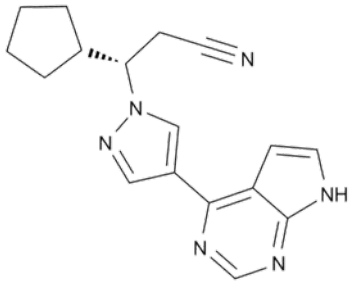


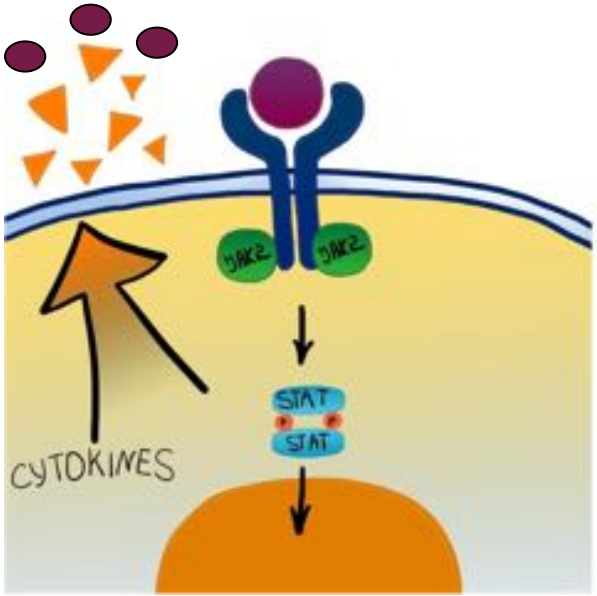


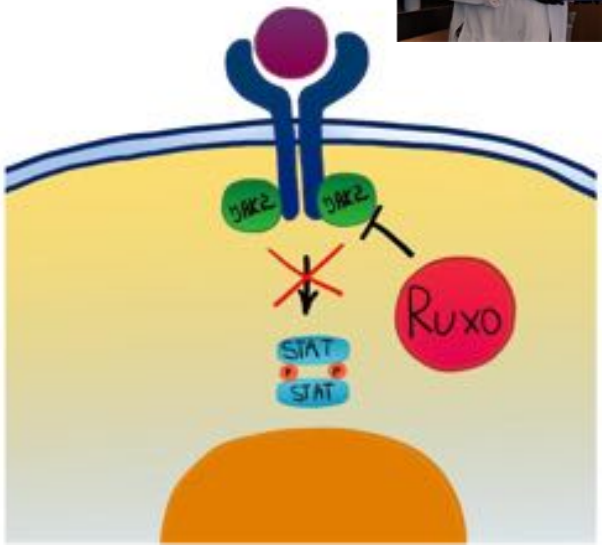
Fenaux, et al Lancet Oncology 2009

Ruxolitinib: a new hope

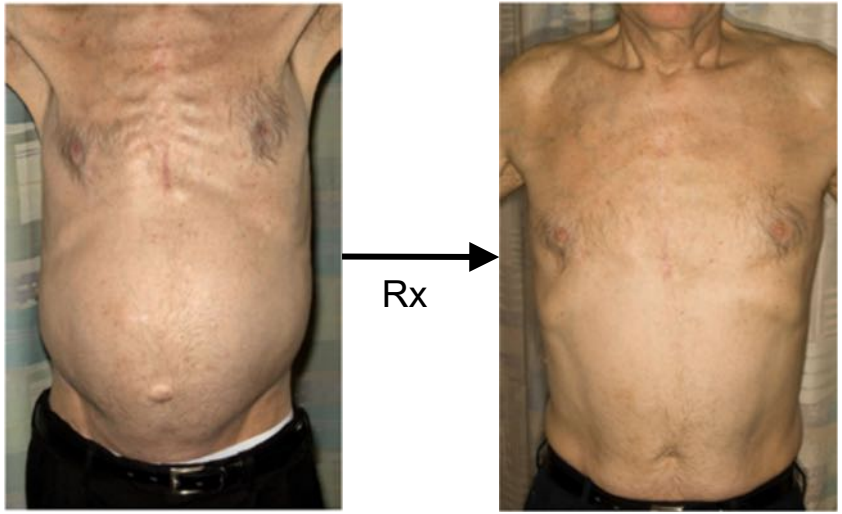
Ruxolitinib



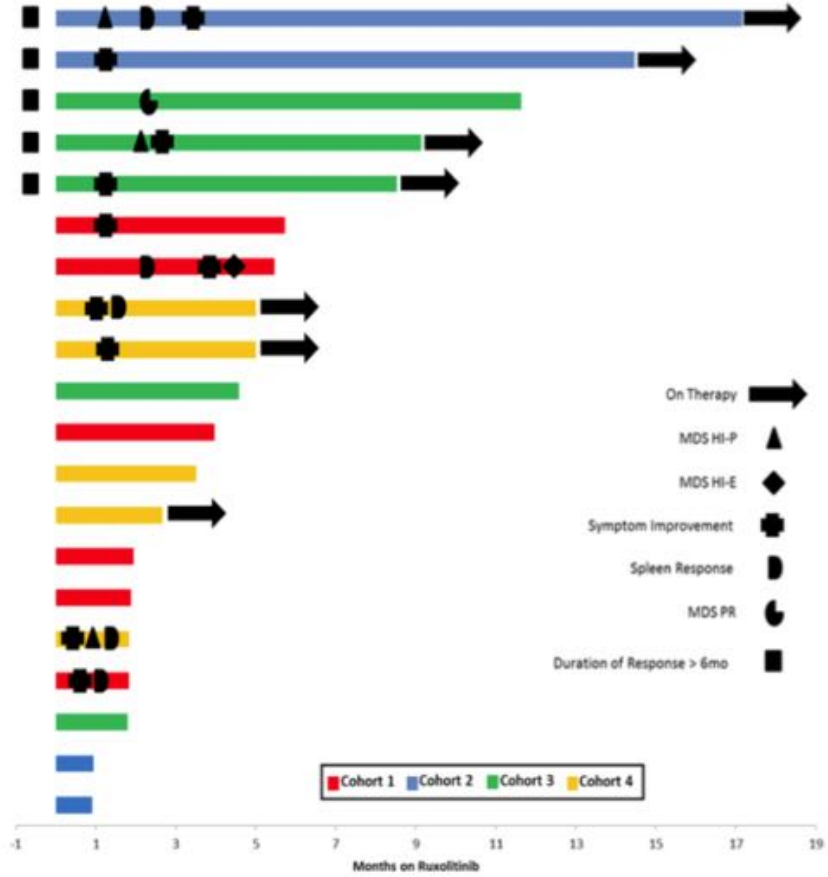




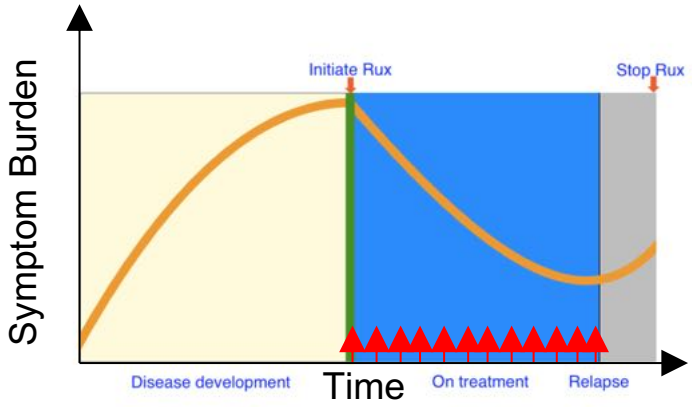
Ruxolitinib: a new hope



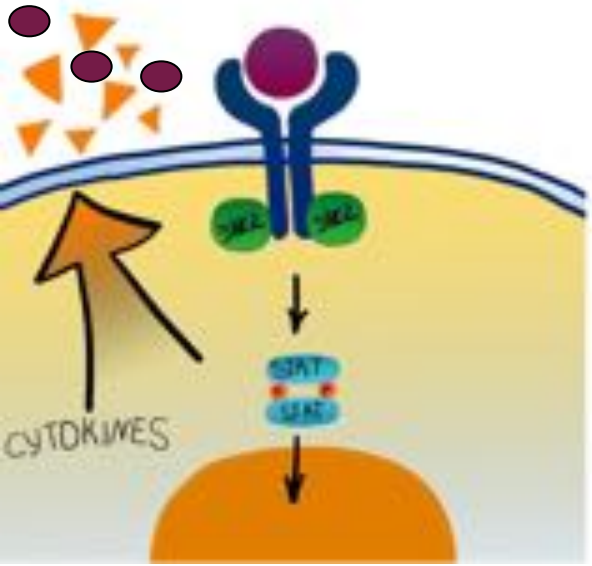
Spleen pre-/post-treatment

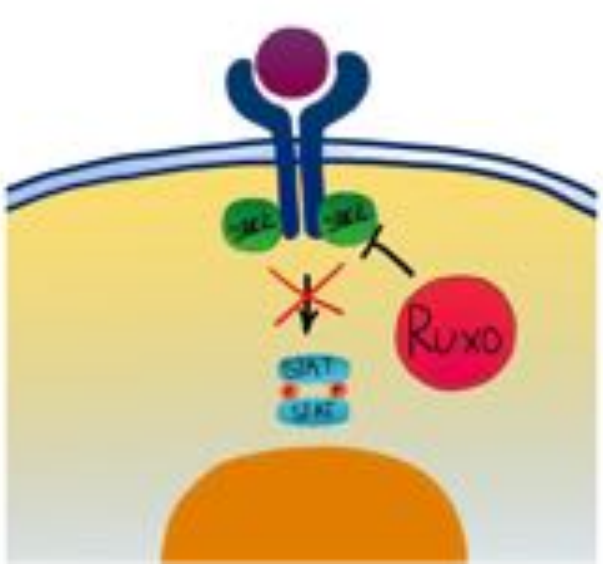


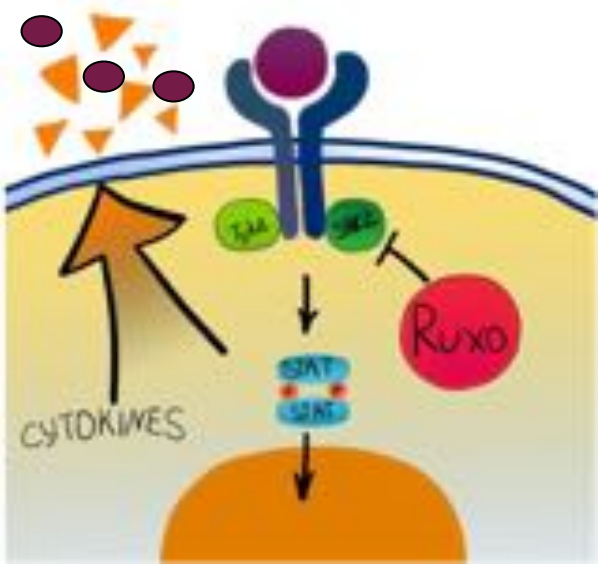
Alas, resistance strikes back



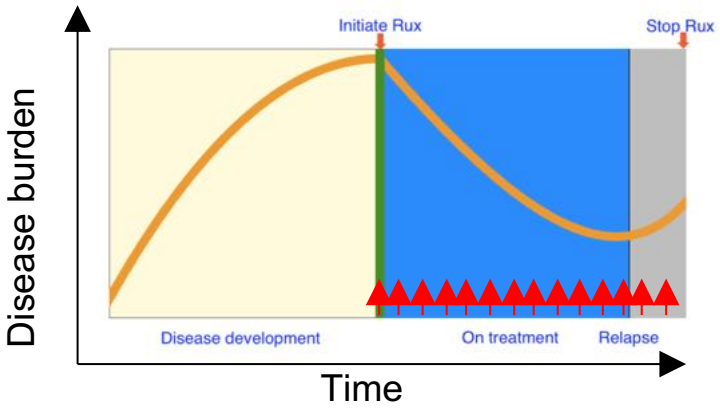
We understand proximal mechanisms

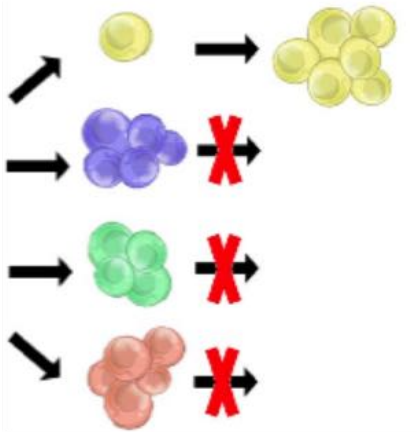


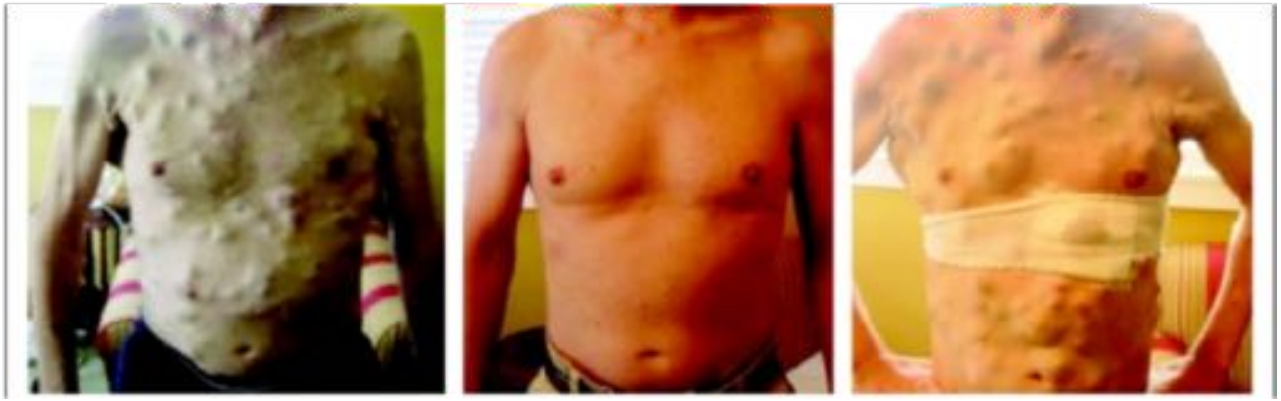




“Normal” resistance: Darwinian selection of resistant (epi)genotypes

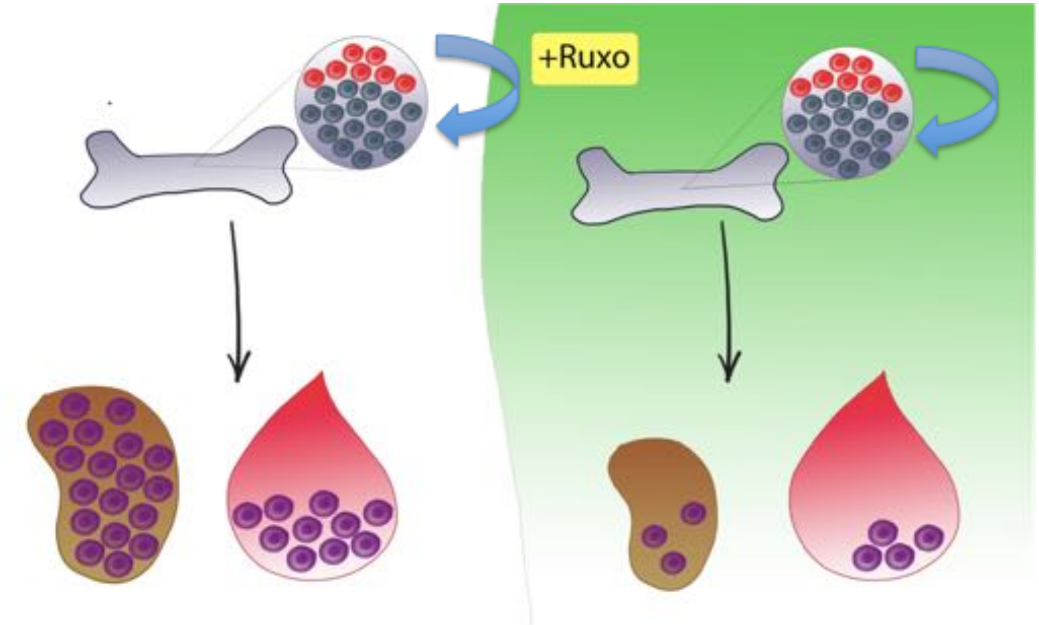




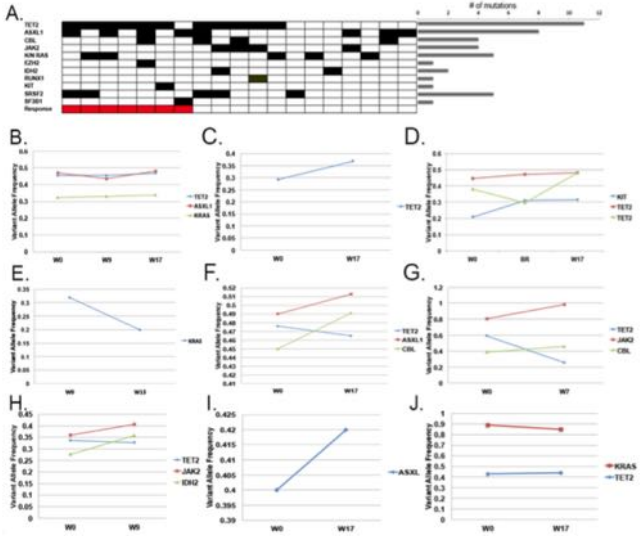


Dark Selection: evolution paradox

No obvious impact on stem/progenitors

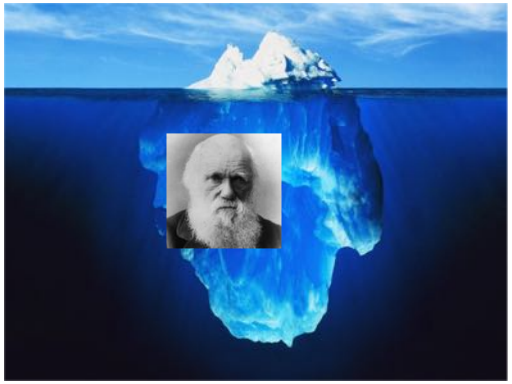


No impact on clonal architecture

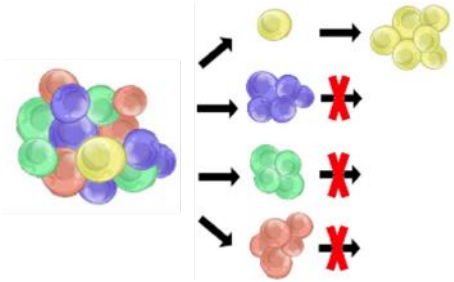


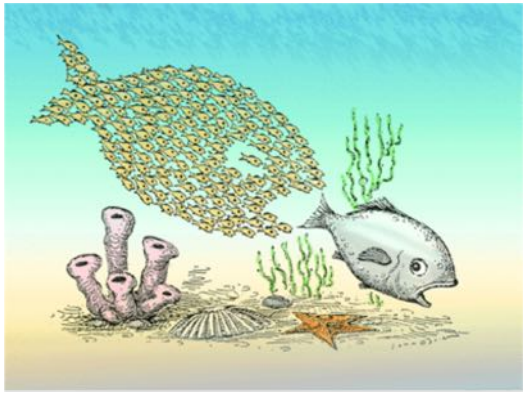
Padron Clin Can Res 2016

Shining light on the paradox of dark selection



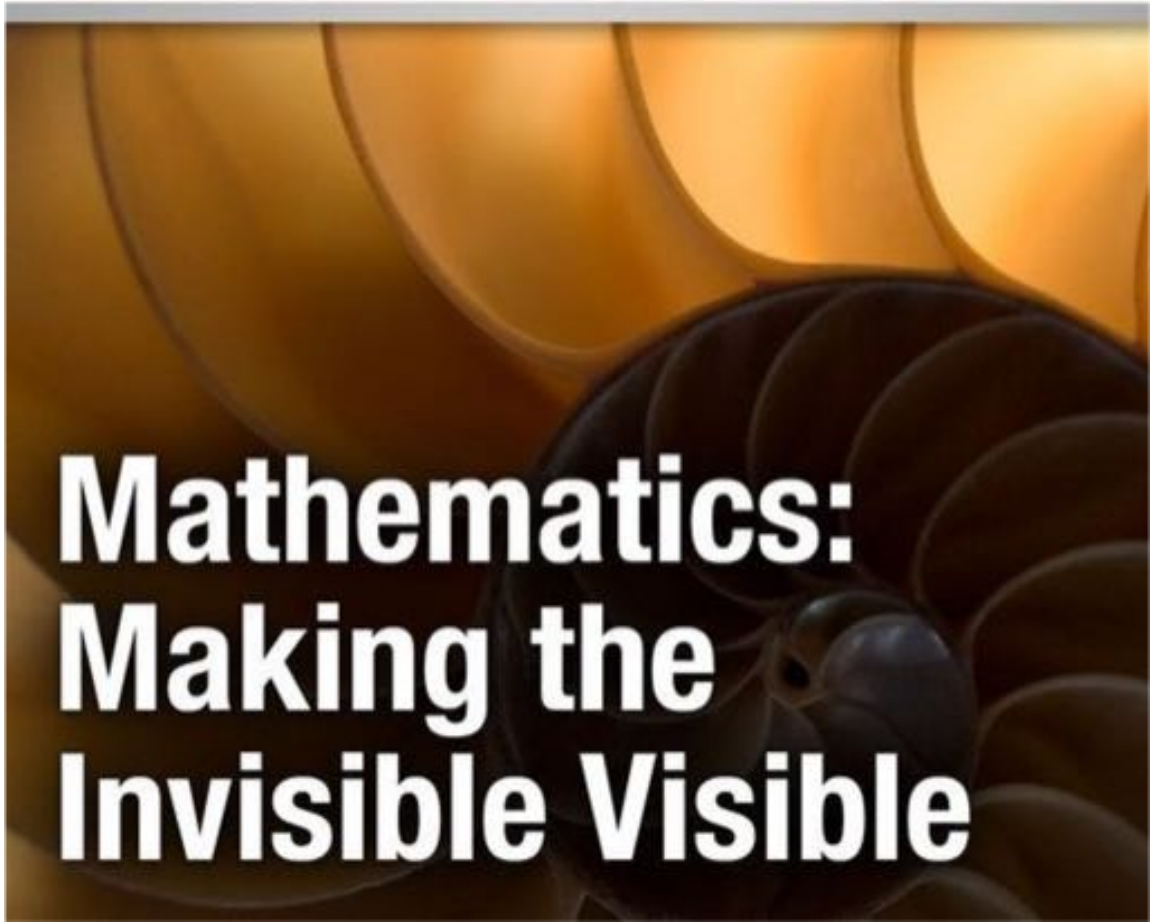
Unusual case of Darwinian selection



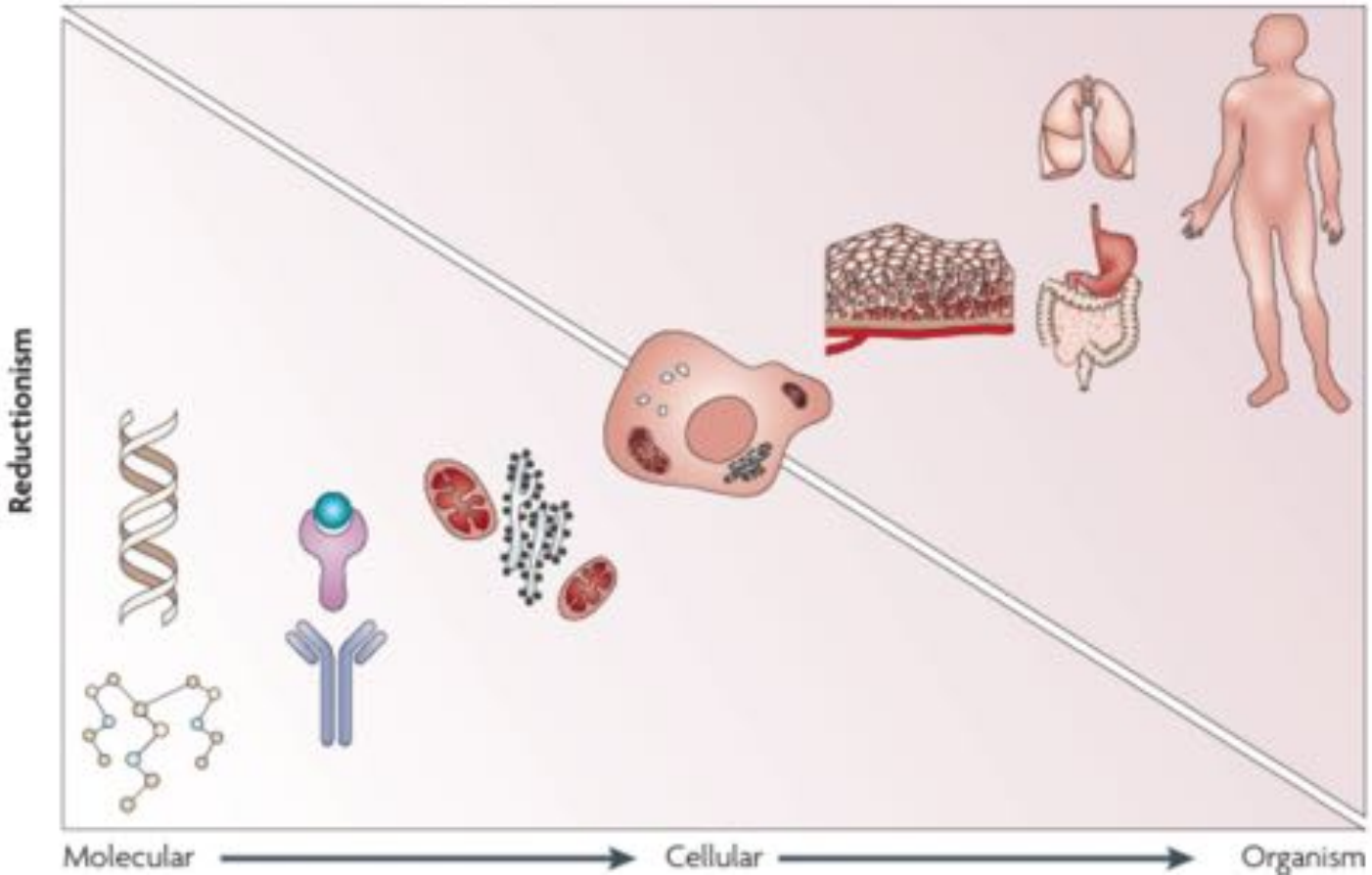


Non-darwinian selection/emergence

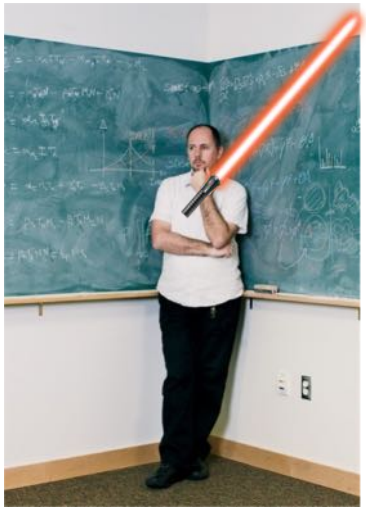
Shining light on the paradox of dark selection



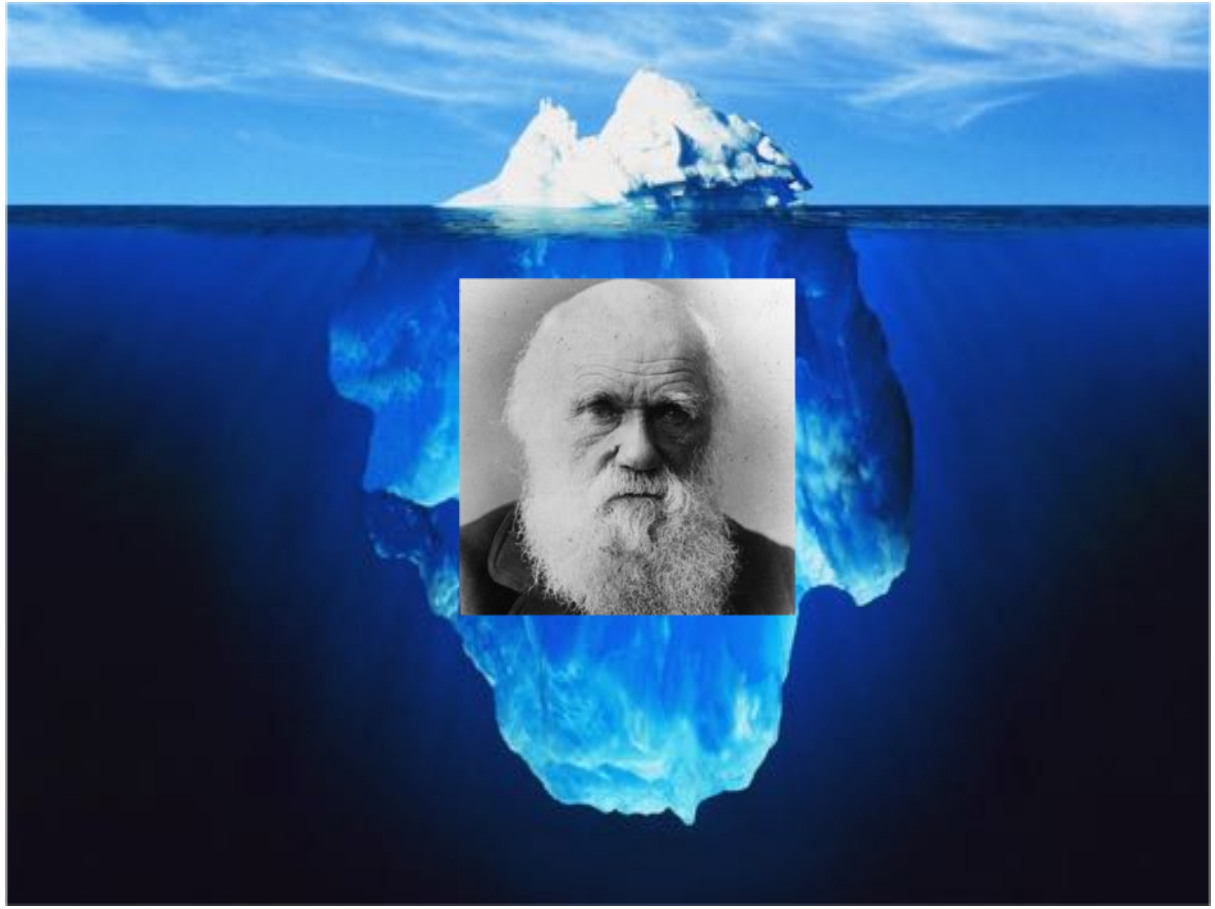
Scales of Cancer



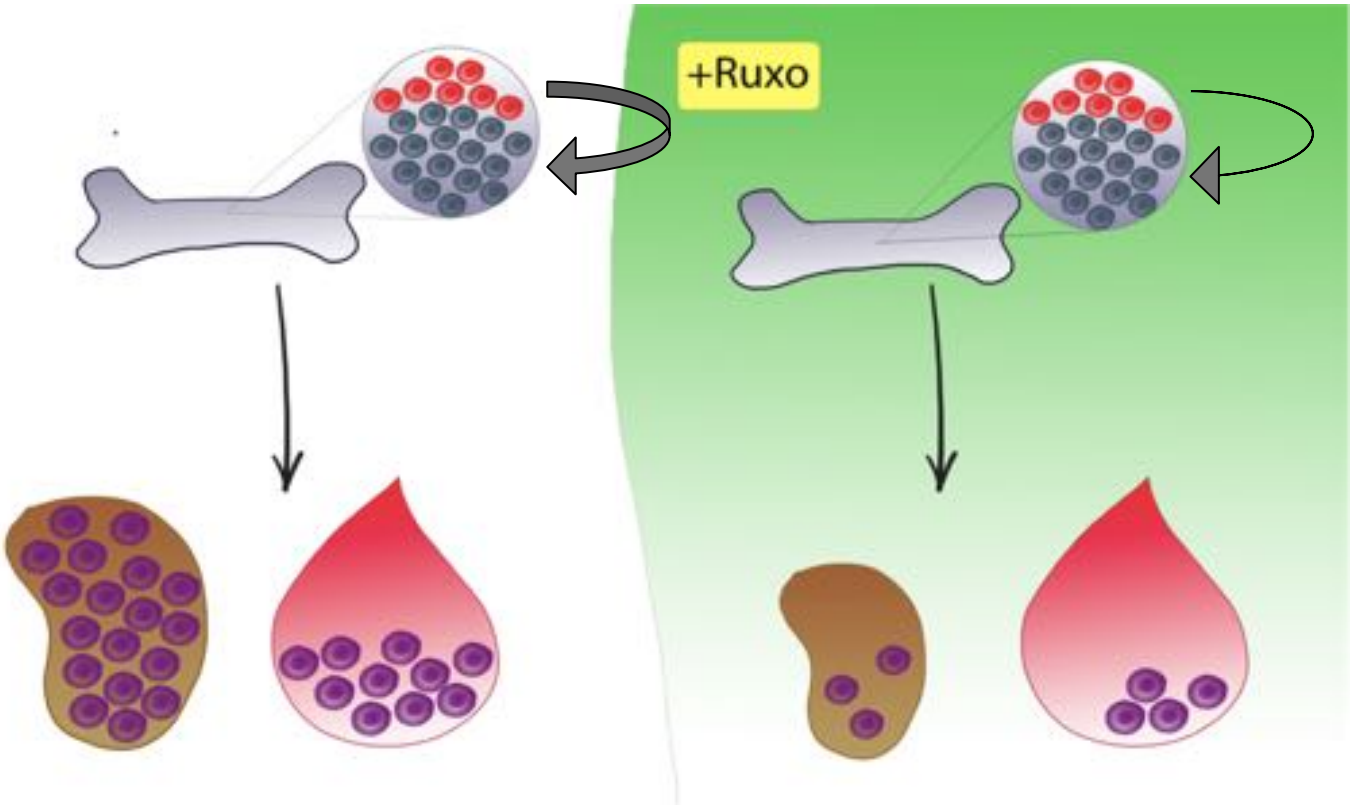
Qolism



Alternative #1: hidden Darwinian selection

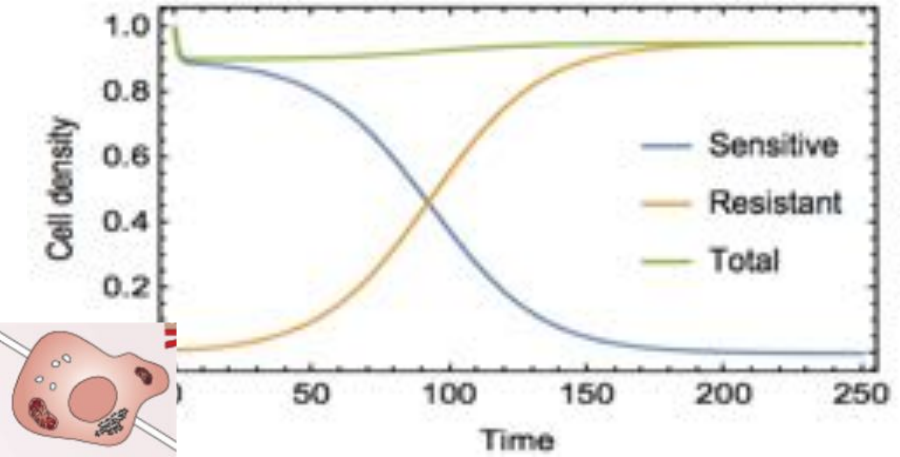


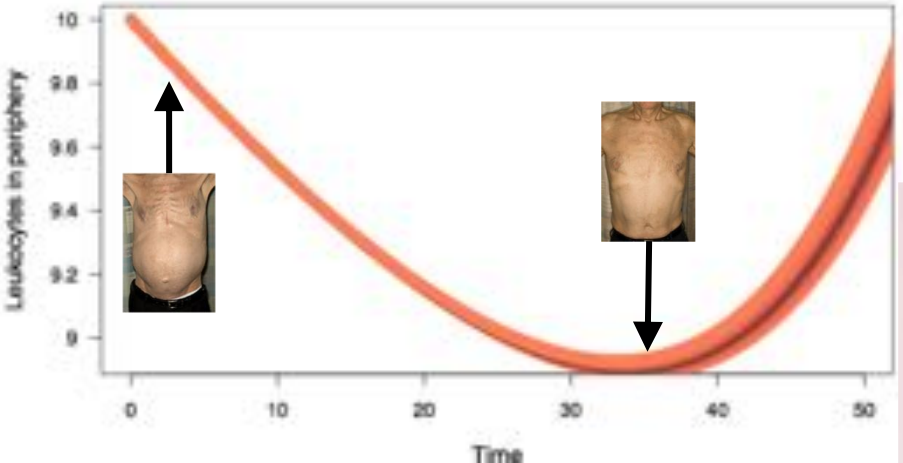
Alternative #1: hidden Darwinian selection



Hidden Darwinian selection is plausible

Left panel: ODE models on disease burden in the Bone Marrow
Right panel: Moran process model on disease output into periphery





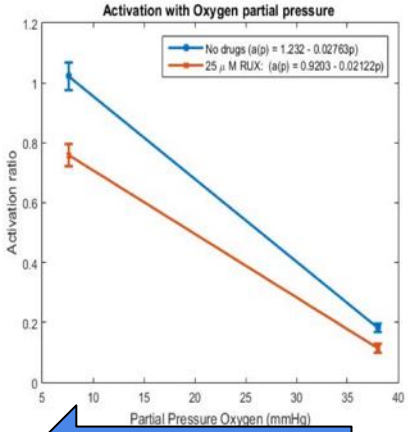
Hidden Darwinian selection is plausible

But:

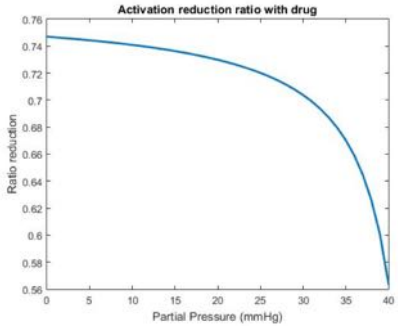
No evidence of genetic changes: must be epigenetic

No evidence for reduced proliferation/increased death

Considering microenvironmental pressure



Increasing Hypoxia



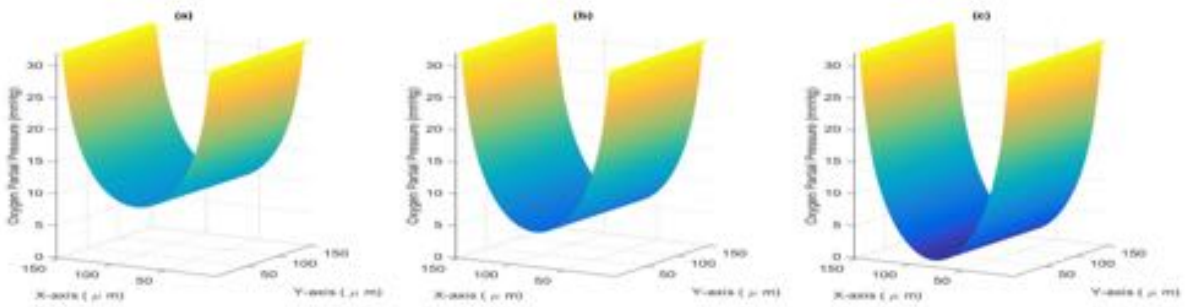
STAT3 activation is inversely related to oxygen partial pressure. Data taken from Wang et al, Cell Biol.. Int., 2005

Oxygen concentration strongly linked to STAT3 expression

- Hypoxia markedly increases STAT3 (and JAK) expression.
- This effect seen both with and without RUX present.

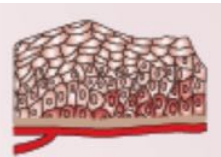
Bone Marrow at low O2 pressure (9-32 mmHg), despite high vascularity

- Oxygen consumption rate (OCR) markedly modulates hypoxia.
- Minor changes in OCR can lead to substantial increases in hypoxia.
- Can minor OCR shifts lead to STAT3 levels > RUX can counteract?

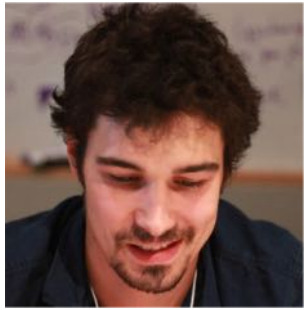


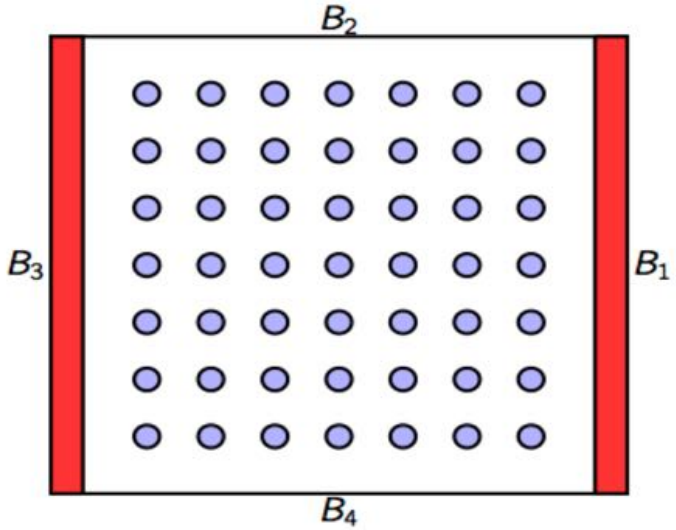
Slight increases in OCR

Slight perturbation of oxygen consumption rate leading to slightly different oxygen distributions (Grimes et al 2016, J R Soc Interface) by two vessels at the boundary. This oxygen map was set in accordance with measured O2 levels in bone marrow (Spencer et al, Nature, 2014).



Hypoxia



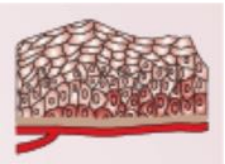


$$\frac{\partial C}{\partial t} - D\nabla^2 C = S(x, y, STAT3) - \delta C$$

$$S(x, y, STAT3) = \Pi STAT3(x, y)\mathbb{1}_{Cells}(x, y)$$

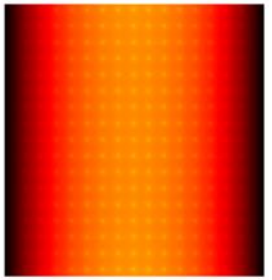
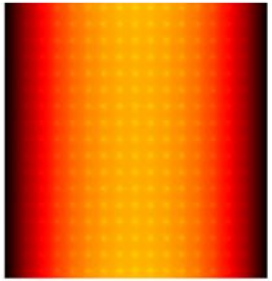
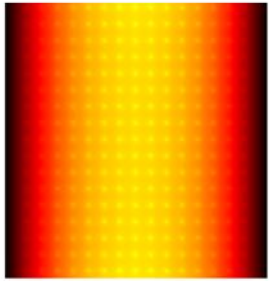
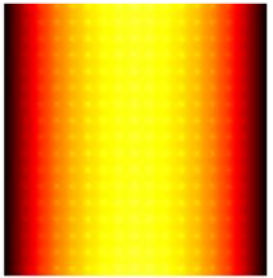
$$C = 0 \text{ on } B_2 \text{ and } B_4$$

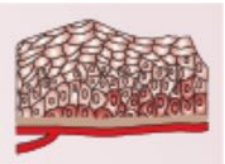
$$C_{|B_3} = C_{|B_1}$$



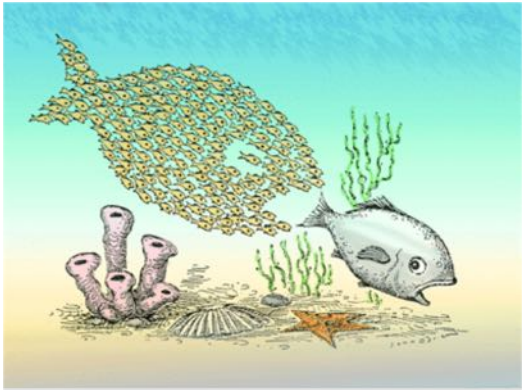
Sustained stabilization of Interleukin-8 mRNA in human macrophages (Mahmoud et al 2014, RNA biology). Diffusion of Interleukin-2 from cells overlaid with cytocompatible enzyme-crosslinked gelatin hydrogen (Yung et al 2010, J Biomed Mater Res A).

Hypoxia

	Situation 1 *15.9 mmHg*	Situation 2 *13.1 mmHg*	Situation 3 *10.1 mmHg*
Drug			
No Drug			



Alternative #2: Dark selection/emergence

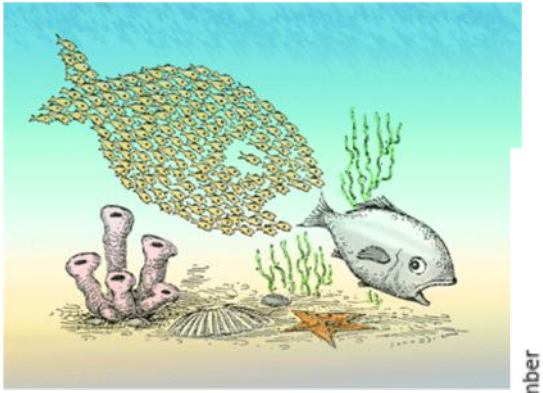


Stochastic ratcheted phenotypic switch

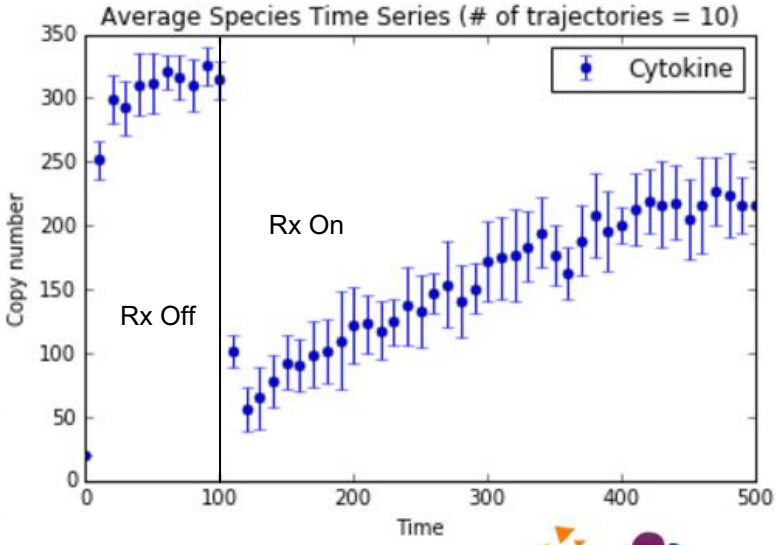
Lamarckian selection

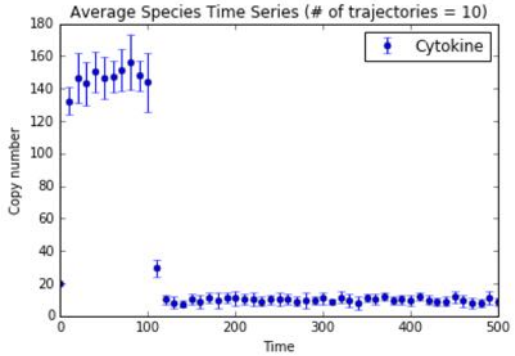
Emerging signaling network behavior

Alt. #2: Molecular basis of dark selection

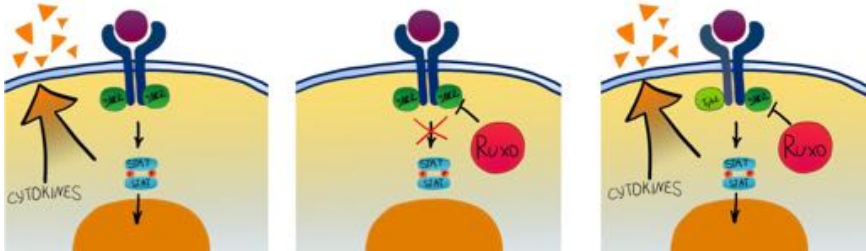


Stochastic ratcheted phenotypic switch
& Lamarkian inheritance

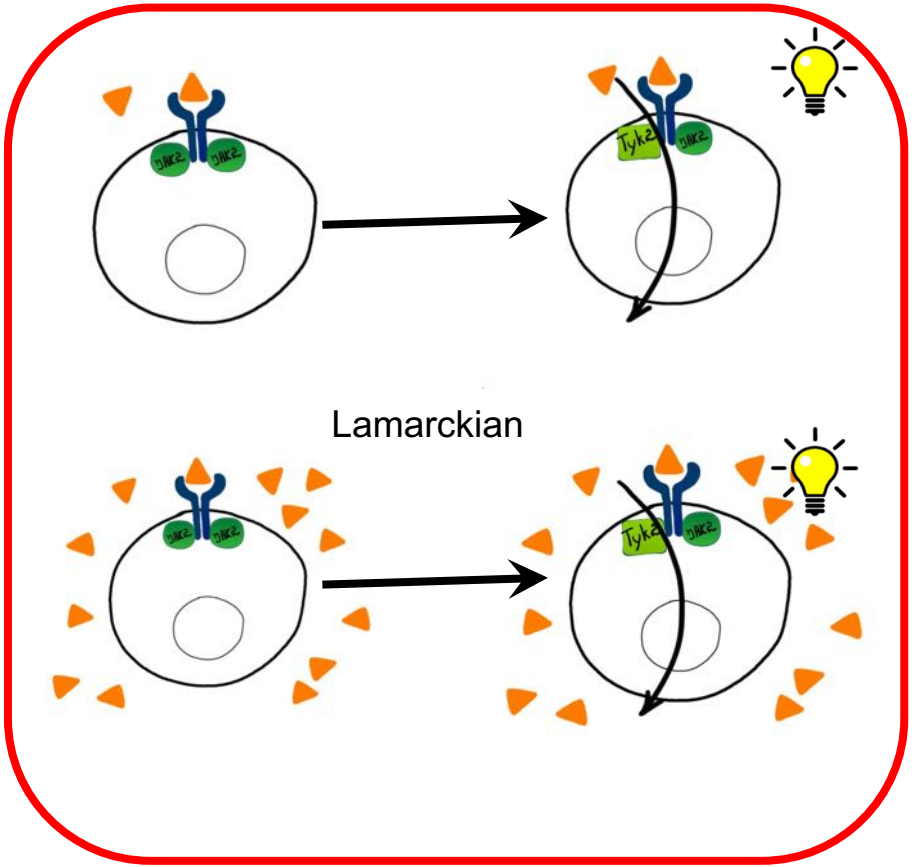


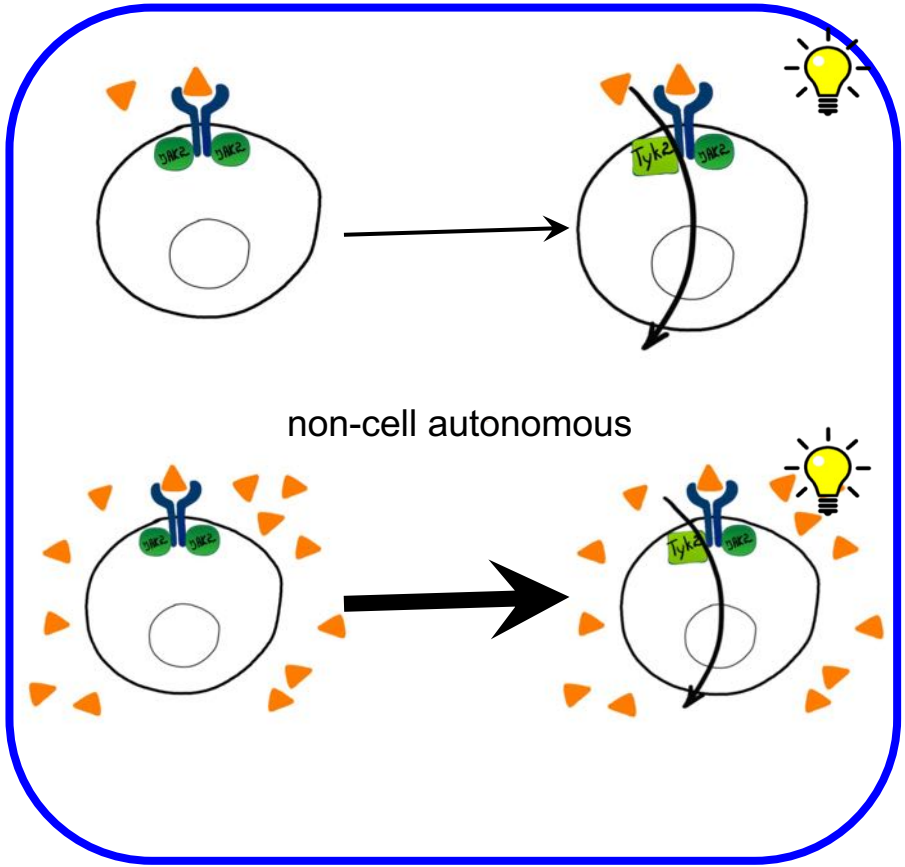



```
STATJJBirth:
    BReceptorJJ > STAT + UReceptor + {2.0}JAK
    BJJ_STAT*BReceptorJJ*Drug

STATJTBirth:
    BReceptorJT > STAT + UReceptor + JAK + TYK
    BJT_STAT*BReceptorJT

BoundReceptorBirth:
    UReceptor + Cytokine > BReceptorZZ
    B_BReceptorZZ*UReceptor*Cytokine

JAKBinding:
    BReceptorZZ + JAK > BReceptorJZ
```



Alternative #2: Dark selection/emergence





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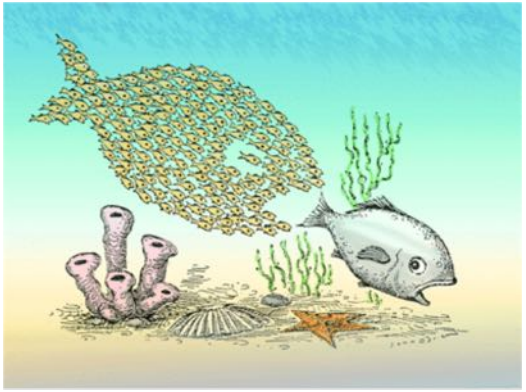
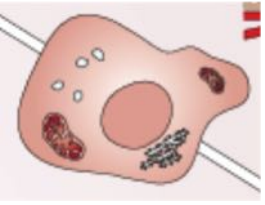
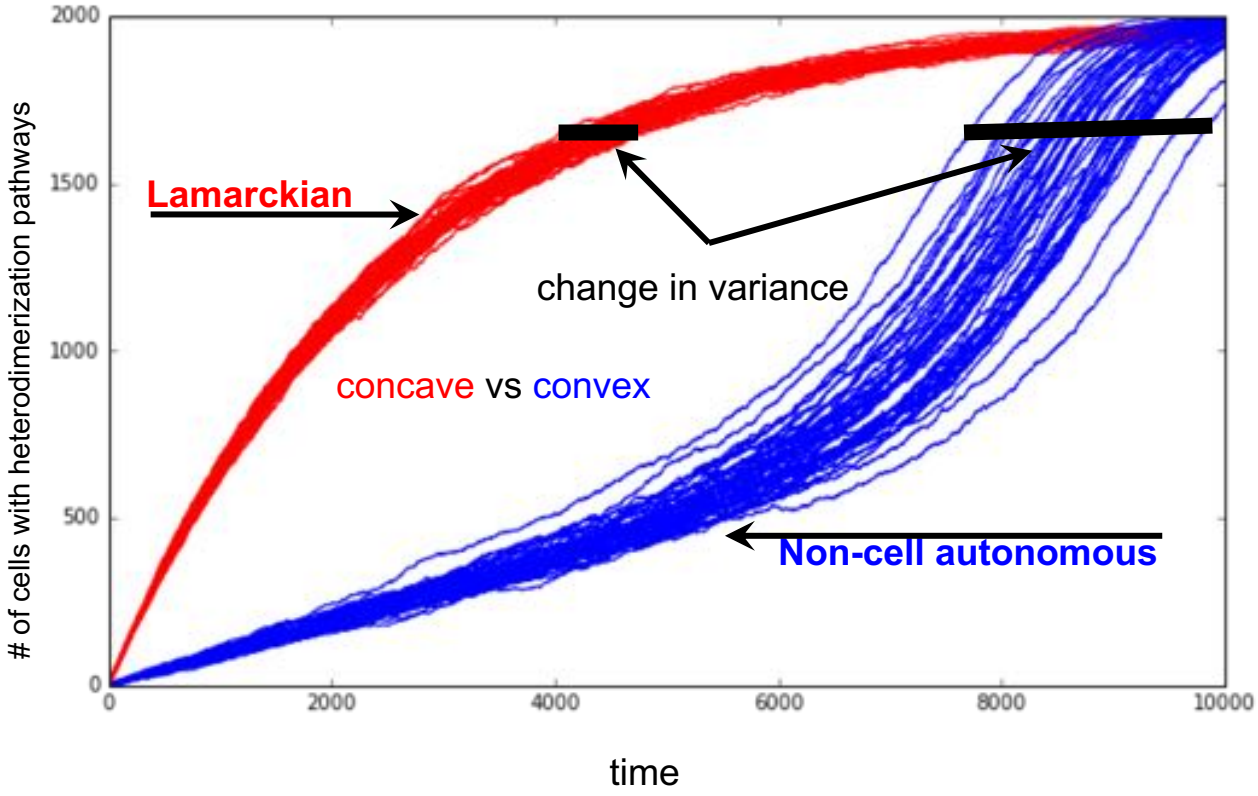
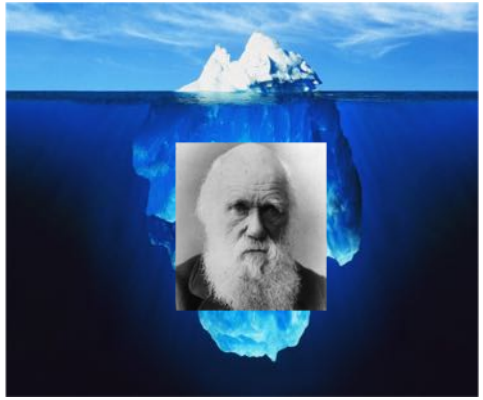
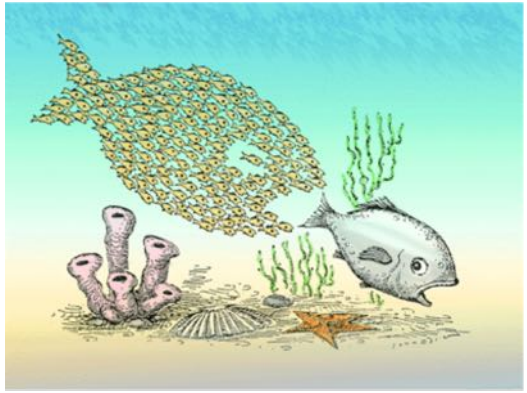






Different sources of “dark selection” lead to different predictions





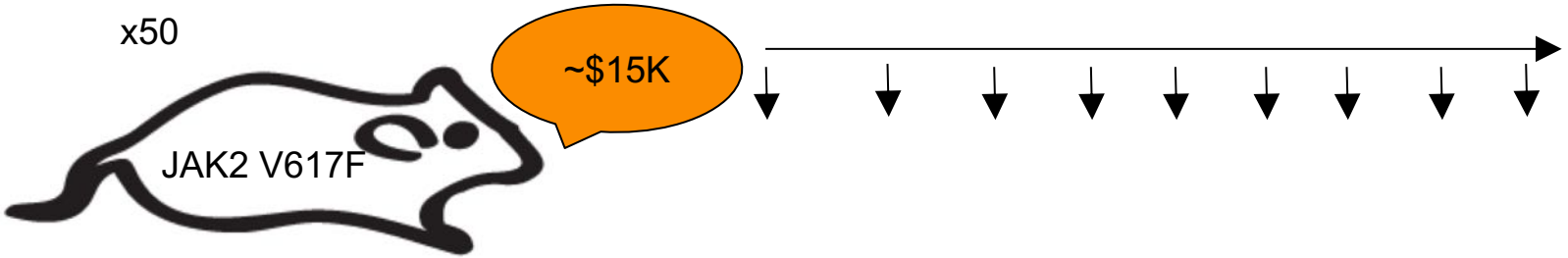
Hidden Darwinian selection

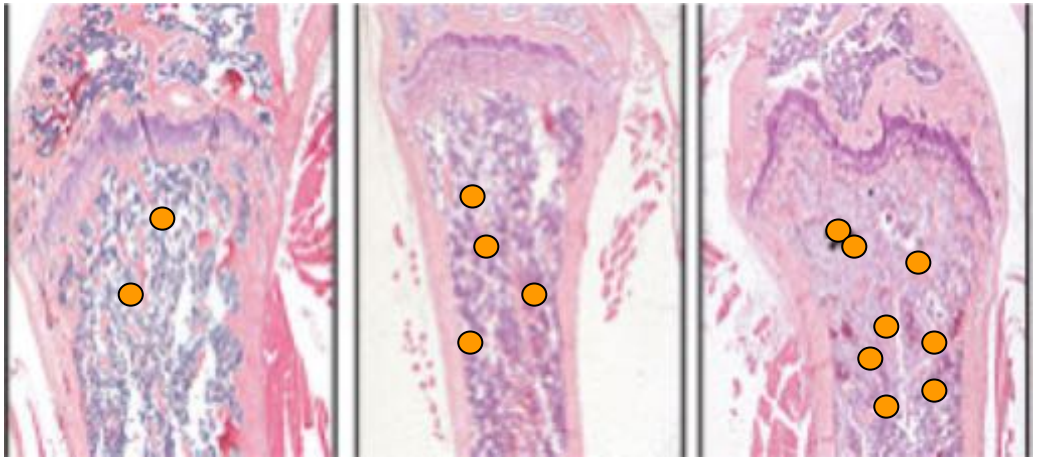
Stochastic ratcheted phenotypic switch

Lamarckian selection

Emerging signaling network behavior

Experiments to differentiate between the scenarios





Analyze in the Bone Marrow:

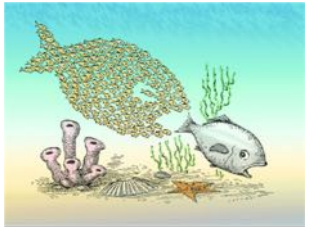
JAK2/Tyk heterodimers (by proximity ligation)
Proliferation
Apoptosis

Blood:

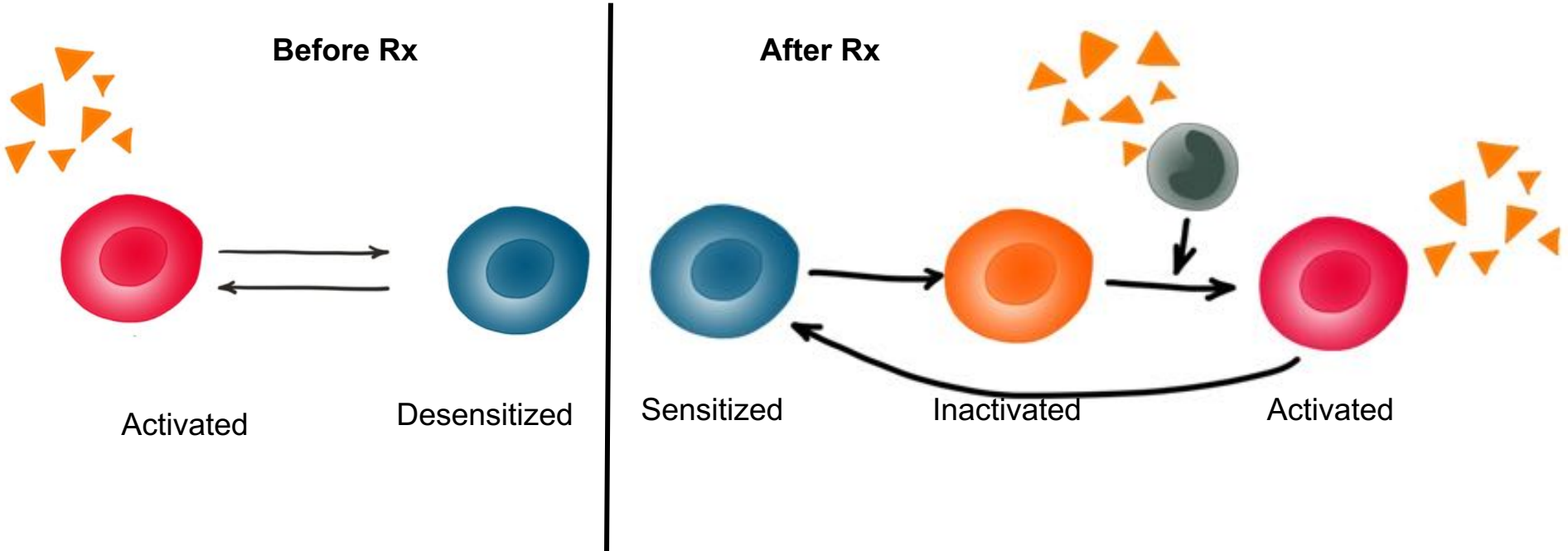
Cytokines

(a) Tumour foci in the metaphysis but not in other regions of long bone (b), tumour foci in epiphysis and metaphysis but not in mid-diaphysis (c). 5x magnification. Figure from Holstead Jones et al, Nature, 2006

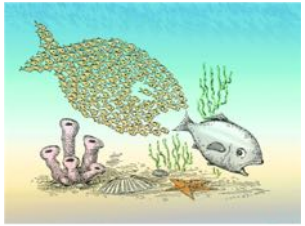
Alternative #2: Dark selection/emergence

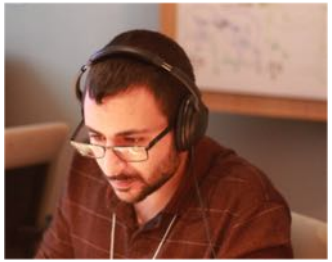


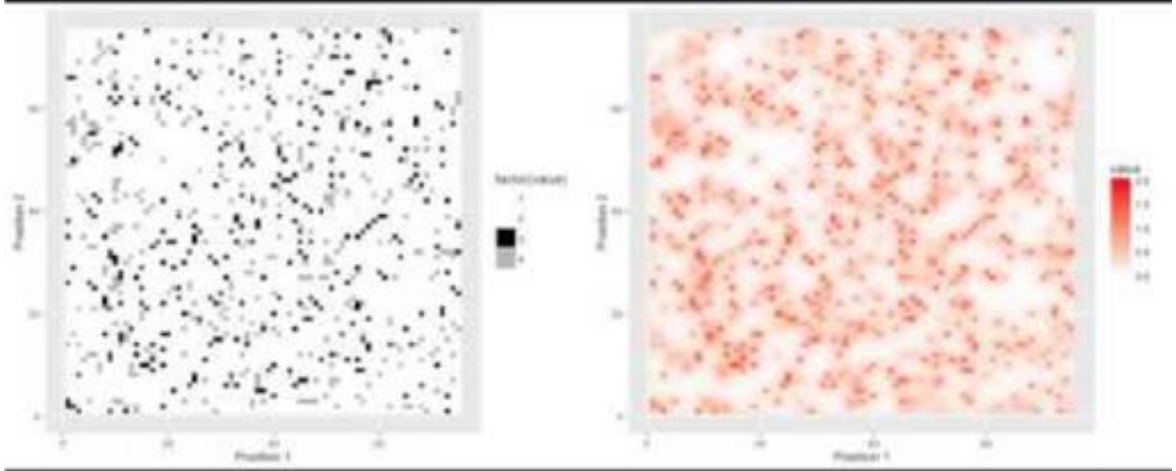
Emerging signaling network behavior

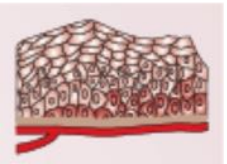


Alternative #2: Dark selection/emergence

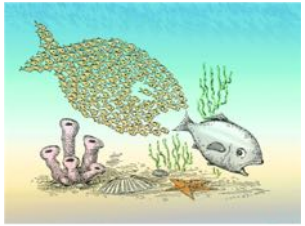


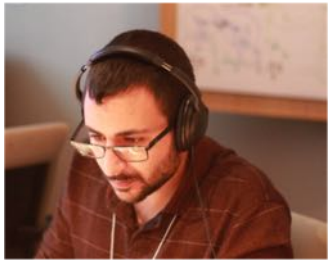


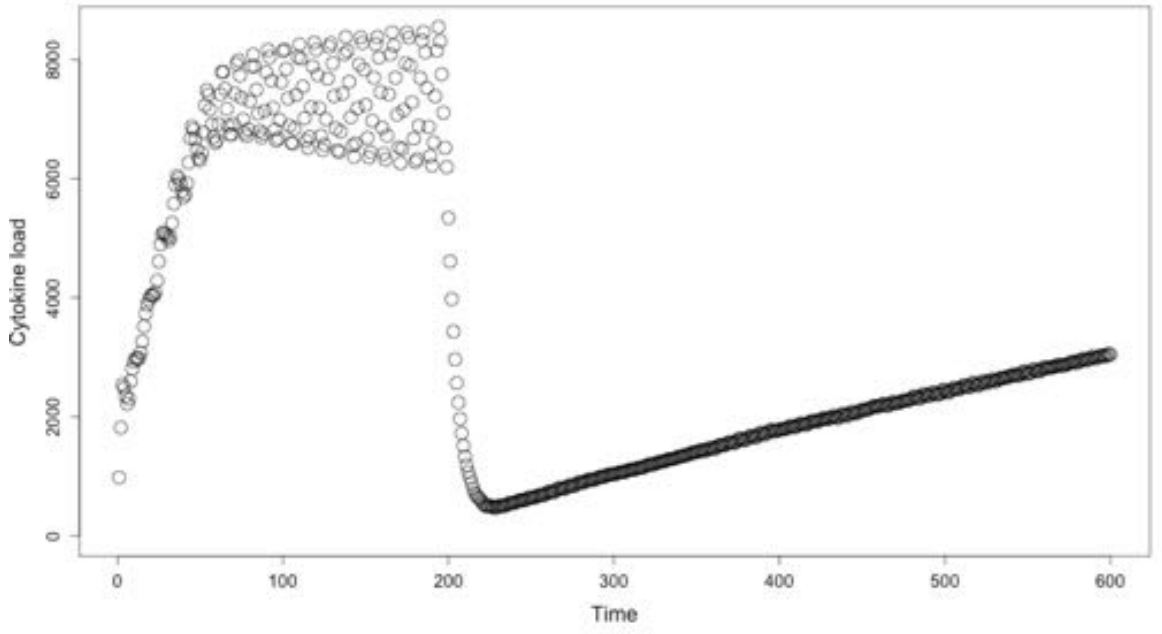


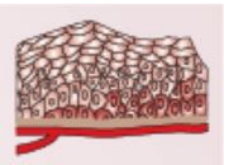


Alternative #2: Dark selection/emergence

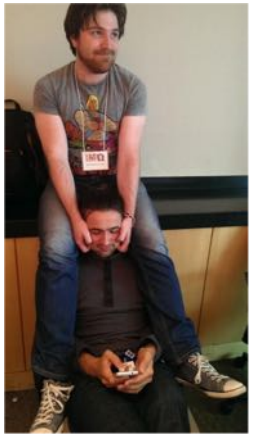


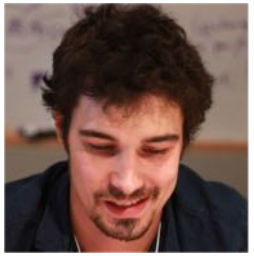


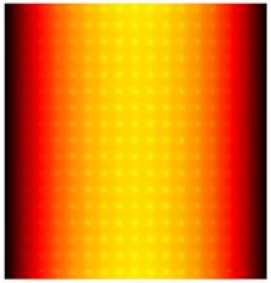


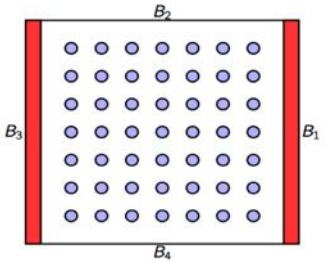


Integrating the continuous and CA models









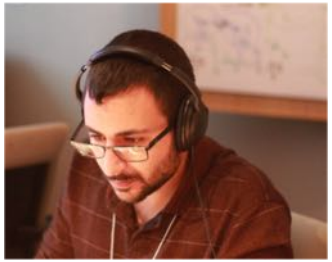
$$\frac{\partial C}{\partial t} - D\nabla^2 C = S(x, y, STAT3) - \delta C$$

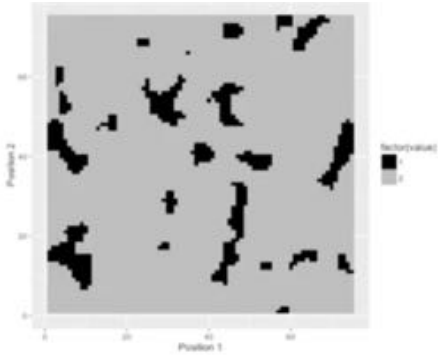
$$S(x, y, STAT3) = \Pi STAT3(x, y)\mathbb{1}_{Cells}(x, y)$$

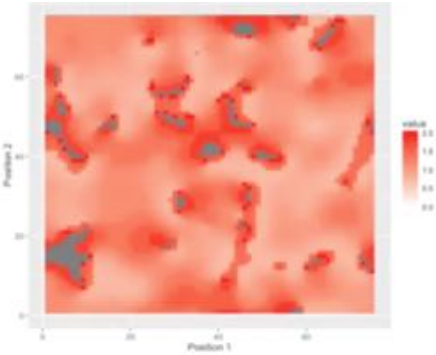
$$C = 0 \text{ on } B_2 \text{ and } B_4$$

$$C_{|B_3} = C_{|B_1}$$

+

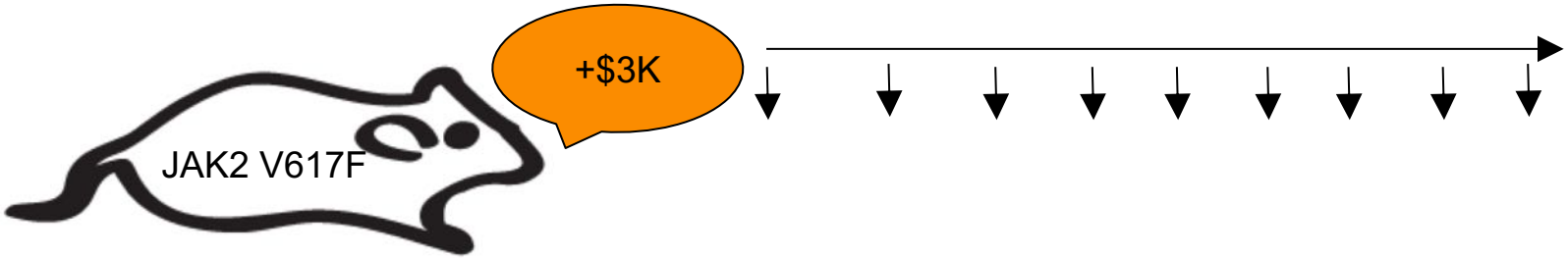




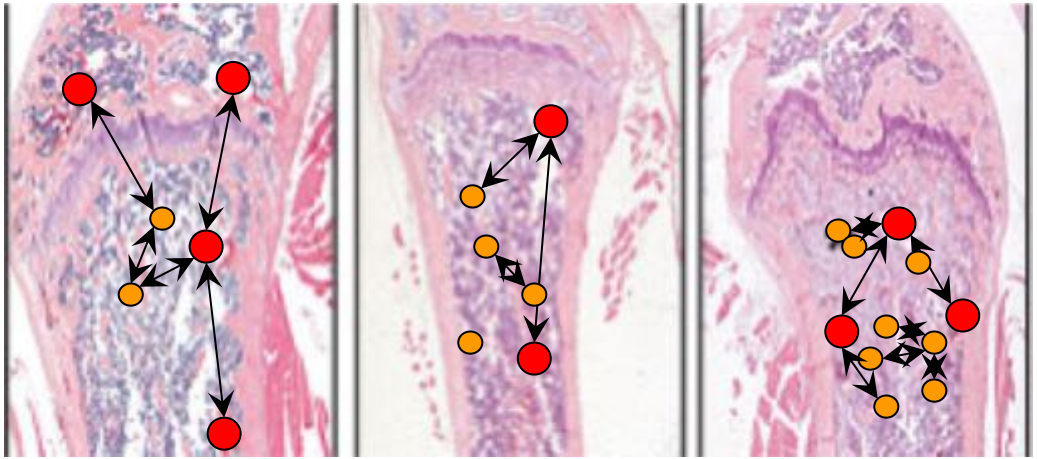


Hybrid Continuous-Discrete Cellular Automaton

Integrating experimental mouse data



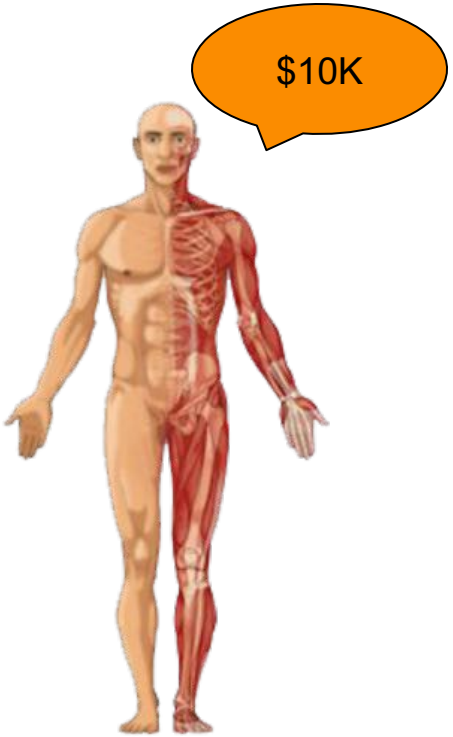
+/- Ruxolitinib



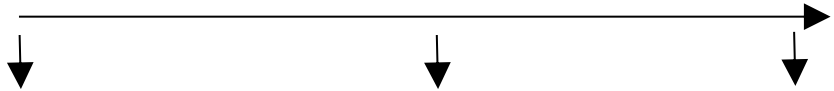
Analyze in the bone marrow:

Spatial analyses:
Blood vessels
STAT3 activation

Experiments to differentiate between the scenarios



x5 patients

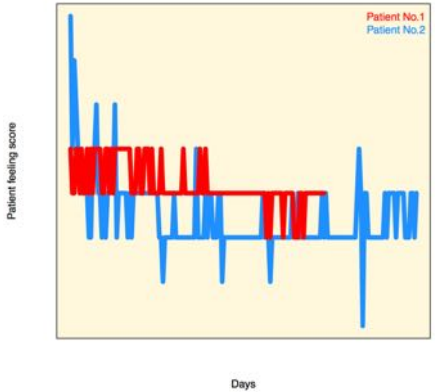


<u>Analyze in the bone marrow</u>

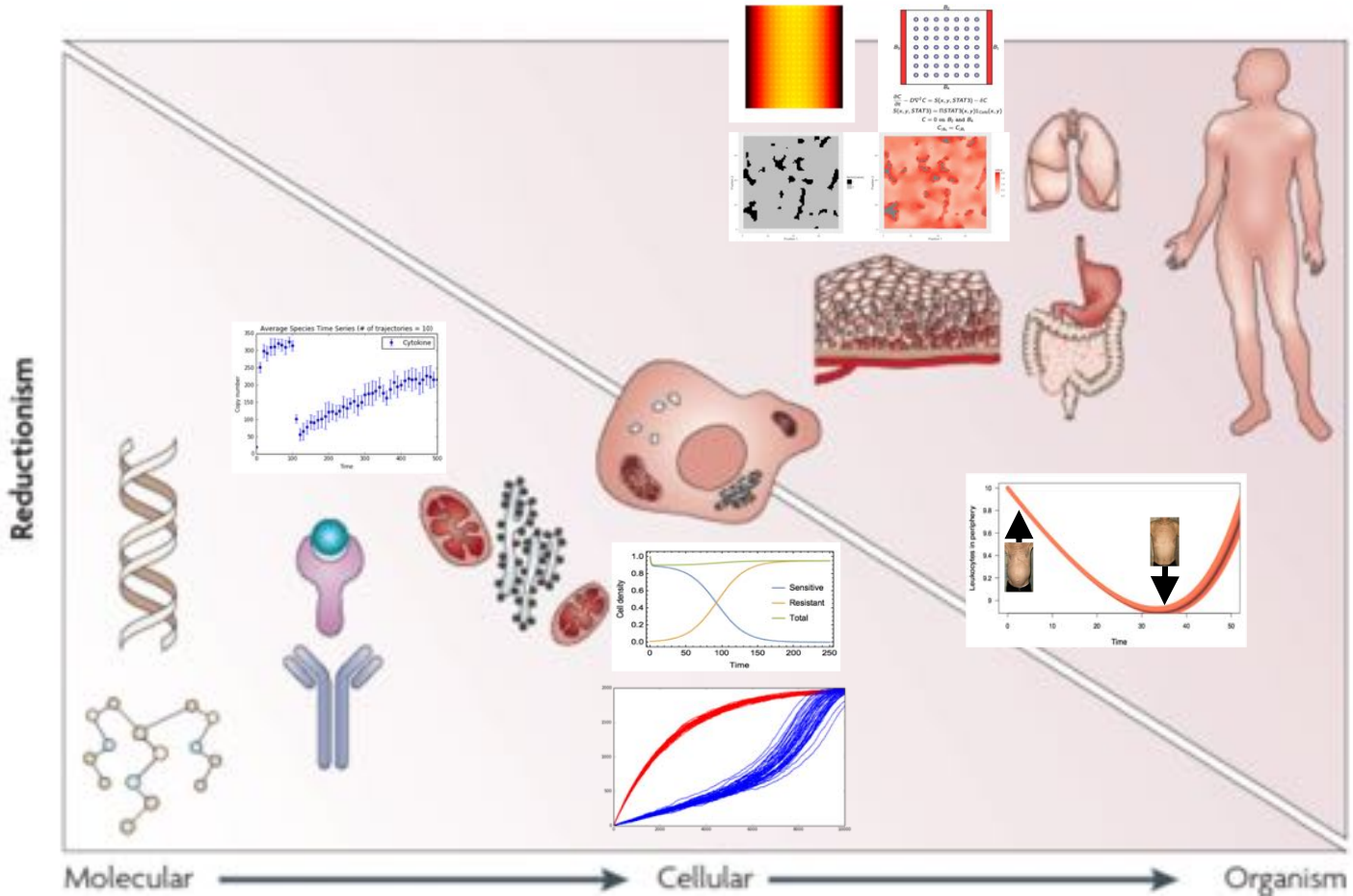
Before treatment
During remission
During response

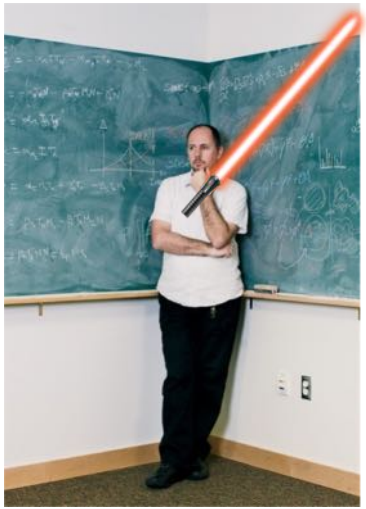
JAK2/TYK heterodimers
Proliferation
Apoptosis

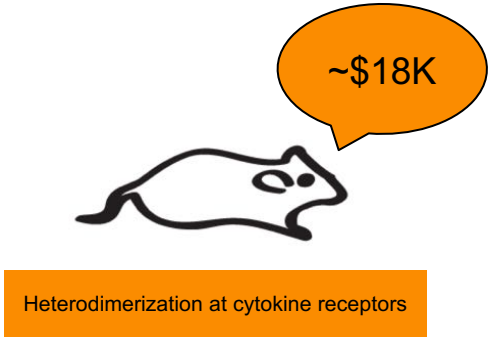
Blood (weekly):
Cytokines



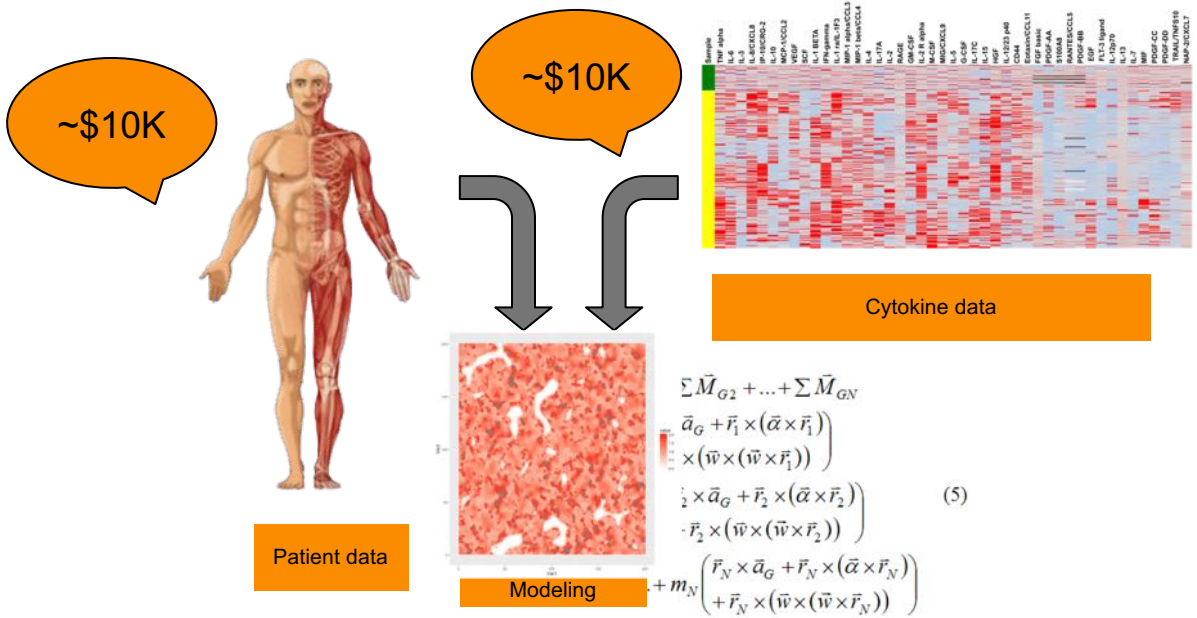
Scales of Cancer







SA1: To determine whether the emergence of ruxolitinib resistance is a Darwinian, Lamarckian, or non-cell autonomous process.



SA2: To determine the kinetics of cytokine expression and resultant symptomatology in patients treated with ruxolitinib..

To summarize

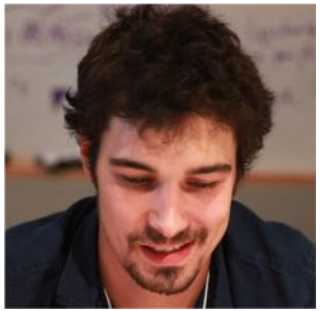
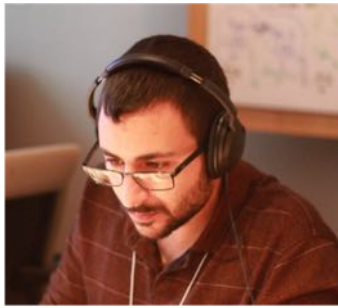
Suite of novel integrated mathematical models test different evolutionary hypotheses and provide experimentally-testable insights

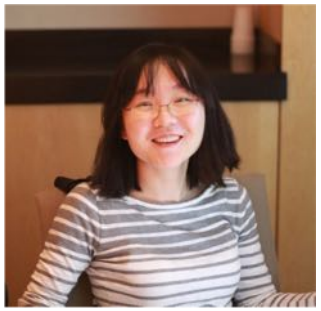
Qualitative cytokine dynamics observed in the clinic can be captured in a simulation that models emergence of resistance

Critically, the resistance paradox may depend on the tumor microenvironment, hidden selection, and network selection



ORANGE

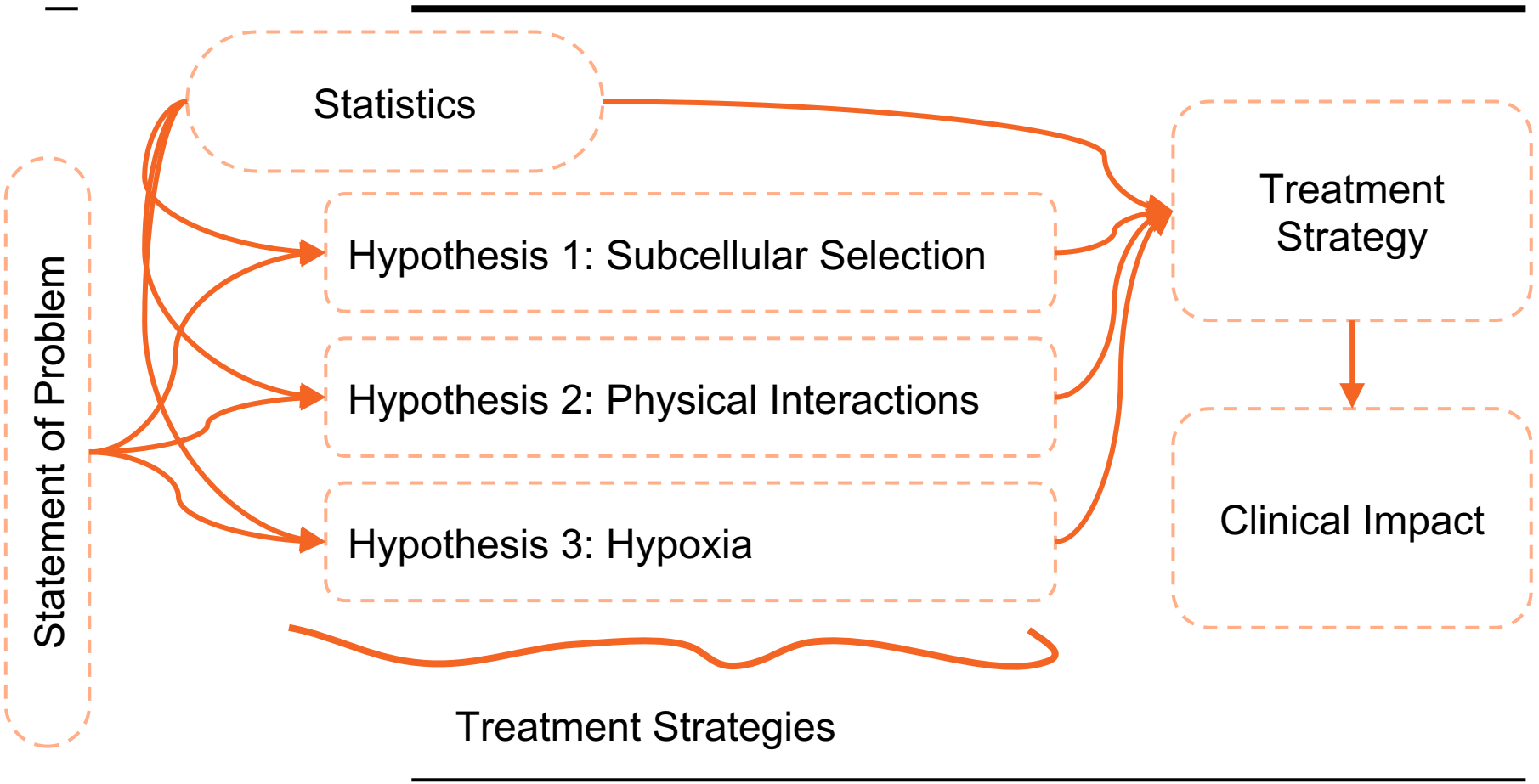








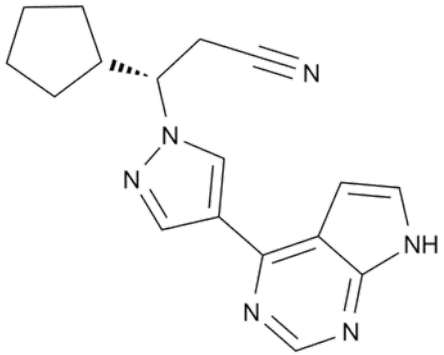

Thanks


Questions?


Statistics
Statement of Problem
Hypothesis 1: Subcellular Selection
Hypothesis 2: Physical Interactions
Hypothesis 3: Hypoxia
Treatment Strategies
Treatment Strategy
Clinical Impact

Ruxolitinib for CMML

Ruxolitinib

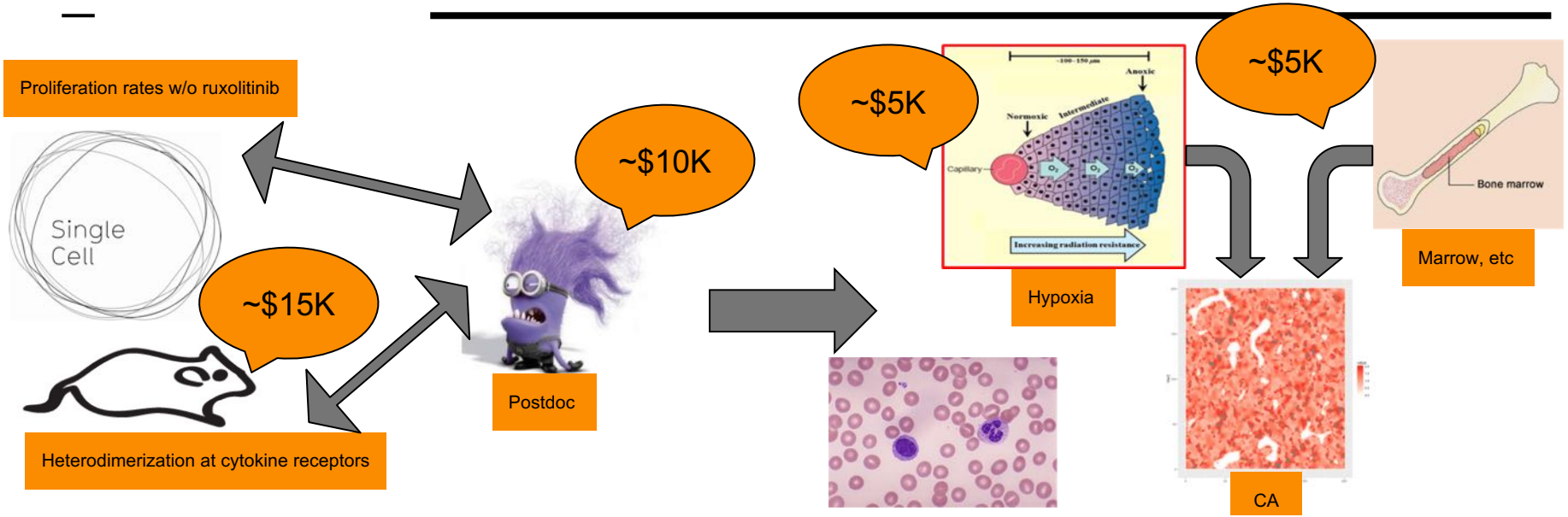


Potent JAK1/2 inhibitor FDA approved for the treatment of primary myelofibrosis

In myelofibrosis, improvements in symptoms, spleen size, and cytokine levels are seen in the majority of patients.

Even in responding patients and mouse models, *no changes in tumor burden or mutational frequencies* are seen while on therapy.

Within months on therapy, symptoms return, spleen enlarges, and disease progresses.



Importance: Our approach addresses an important and unresolved evolutionary question that is applicable to CMML but also to a wide range of cancers.
Originality: Suite of novel mathematical tools to explore a potentially novel mechanism of treatment resistance.
Feasibility: Padron and Marusyk's lab and the young talent at #teamOrange

CMML: therapy does not visibly impact relevant population