Mathematical modeling of heterogeneity and clonal selection in acute leukemias

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April 28, 2017

Interdisciplinary collaboration

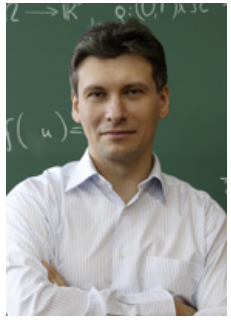
Maintenance and Differentiation of Stem Cells in Development and Disease

SFB 873

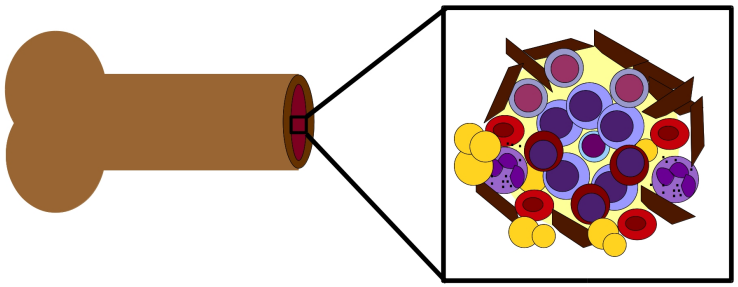
- Collaborative Research Center (SFB) "Maintenance and Differentiation of Stem Cells in Development and Disease"
- Collaboration with Anthony Ho, Natalia Baran and Christoph Lutz (Department of Medicine V, Heidelberg Univ.)
- Multicompartment models of hematopoiesis and leukemia: with Thomas Stiehl (IWR/IAM, Heidelberg Univ.)
- Models of fitness selection: with Piotr Gwiazda (University of Warsaw)



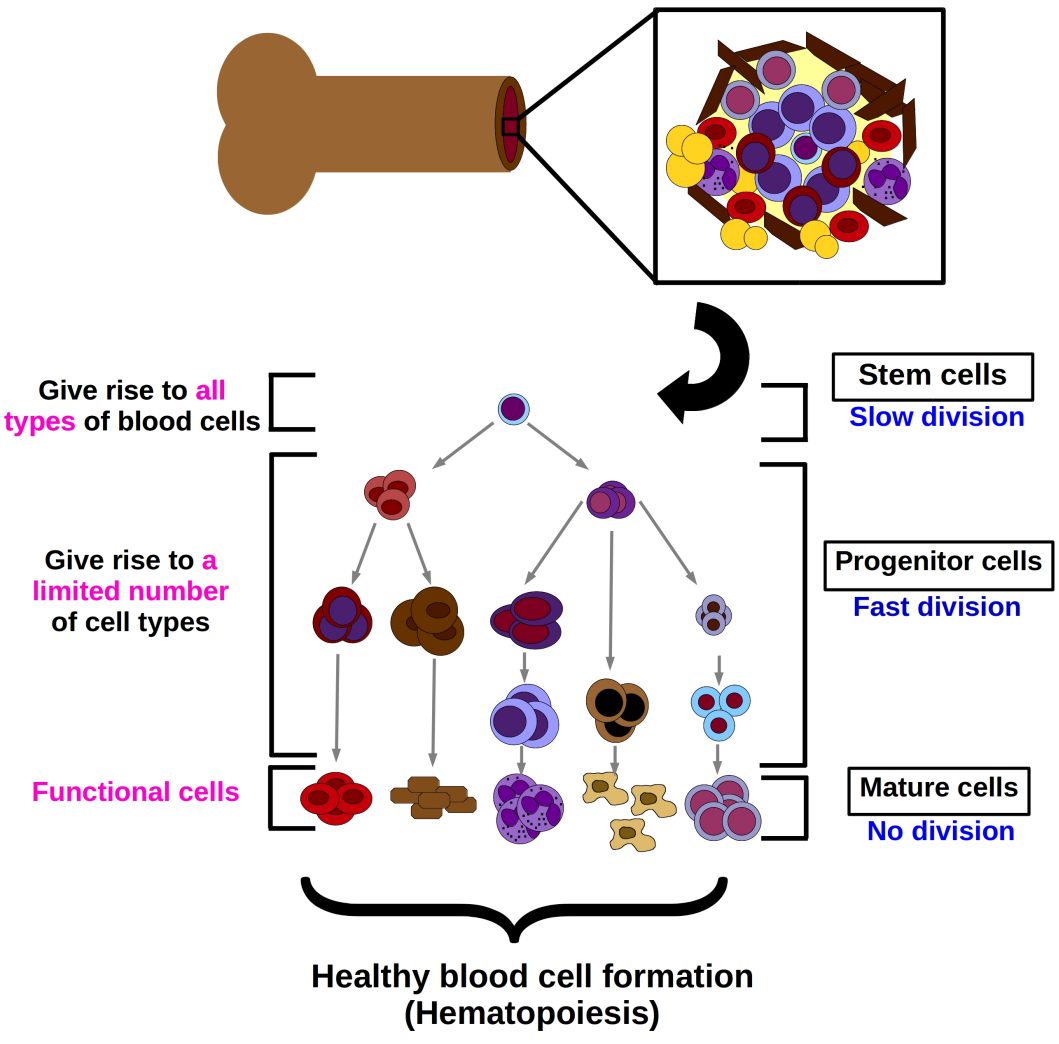




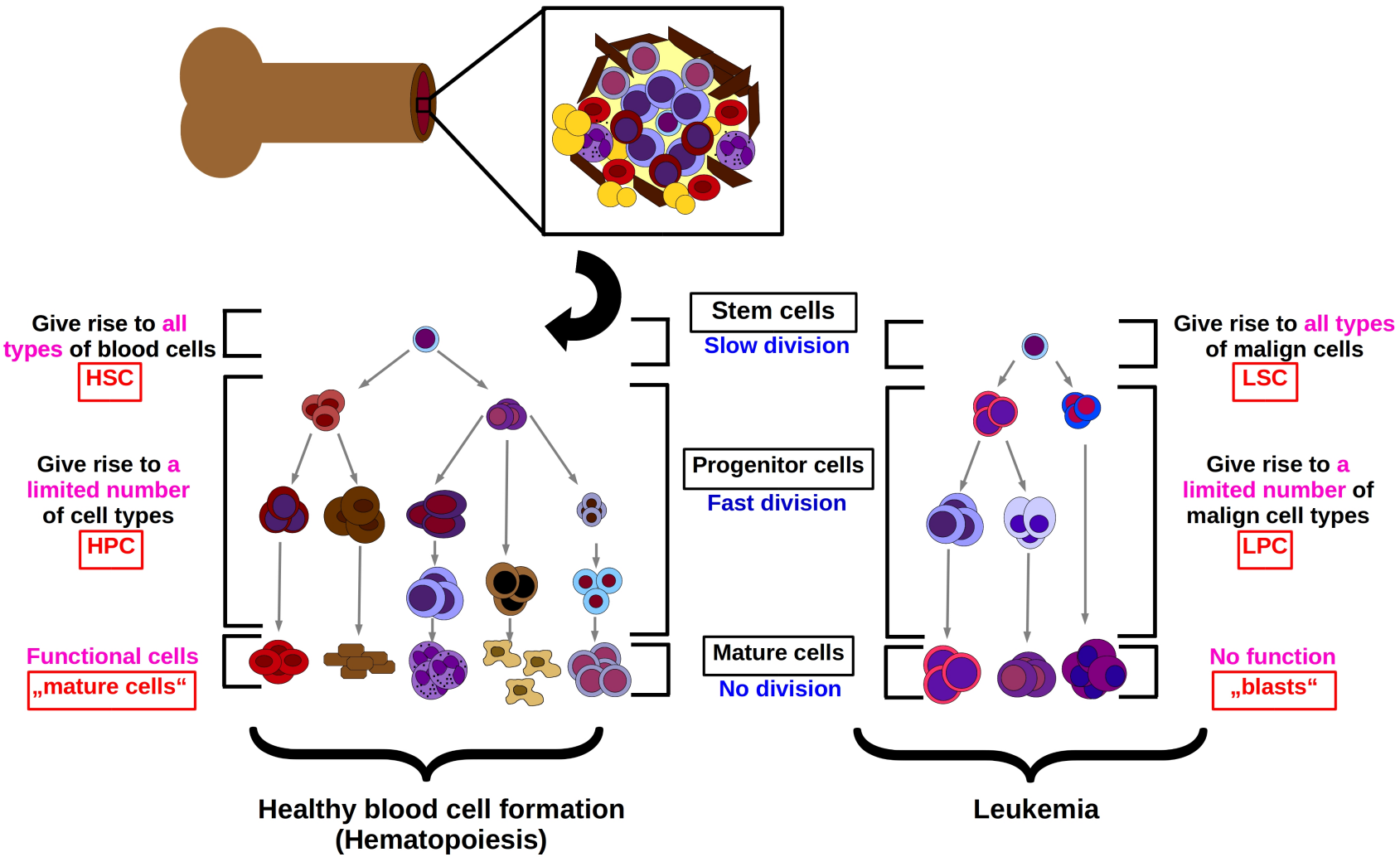
Hematopoiesis



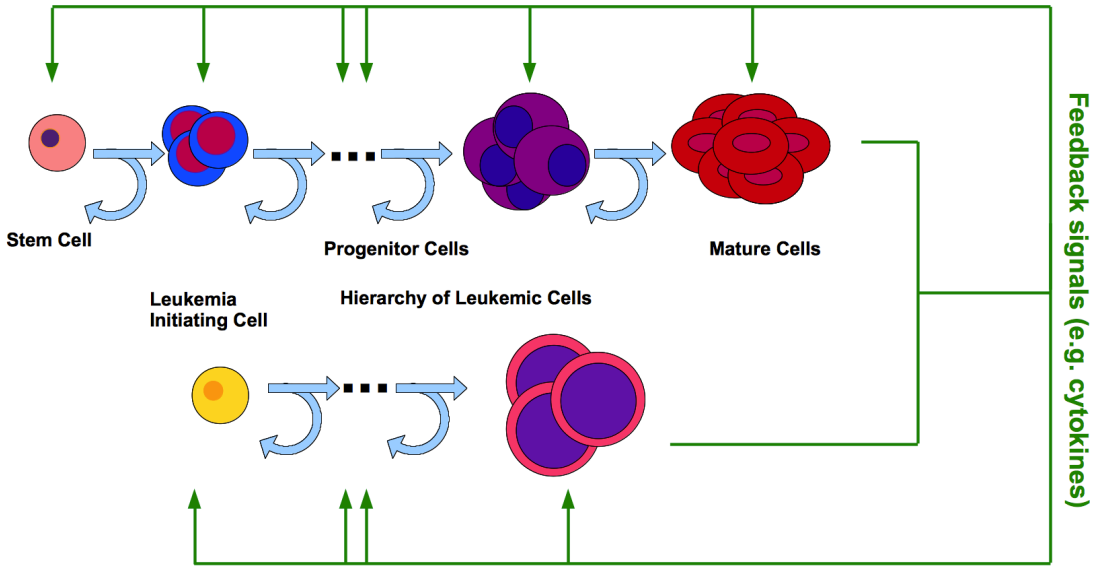
Hematopoiesis



Hematopoiesis and Leukemia



Model of leukemia



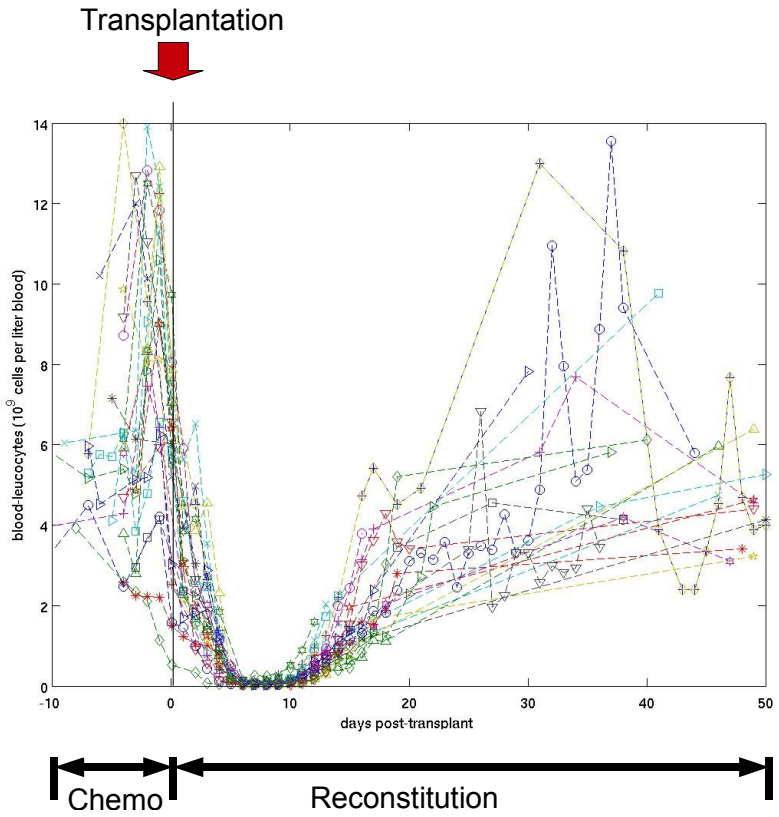
Model ingredients

- Transitions between different differentiation stages
- Regulation of the self-renewal vs. differentiation process
- Clonal heterogeneity of cancer
- Mutations?

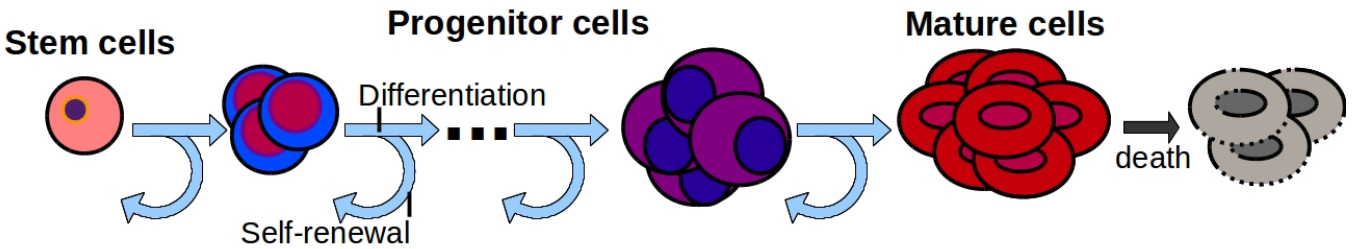
Model of the healthy cell line

Patients data

- Stress conditions (chemotherapy)
- Bone marrow transplantation (CD34+ cells)
- Blood regeneration



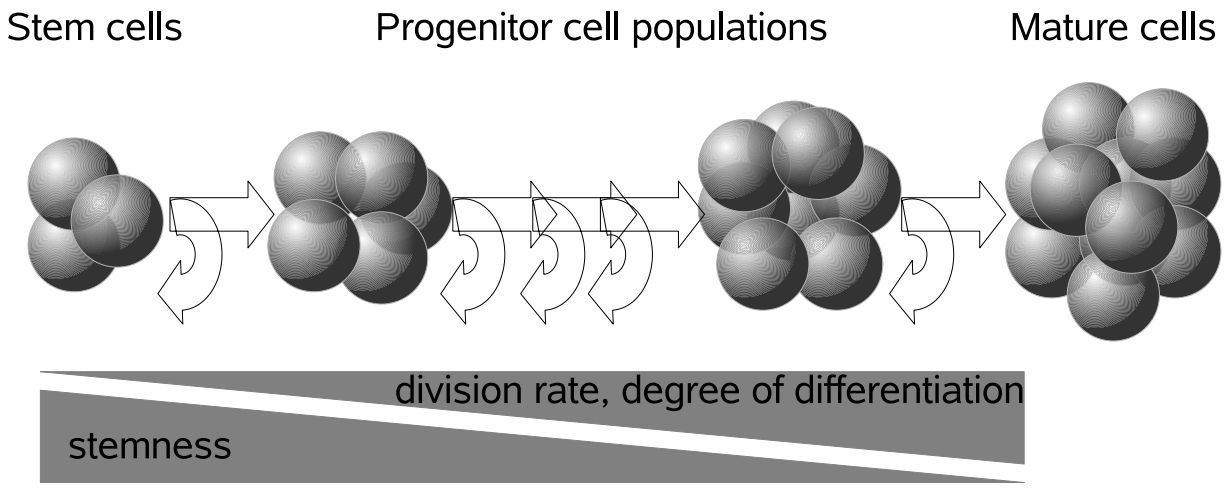
Model - Hematopoiesis



Key parameters

- Proliferation rates p_i
- Fractions of self-renewal a_i
- Death rates d_i

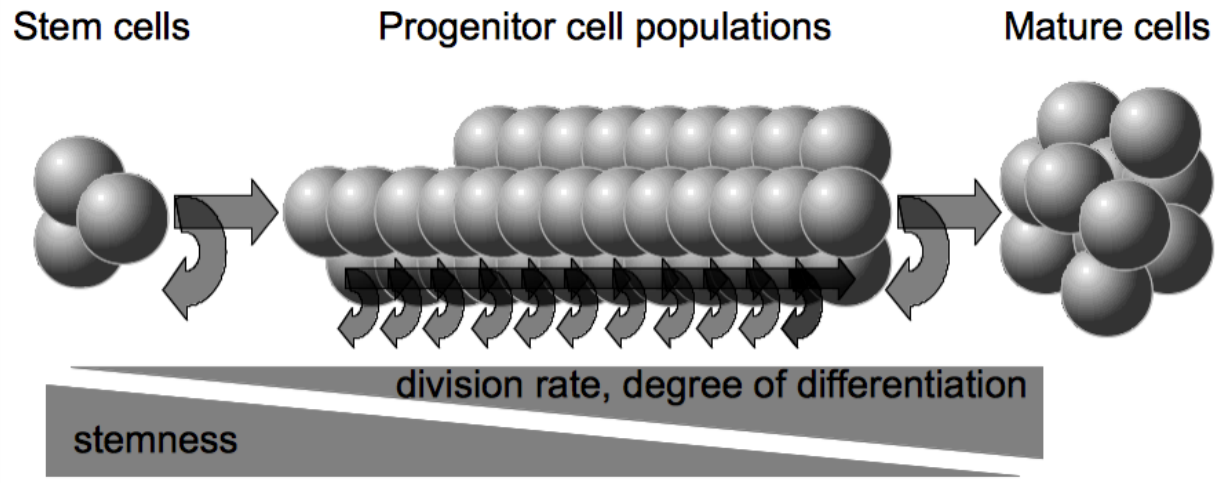
Cell differentiation model



$$
\begin{aligned}
\frac{du_1}{dt} &= (2a_1 - 1)p_1 u_1, \\
\frac{du_i}{dt} &= (2a_i - 1)p_i u_i + 2(1 - a_{i-1})p_{i-1} u_{i-1}, \\
\frac{du_n}{dt} &= 2(1 - a_{n-1})p_{n-1} u_{n-1} - d_n u_n.
\end{aligned}
$$

M-C, Stiehl, Jäger, Ho, Wagner, SC Dev 18, 2009

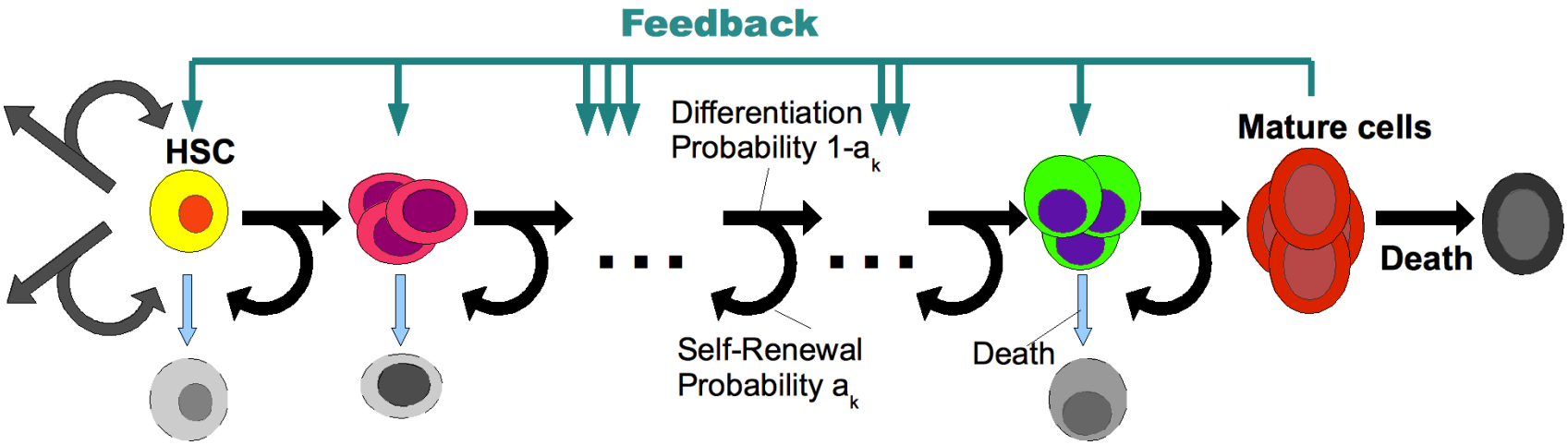
Structured population model: continuous structure



$$\partial_t u(x,t) + \partial_x [g(x, v(t)) u(x,t)] \quad = \quad p(x) u(x,t)$$

Doumic, M-C, Perthame, Zubelli, SIAM J.Appl.Math., 2011

Model of the feedback



Dynamics of signalling molecules (cytokines; G-CSF)

$$\frac{dS(t)}{dt} = \alpha - \mu S(t) - \beta u_n(t) S(t)$$

Quasi steady state approximation (Tikhonov Theorem)

$$s(t) = \frac{1}{1 + k u_n(t)} \in [0, 1],$$

where $s(t) := \frac{\mu}{\alpha} S(t)$ and $k := \frac{\beta}{\mu}$.

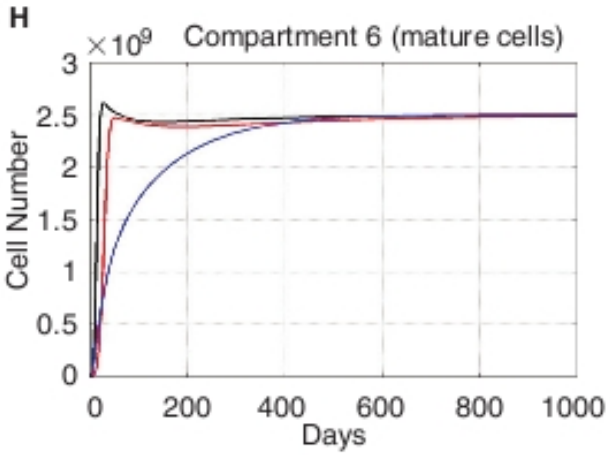
Assumptions on cytokines

Regulation modes

- All regulated cell properties depend linearly on the cytokine concentration

1. Regulation of proliferation: $p_i(s(t)) := p_i s(t) = \frac{p_{i,max}}{1+k u_n(t)}$
2. Regulation of self renewal versus differentiation
   $a_i(s(t)) := a_i s(t) = \frac{a_{i,max}}{1+k u_n(t)}$

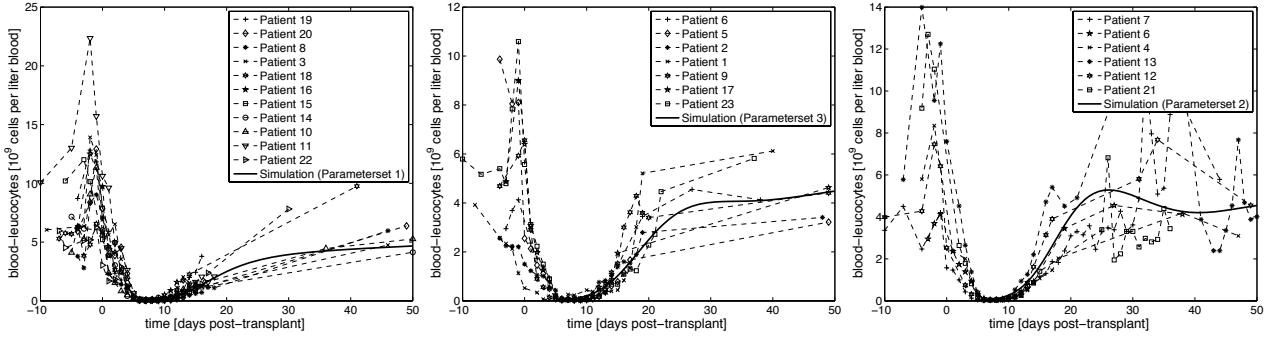
Application to hematopoietic reconstitution



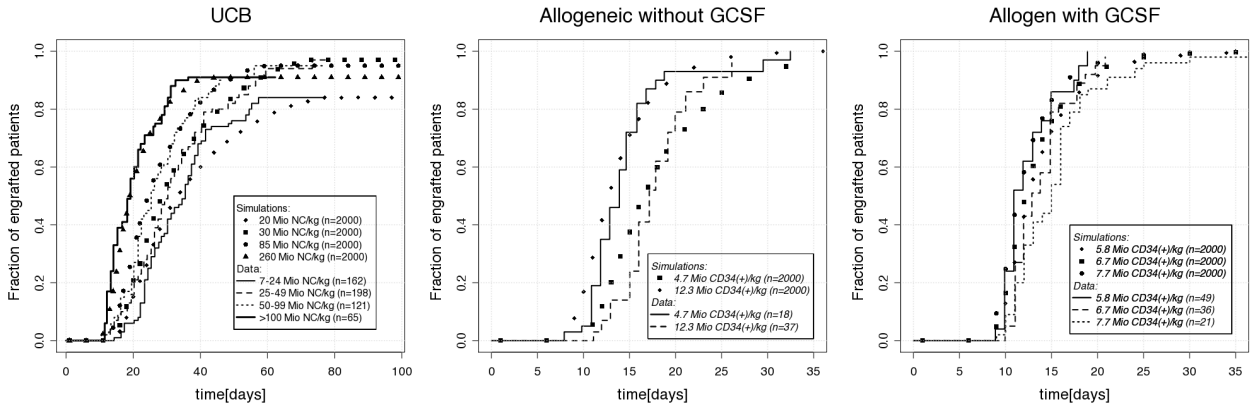
- Regulation of self-renewal fractions is the most effective mechanism of hematopoietic reconstitution

Model validation: Comparison to patients data

- Individual patients



- Large patient groups



Stiehl, Ho, M-C, Bone Marrow Transplantation 49, 2014

Dynamics of the model

- Trivial steady state - unstable (unless it is the only equilibrium)
- Semi-trivial steady state: $(0, ..0, \bar{u}_k, .., \bar{u}_n)$ - linearly unstable iff there exists a steady state with more positive components
- Unique positive steady state: $(\bar{u}_1, .., \bar{u}_n)$ - globally stable ?
  - Global stability for the 2-compartment model

$$L(u_1(t), u_2(t)) := \frac{1}{p_1 G(\bar{u}_2)} L_{21}(t, u_1(t), u_2(t)) + \frac{1}{d_2} L_{22}(t, u_1(t), u_2(t))$$

with $G(\xi) = 2(1 - a_1/(1 + k u_2))$ and

$$L_{21}(t, u_1, u_2) := \frac{u_1}{\bar{u}_1} - 1 - \ln \frac{u_1}{\bar{u}_1},$$

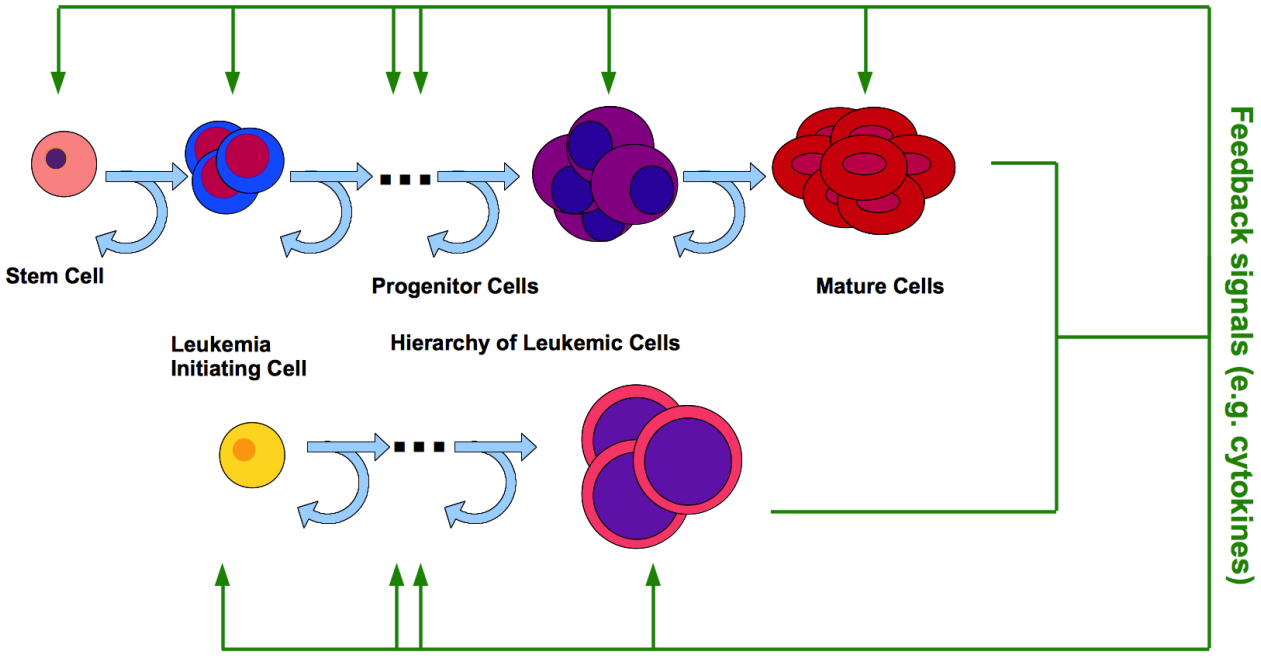
$$L_{22}(t, u_1, u_2) := \frac{u_2}{\bar{u}_2} - 1 - \frac{1}{\bar{u}_2} \int_{\bar{u}_2}^{u_2} \frac{G(\bar{u}_2)}{G(\xi)} d\xi.$$

- Hopf bifuraction and oscillations in the 3-compartment model and in the structured population model.

Stiehl and Marciniak-Czochra, Math. Comp. Models., 2010
Nakata, Getto, M-C and Alarcon, J. Biol. Dynamics, 2012
Getto, M-C, Nakata and dM Vivanco, Math. Biosciences, 2013

Model of leukemia development

Model of leukemia

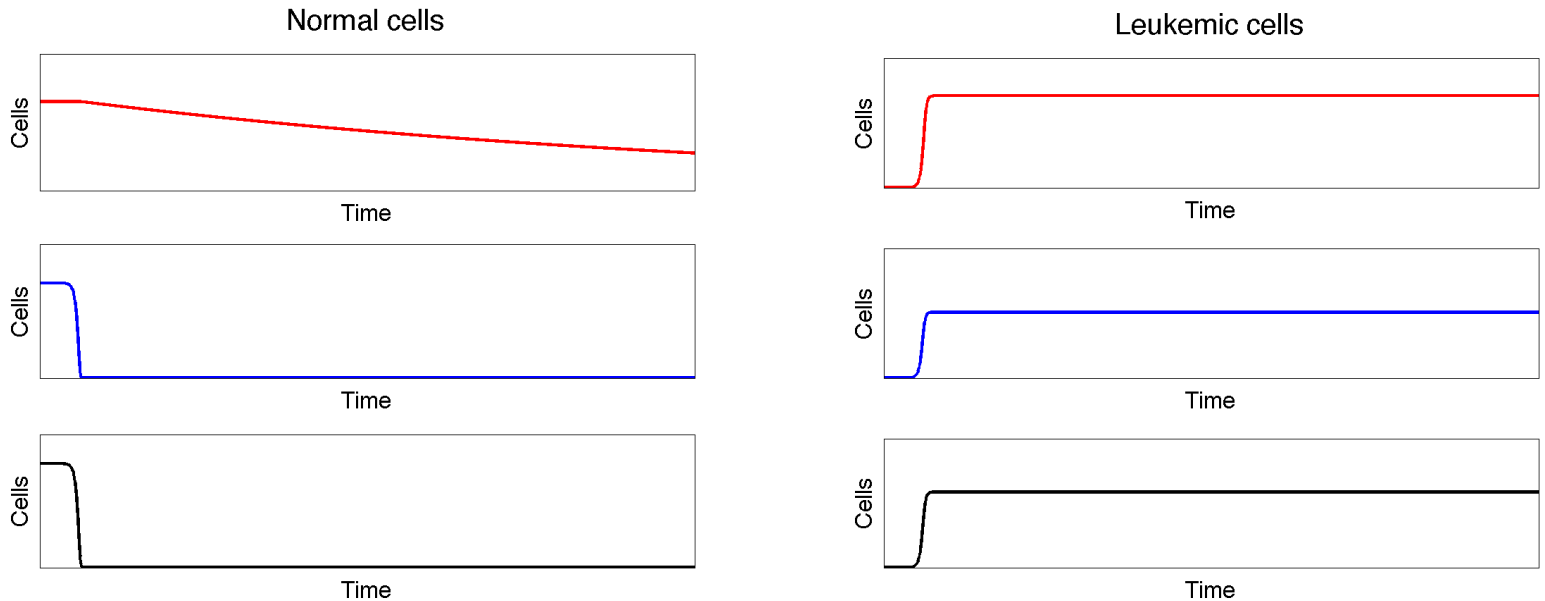


- Cells compete for spatial (bone marrow niches) or environmental resources (cytokines).
- Leukemic cells have better fitness (larger self-renewal and/or larger proliferation...)

Stiehl and Marciniak-Czochra, Math. Mod. Nat. Phenomena, 2012

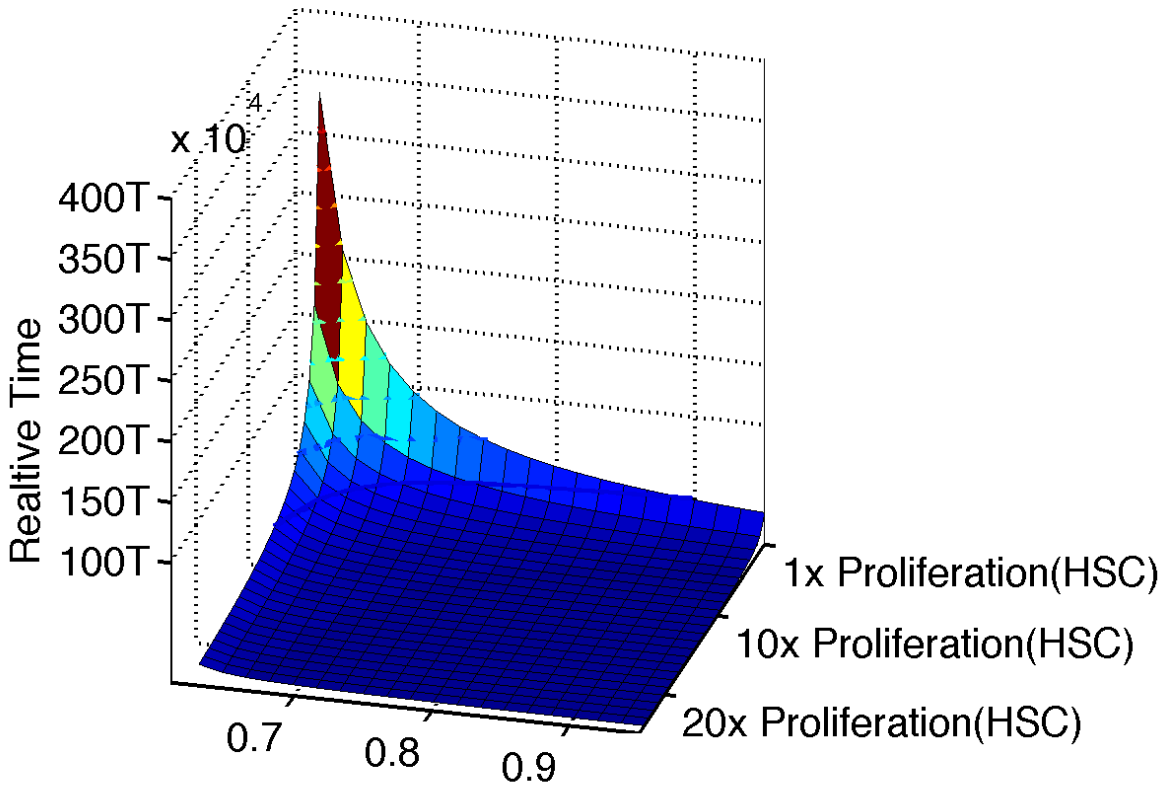
Development of leukemia

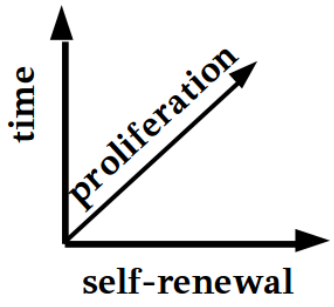
- We start in hematopoietic equilibrium with a small number of leukemic stem cells (LSC)
- We measure how long it takes until mature hematopoietic cell counts are reduced by a certain percentage.
- Theorem: Larger self-renewal of LSC always leads to development of leukemia.



Impact of LSC Properties

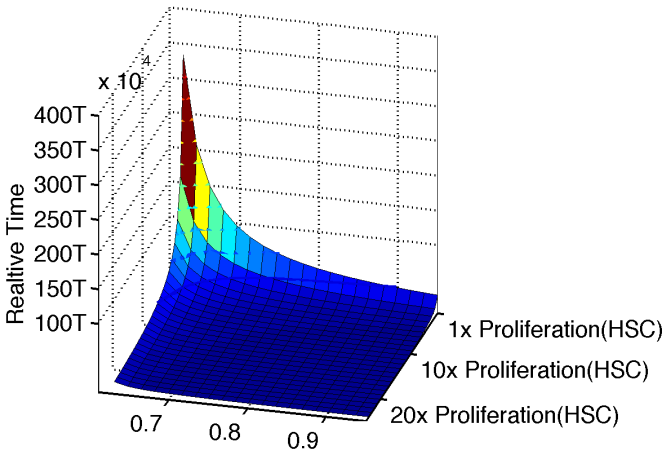
Time needed for reduction of mature blood cells by 20%



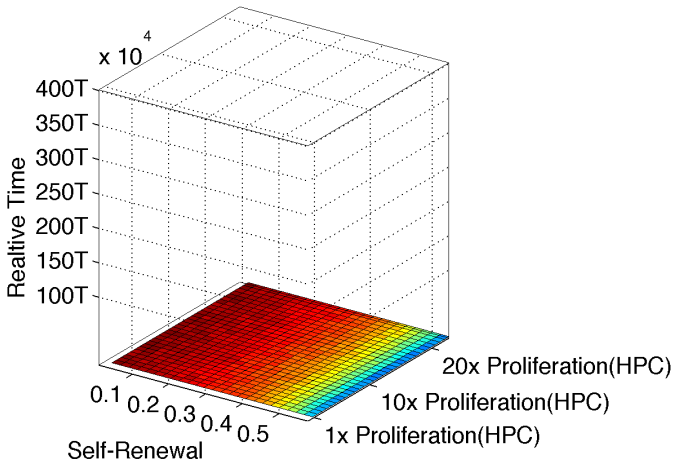


Impact of LSC and non-LSC Properties

Different LSC Properties
others fixed

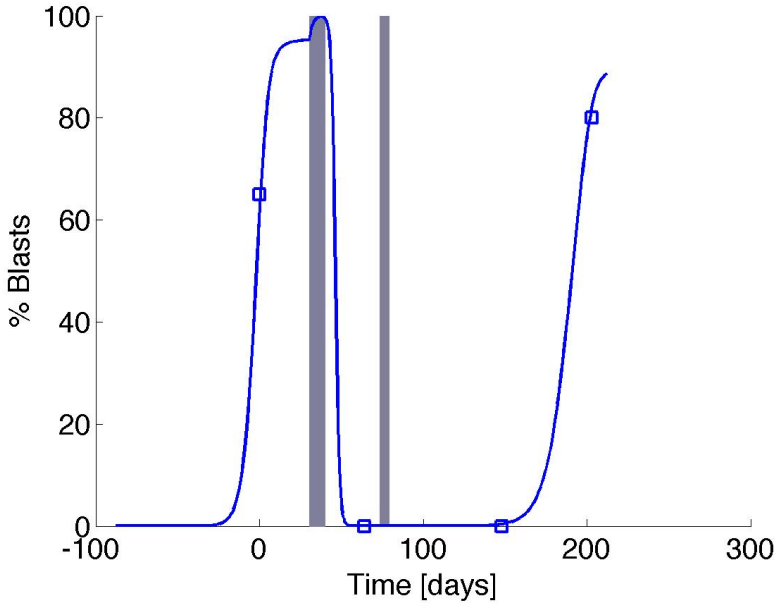


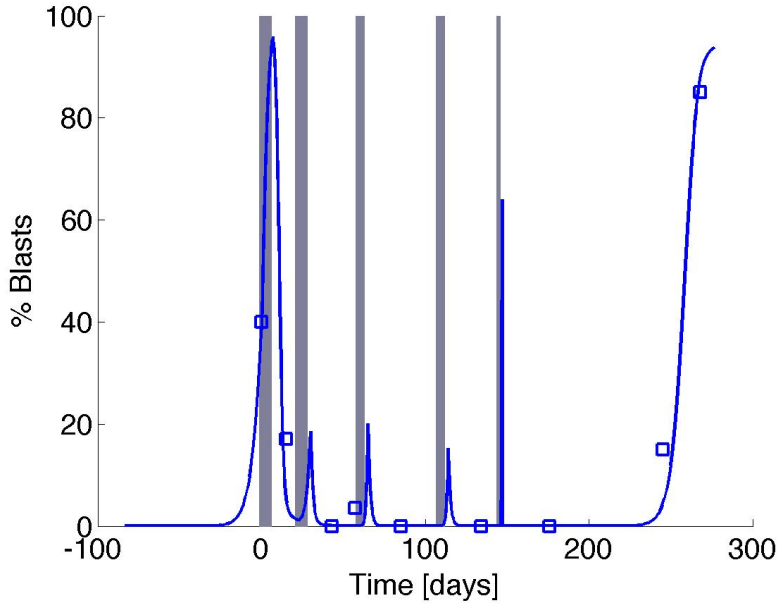
Different non-LSC Properties
LSC fixed

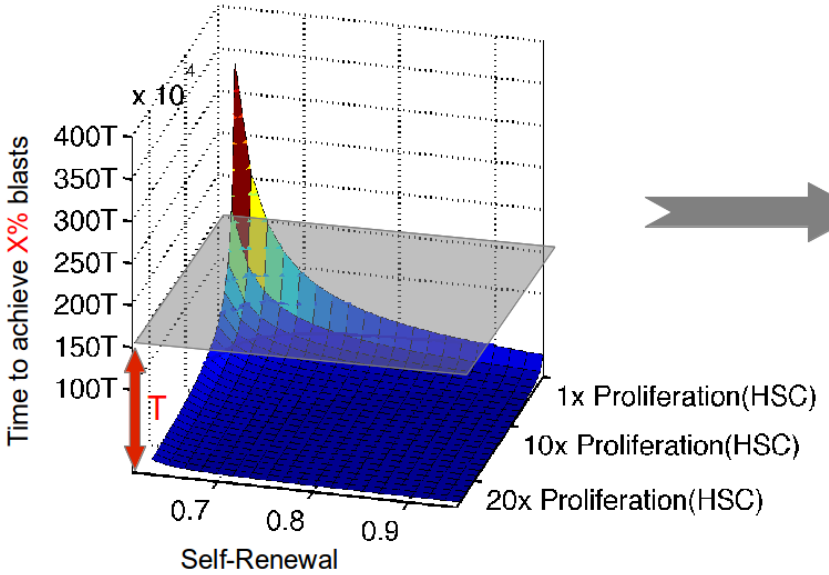


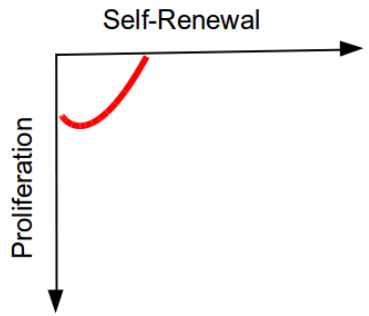
Dynamics does not depend on non-LSC properties.

Estimation of LSC properties using patients data





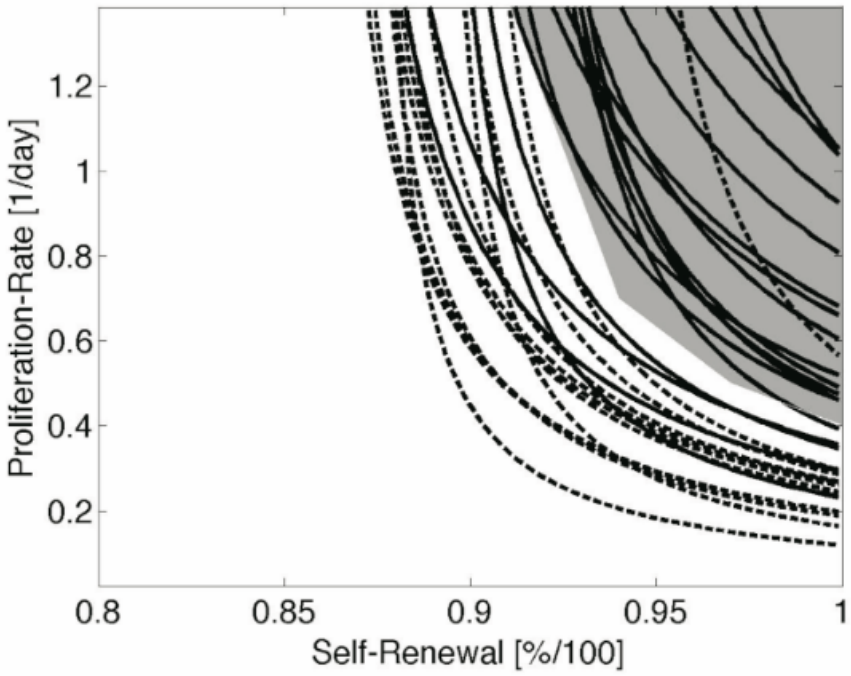


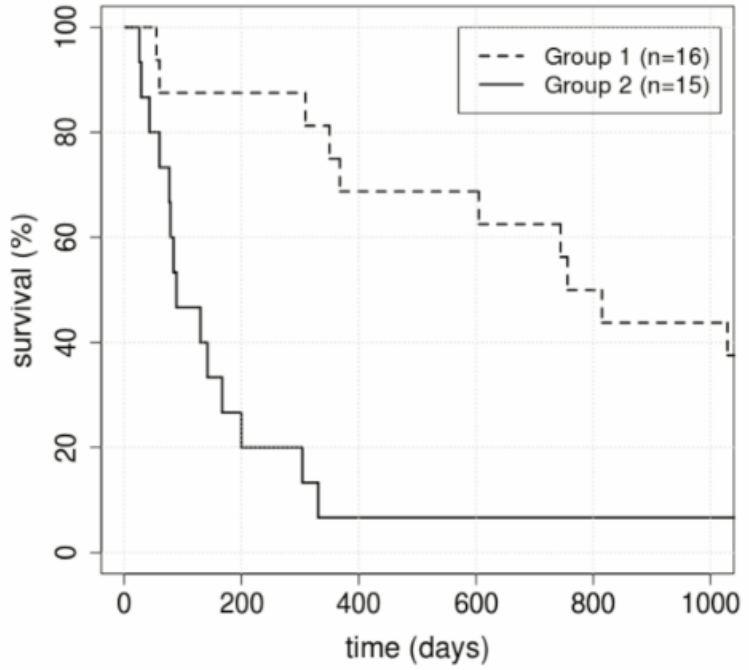


Cell properties fitting to an individual patient

Estimated LSC properties and prognosis

Estimated cell properties correlate with patient survival.

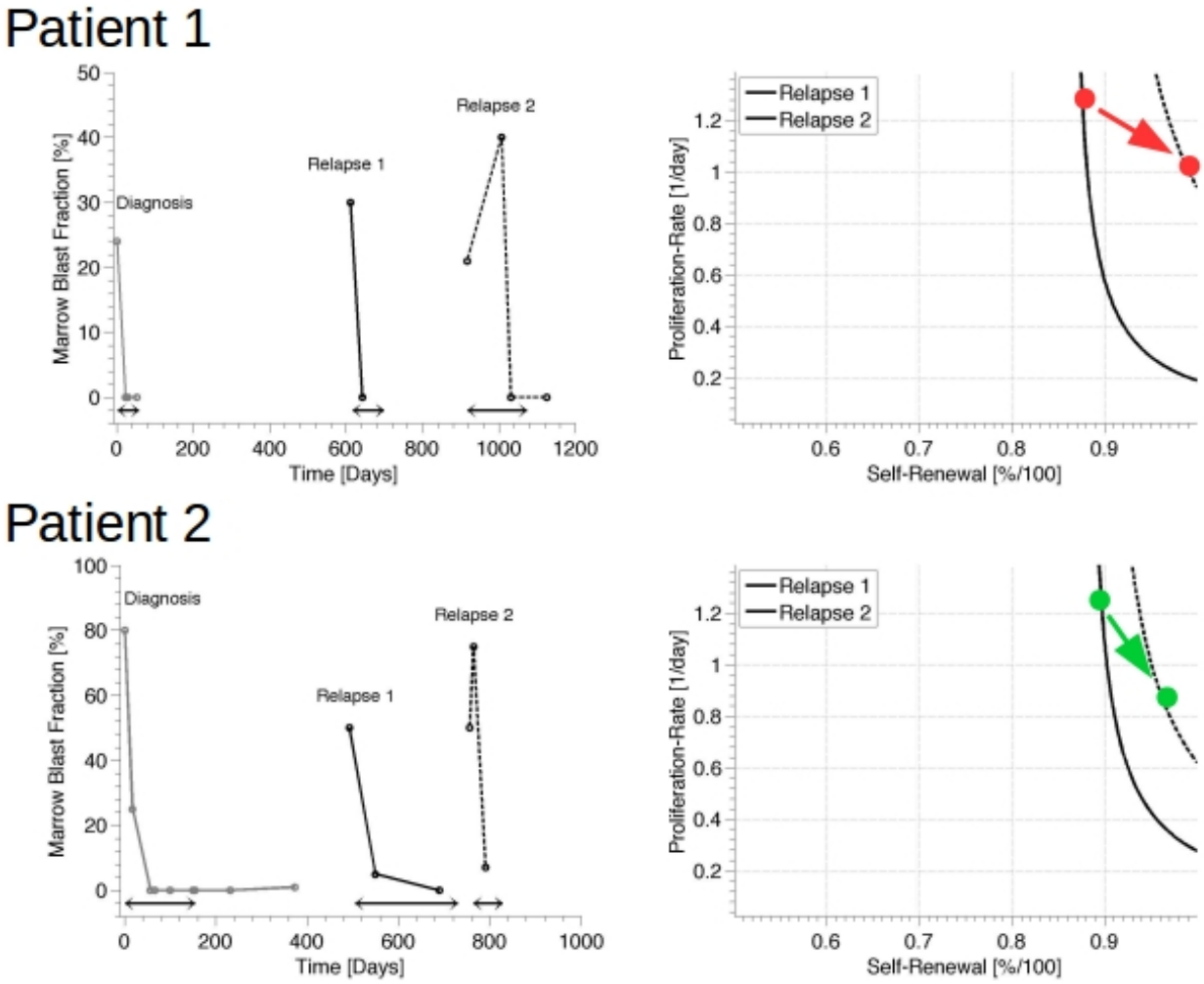




Stiehl, Baran, Ho, M-C, Cancer Research 2015

Development of resistance

LSC properties change between multiple relapses

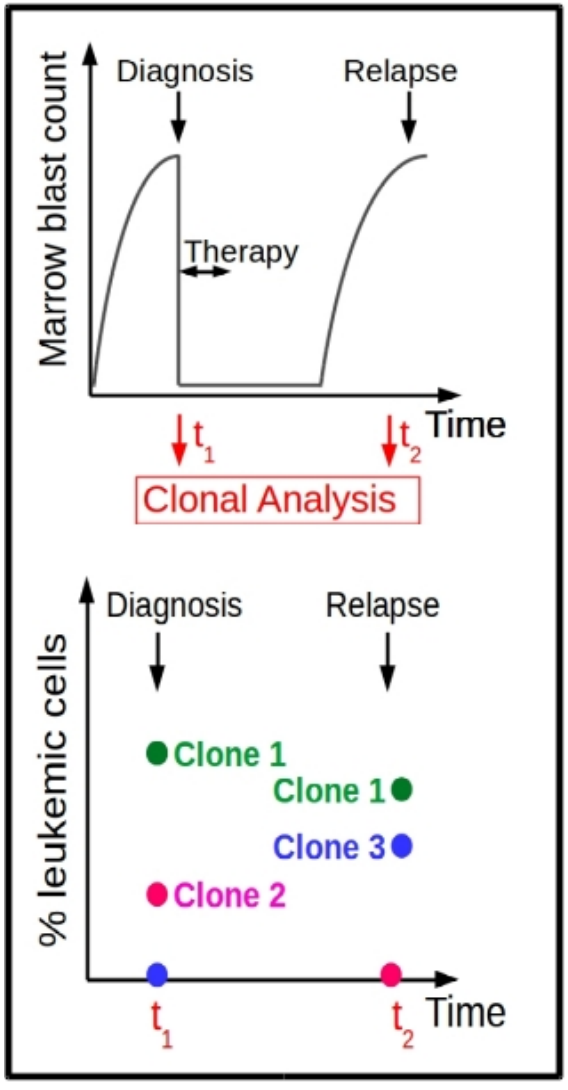


Models of heterogenous (multiclonal) AML

Clonal evolution (AML and ALL)

Recent Experimental Findings

- Deep sequencing techniques allow to study the clonality and clonal evolution patterns in leukemias (Ding et al, Nature 2012 and Anderson et al Nature 2011)
- Primary manifestation as well as relapses involve only few clones
- 2 major evolution patterns have been defined:
  1. Repeating clones
  2. Related but different subclones.



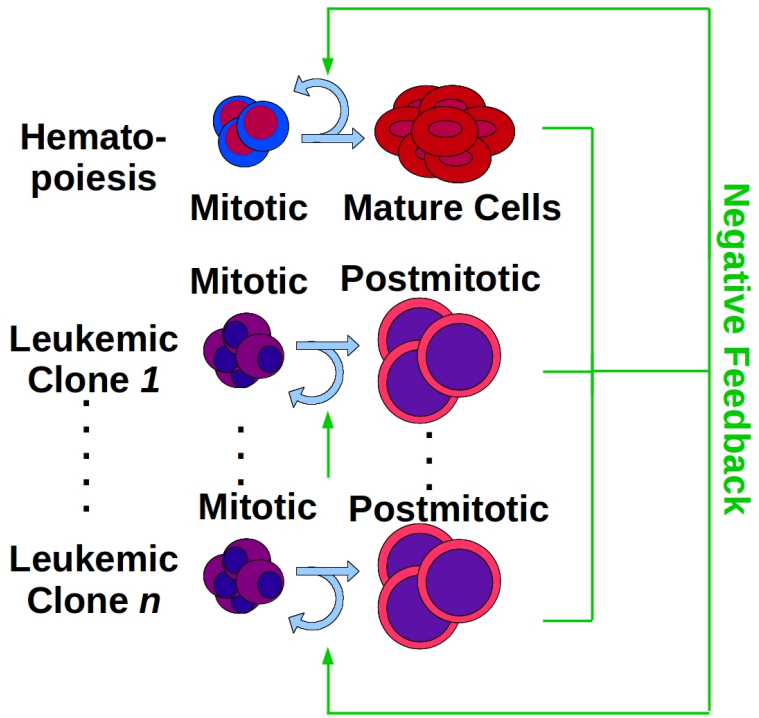
Multiclonality

Observation:

- Leukemic cell mass consists of multiple clones
- Size of different clones varies over time

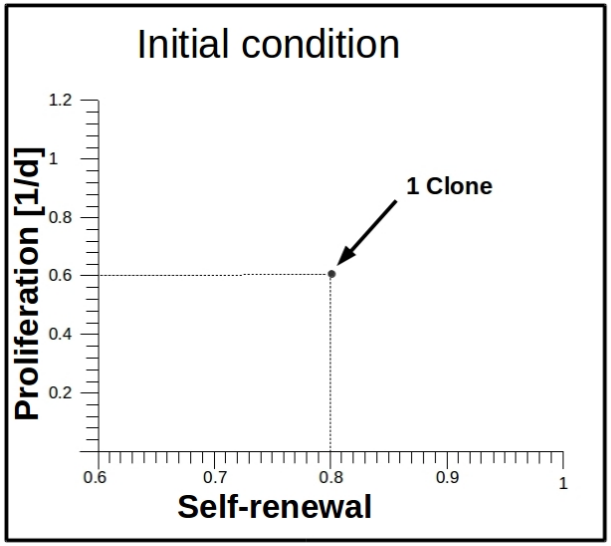
Model:

- 1 healthy cell line
- n leukemic clones
- **Simplification:**
  2 compartments

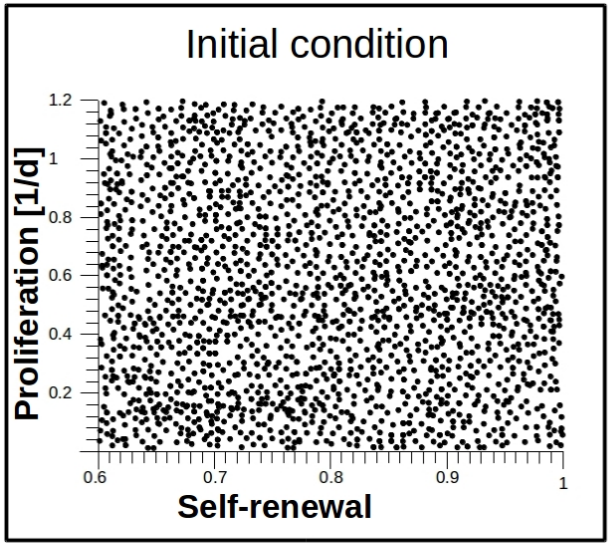


Stiehl, Baran, Ho, M-C, JRS Interface 11, 2014

Clonal selection

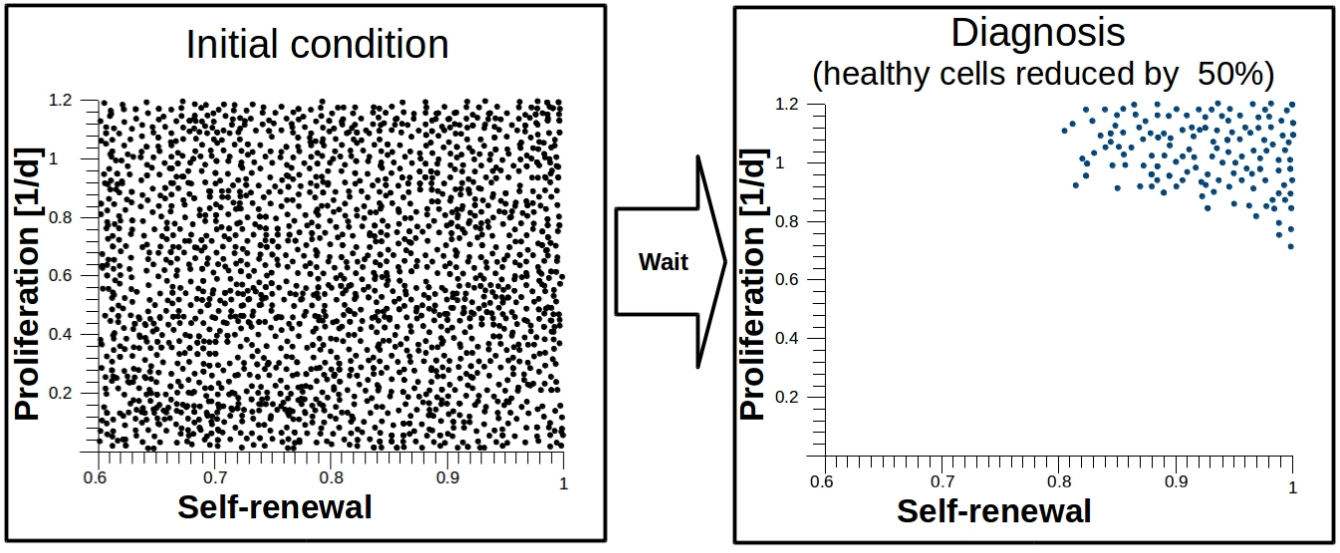


Clonal selection



- Clones of **50 „virtual Patients“**
- Only clones contributing **at least 1% to total cell mass** are depicted

Clonal selection



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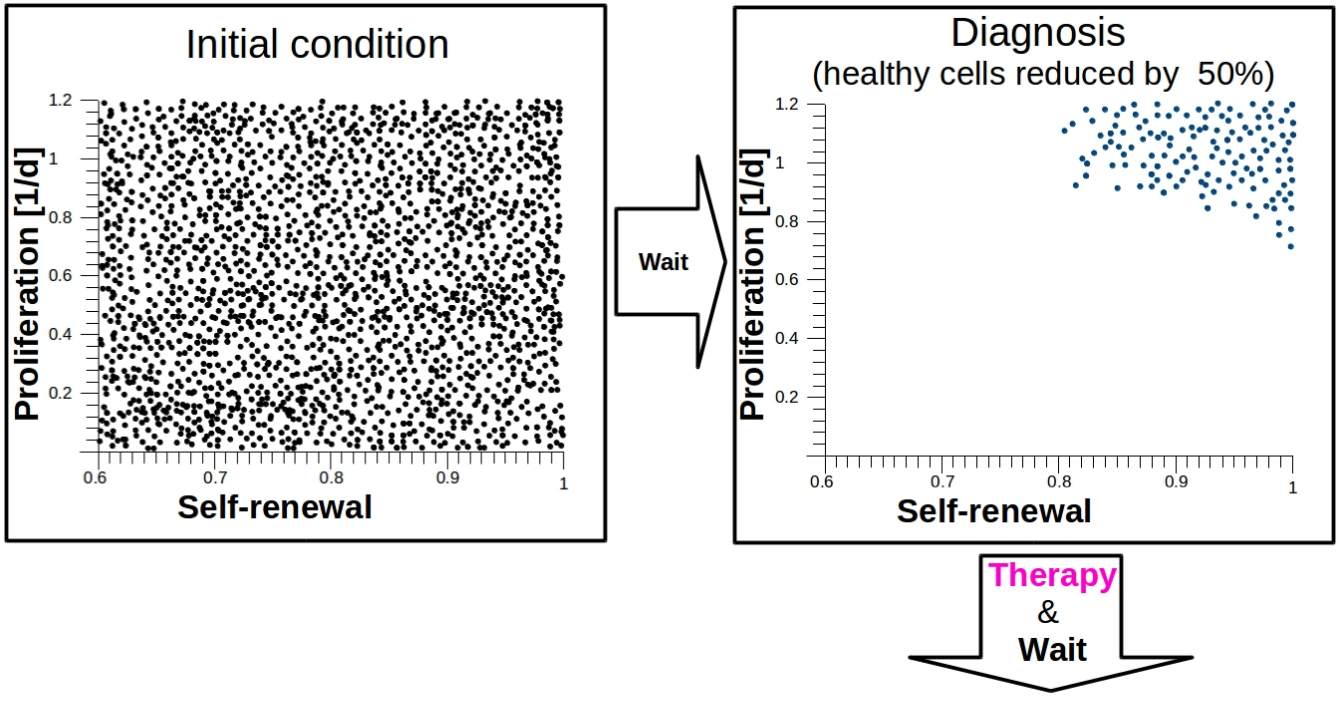
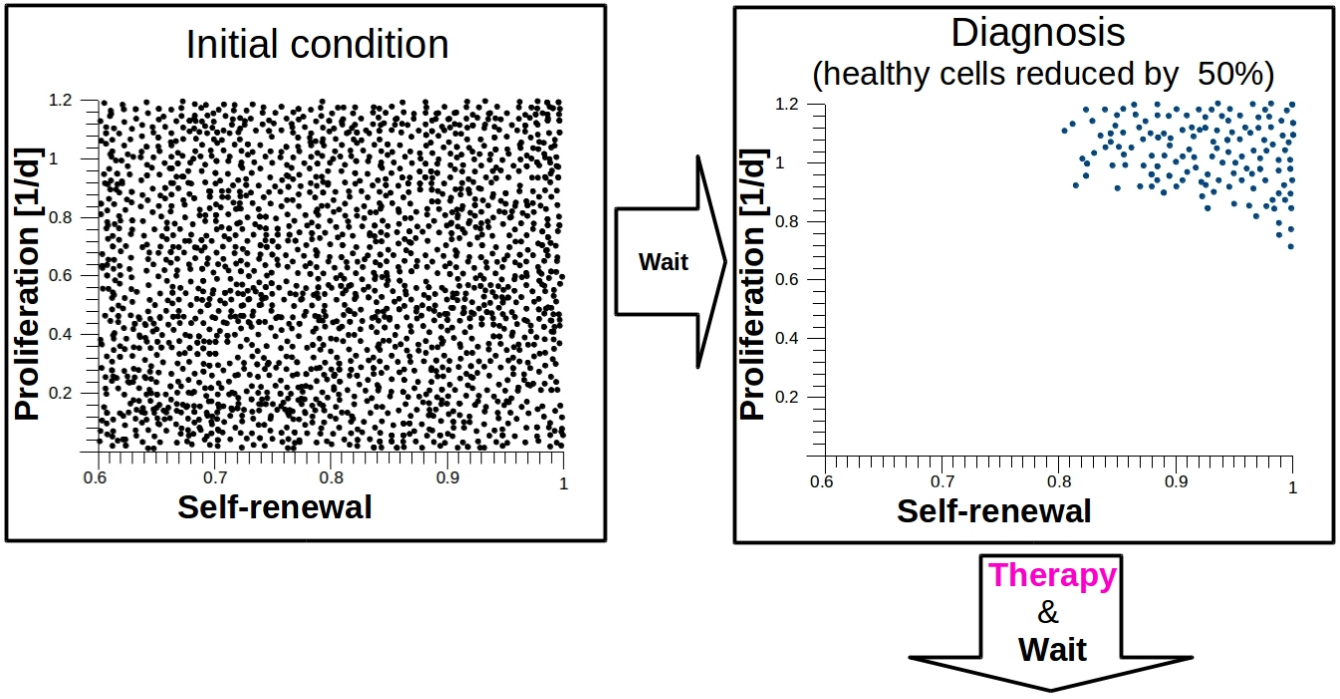


- Clones of **50 „virtual Patients“**
- Only clones contributing **at least 1% to total cell mass** are depicted

Therapy: Toxicity increases with increasing proliferation:

$$-k \cdot p_i \cdot I_i$$

Clonal selection



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Clonal selection as a dynamical process

- What are cell properties at diagnosis and relapse?

Answer:

- **Diagnosis:** high proliferation + high self-renewal
- **Relapse:** low proliferation + high self-renewal
- Low proliferation causes **resistance** to therapy, high self-renewal guarantees **expansion**.
- **Selection** explains different cell properties
- No mutations are required!

Clonal selection as a dynamical process

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- **Relapse:** low proliferation + high self-renewal
- Low proliferation causes **resistance** to therapy, high self-renewal guarantees **expansion**.
- **Selection** explains different cell properties
- No mutations are required!

- What is the number of clones at diagnosis and relapse?

Answer:

- The number of large clones at diagnosis and relapse is relatively small.
- The **nonlinear and nonlocal feedback** underlying the competition limits the number of large clones.

Results are conserved for different feedback mechanisms and independent on the number of clones

Structured population model of clonal evolution

Model structured by a self-renewal potential

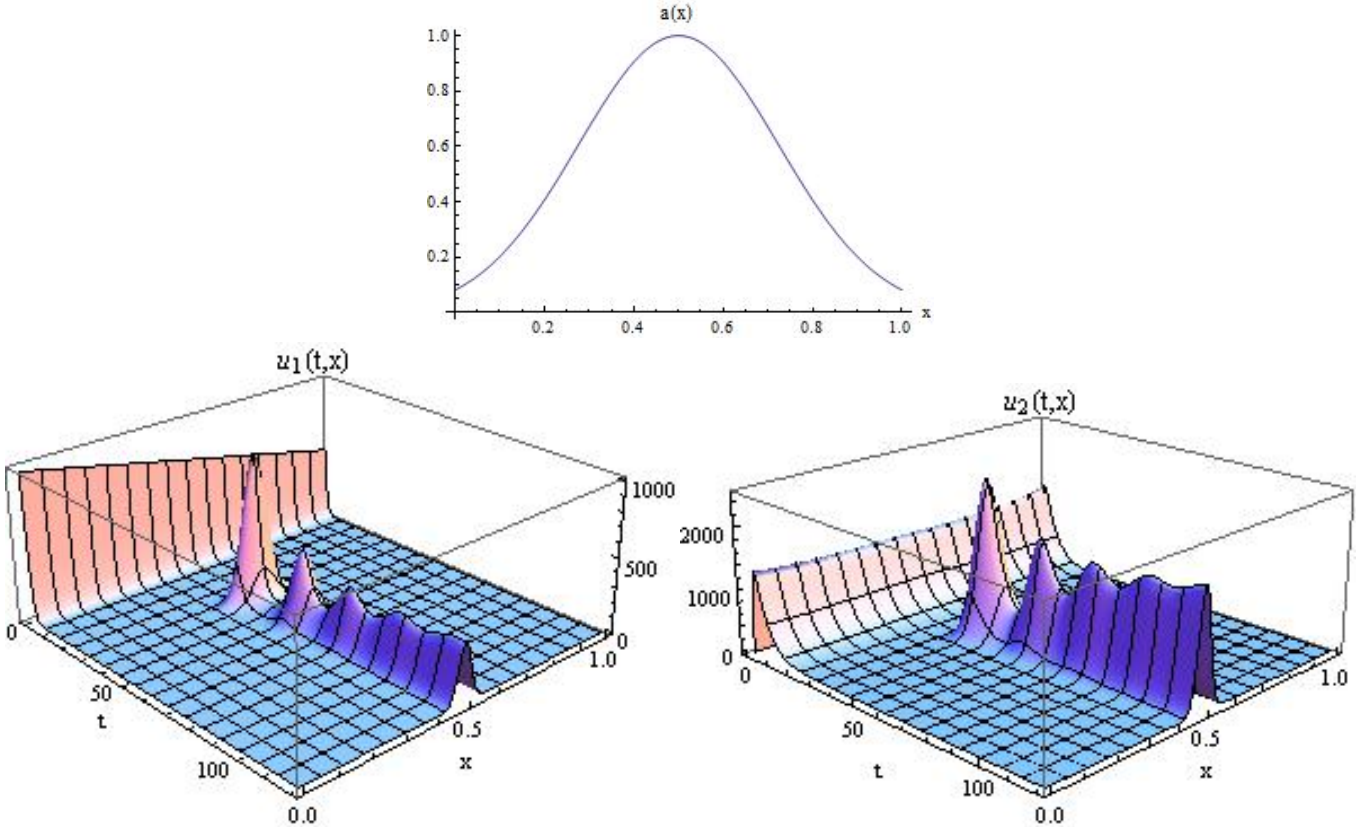
- Let $u(x,\ t)$ be a clone characterized by an internal parameter:
  - $x \in \{x_1, ..., x_N\}$ (a discrete structure)
  - $x \in \overline{\Omega}$ (a continuous structure)

$$\begin{aligned}
\frac{\partial}{\partial t}u(t,x) &= \left(\frac{2a(x)}{1+K\rho_2(t)} - 1\right) p(x)u(t,x), \\
\frac{\partial}{\partial t}v(t,x) &= 2\left(1 - \frac{a(x)}{1+K\rho_2(t)}\right) p(x)v(t,x) - dv(t,x),
\end{aligned}$$

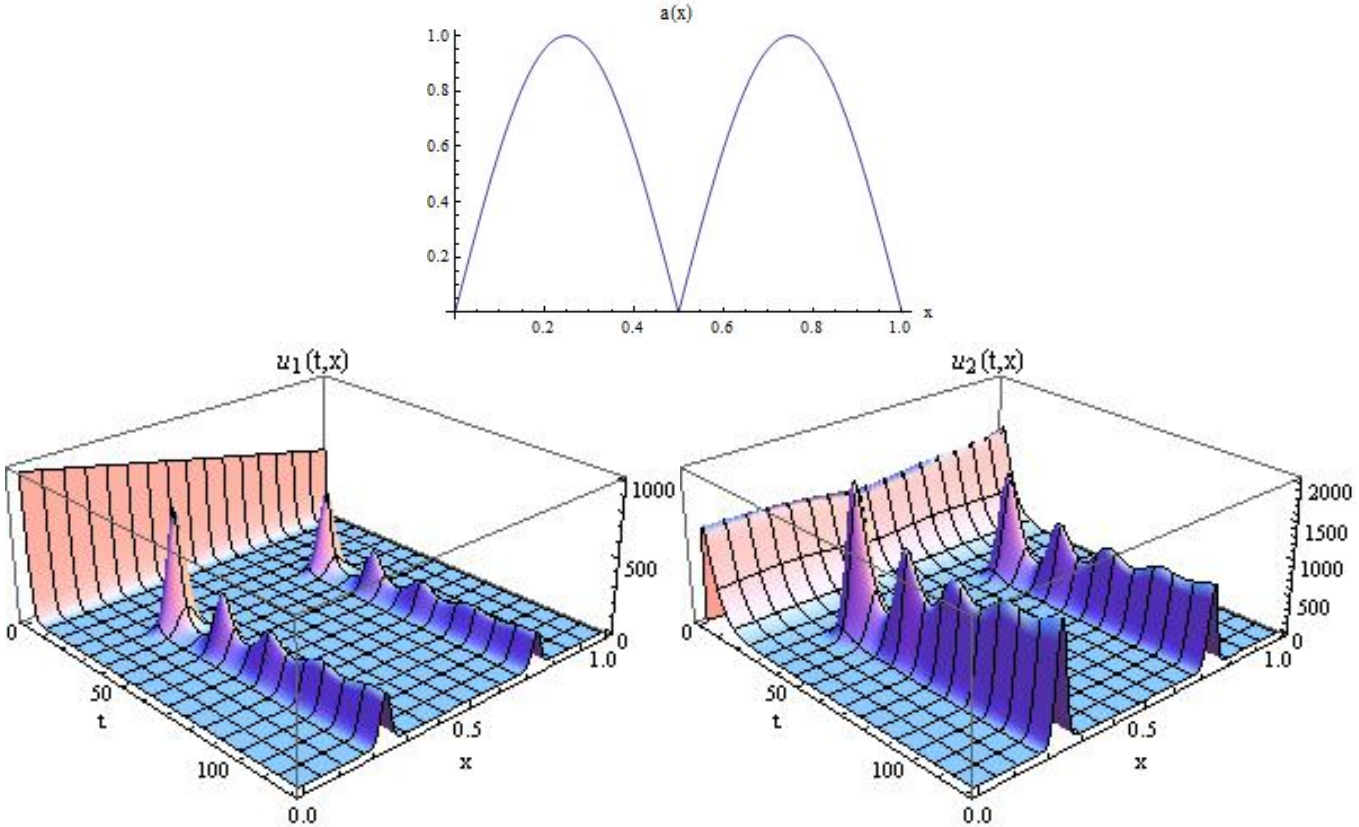
where $\rho_2(t) = \int_\Omega v(t,x)dx$

- Assumptions: $p(x) = p$, d and K are positive constants
- $a \in C(\overline{\Omega})$ with $\frac{1}{2} < a < 1$

Simulations of a single clone selection



Simulations of multiple clones selection



Main result: Clonal selection

Theorem

(i) *Both u_1 and u_2 converge to measures with support contained in the set*

$$\Omega_a = \arg\max_{x\in\overline{\Omega}} a(x) = \left\{ \bar{x} \in \overline{\Omega} \,\middle|\, a(\bar{x}) = \max_{x\in\overline{\Omega}} a(x) \right\}$$

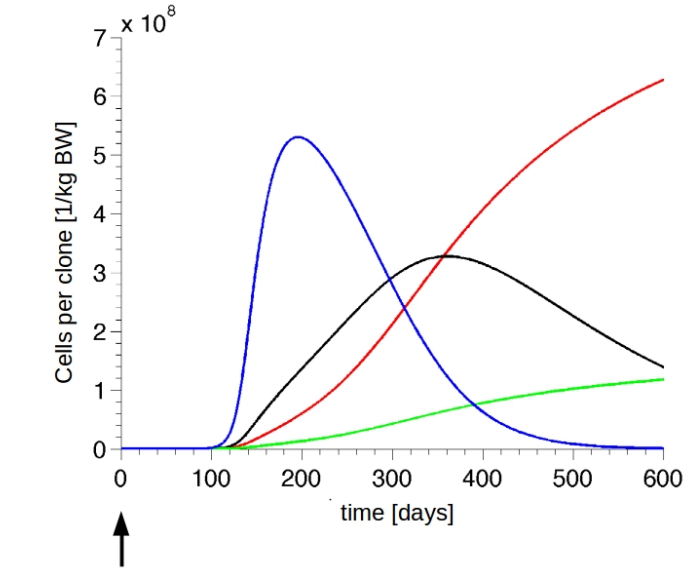
as t tends to infinity.

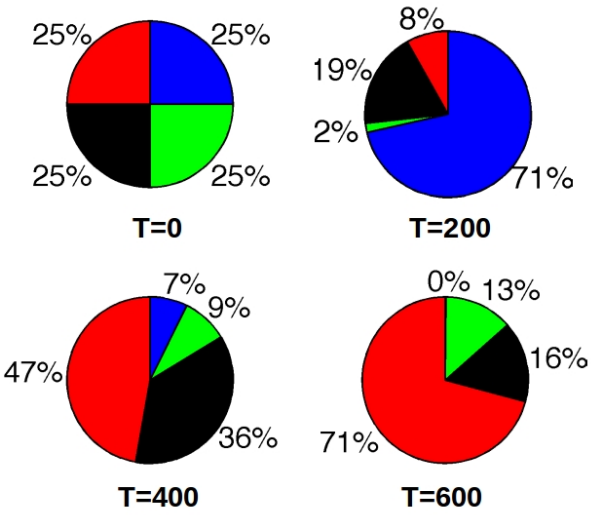
(ii) *If Ω_a consists of a single point $\bar{x}$, then the solution converges to a stationary measure (Dirac measure multiplied by a positive constant) concentrated in $\bar{x}$.*

(iii) *If Ω_a is a set with positive measure, then the solution converges to a discontinuous bounded function.*

`Busse, Gwiazda, M-C. J. Math. Biol., 2015`

Dynamics of the clones with heteoregenity in (a, p)



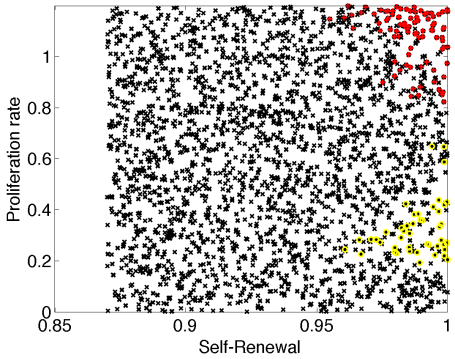


- Dynamically changing maximal growth rate: $\max\{(\frac{2a(x)}{1+k\rho_2(t)} - 1)p(x)\}$, but the fitness corresponds to $\max a(x)$

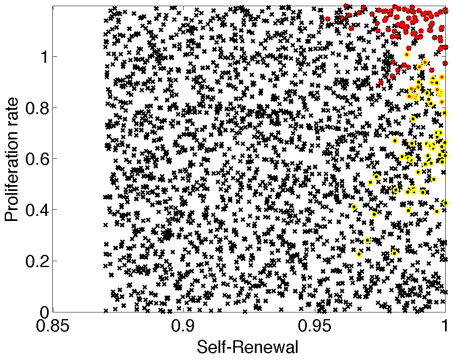
Application to therapy and cancer relapse

Cellular Properties at Relapse

Strong Chemotherapy



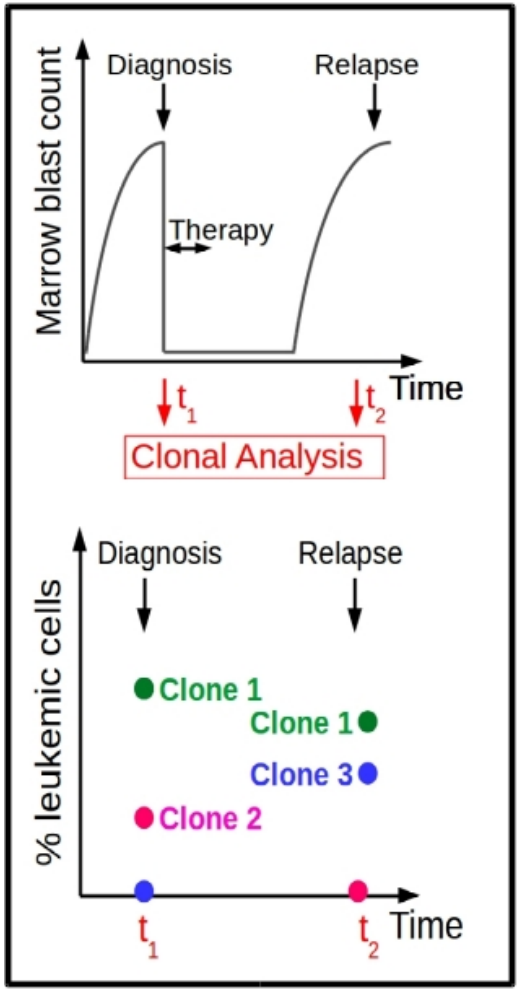
Weak Chemotherapy

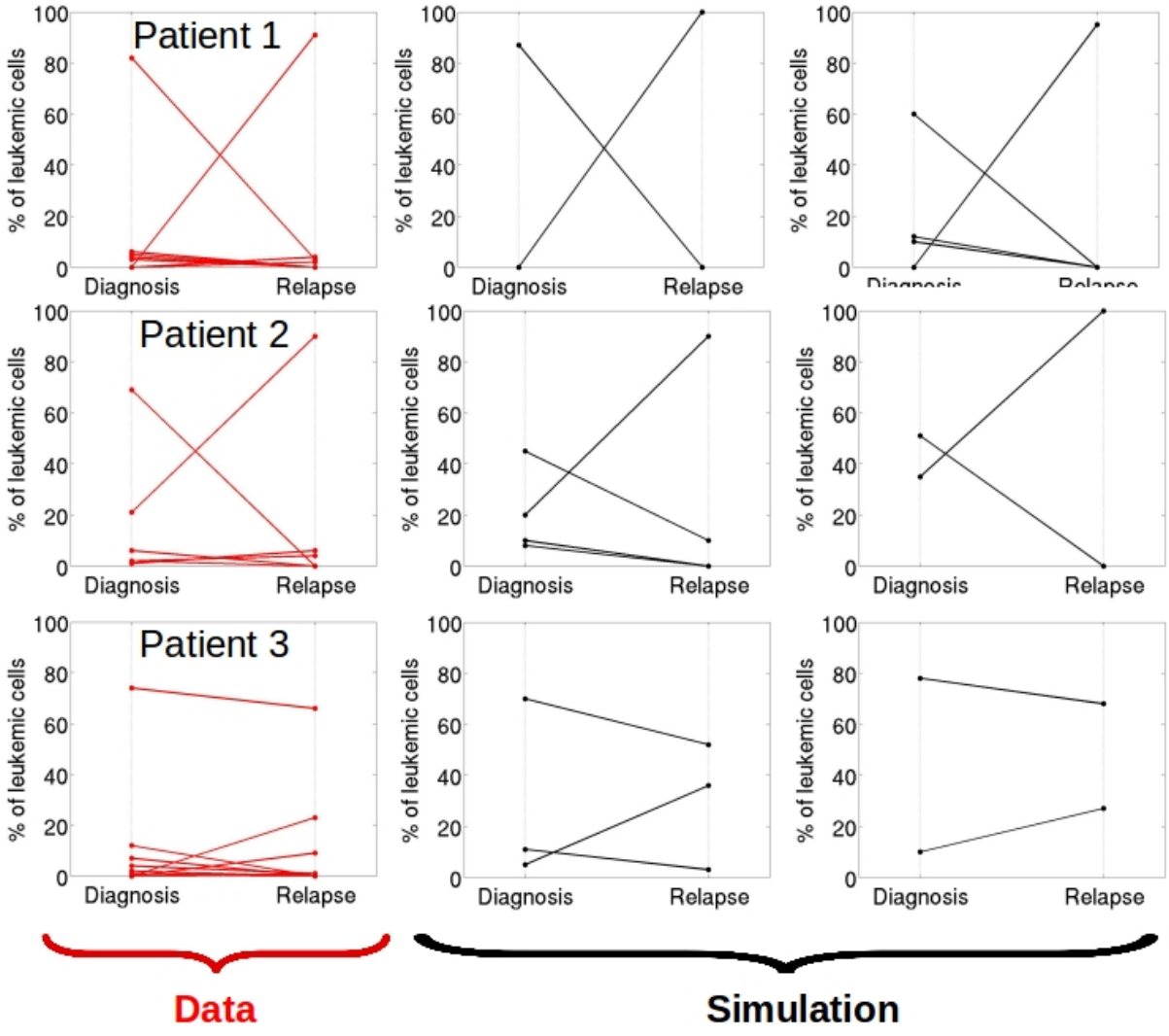


- (Sub-)clones already present at diagnosis but not contributing to cell mass can survive therapy and trigger relapse
- Chemotherapy selects for slowly proliferating cells with high self-renewal

`Stiehl, Baran, Ho, M-C, JRS Interface 11, 2014`

Change of clonal size





Data from Anderson et al Nature 2011

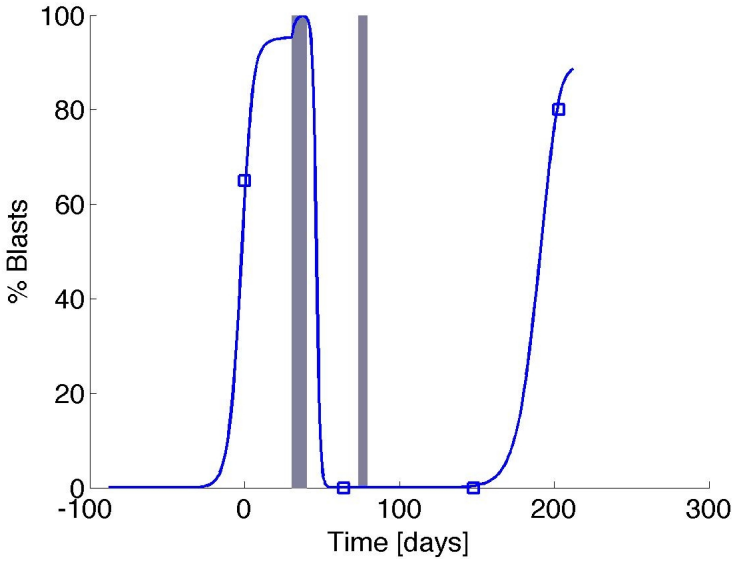
Fitting to patient data

The model can be fitted to patient data:

Genetic Data

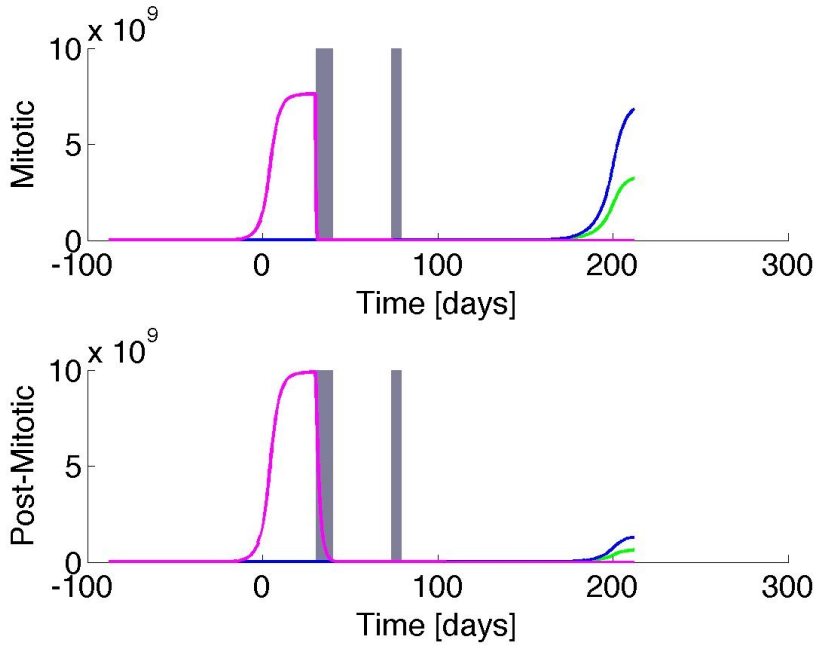
	Diagnosis t=0		Control t=150		Relapse t=200
Clone 1 (FLT3-ITD, 39 bp)	present	→	0	→	0
Clone 2 (FLT3-ITD, 42 bp)	0	↗	present	↗	present
Clone 3 (FLT3-ITD, 63 bp)	0	→	0	↗	present

Marrow Blast Fraction



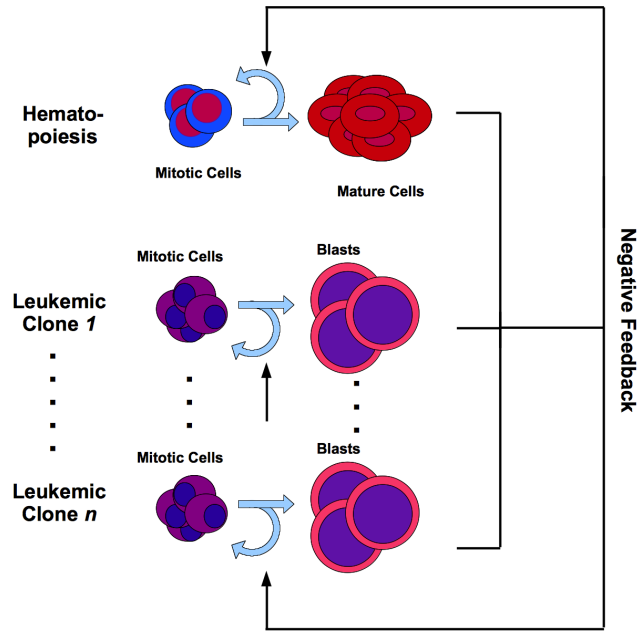
□ = Marrow aspiration data

Clonal Contribution

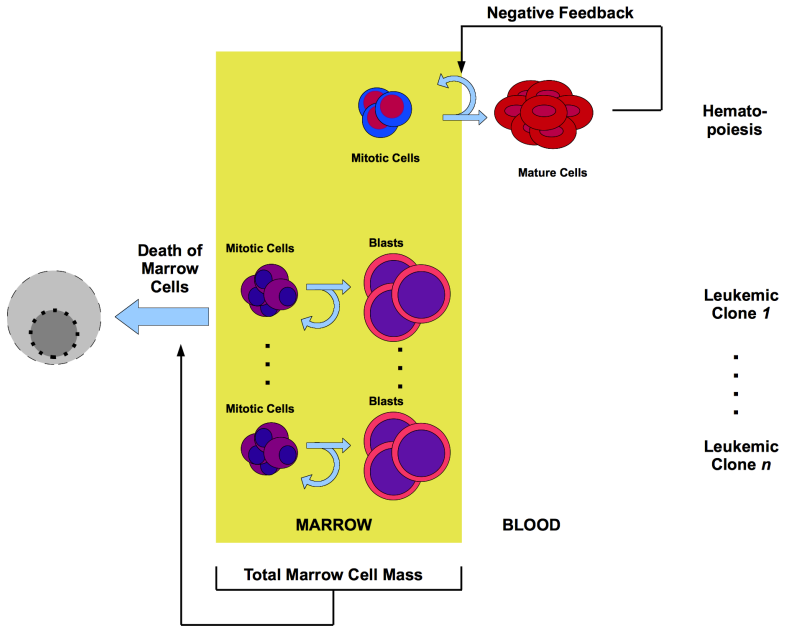


What is the mechanism of selection?

Two regulatory mechanisms



Model 1: Competition for surviving factors



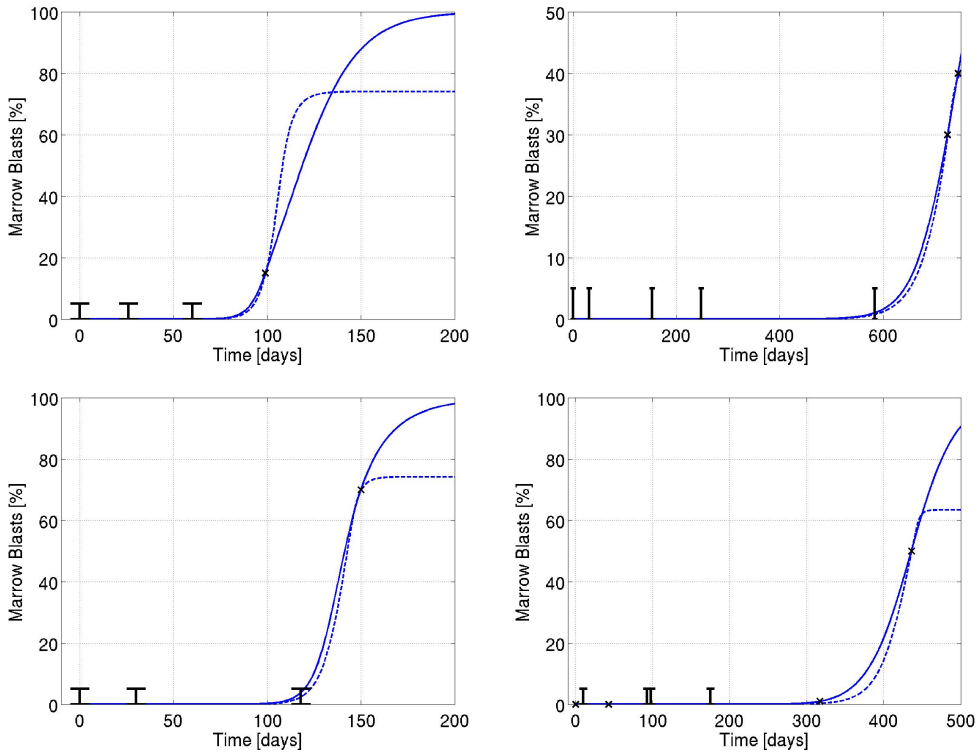
Model 2: Competition for space

- The selection takes place in both models.
- How to distinguish between the mechanisms?

`Stiehl, Baran, Ho, M-C, JRS Interface 11, 2014`

System dynamics for both models

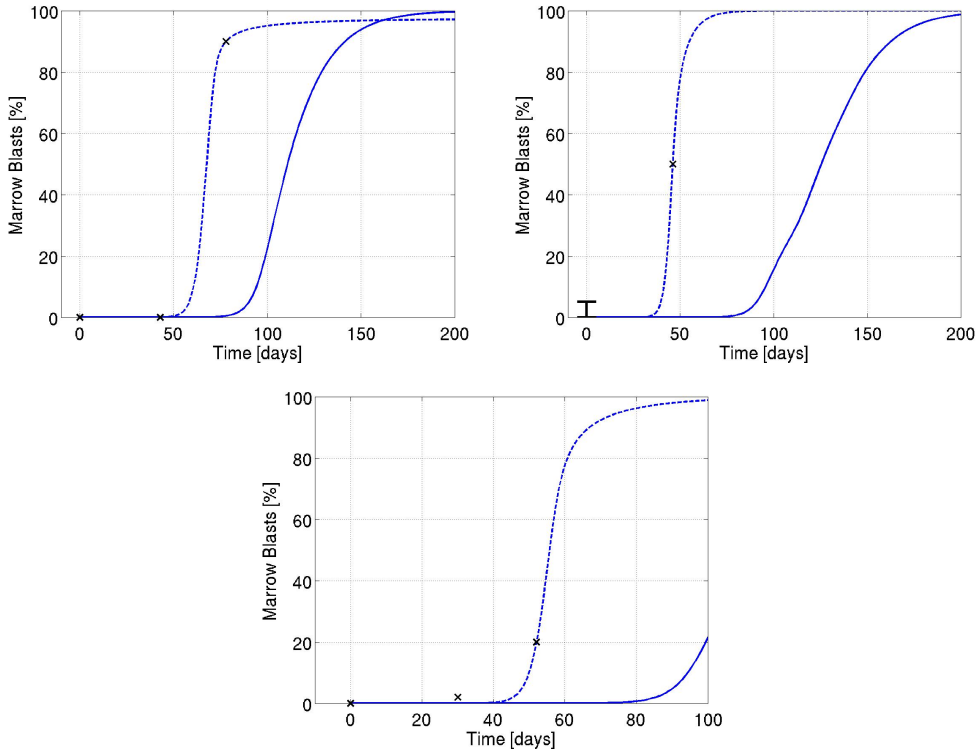
- We fit Models 1 and 2 to the patients data (bone marrow data + time between treatment and relapse)
- In most cases both models are compatible with observed dynamics



straight line: Model 1, dotted line: Model 2

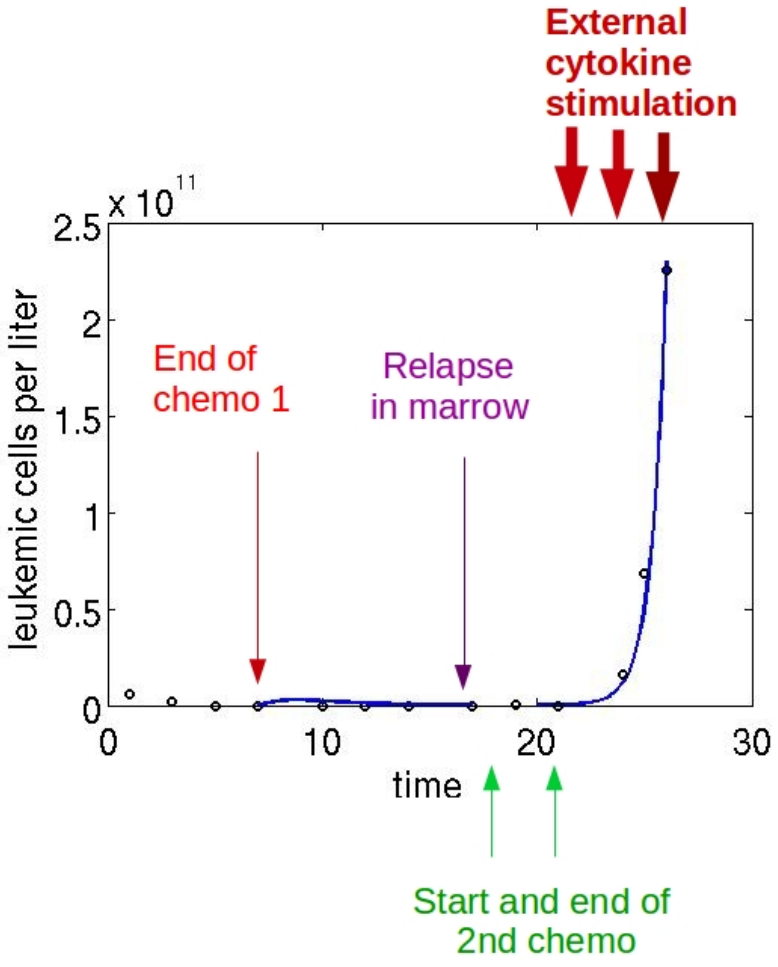
Model discrimination

- Fast increase of leukemic cell counts is compatible only with Model 2.



straight line: Model 1, dotted line: Model 2

Fit to data: Special case



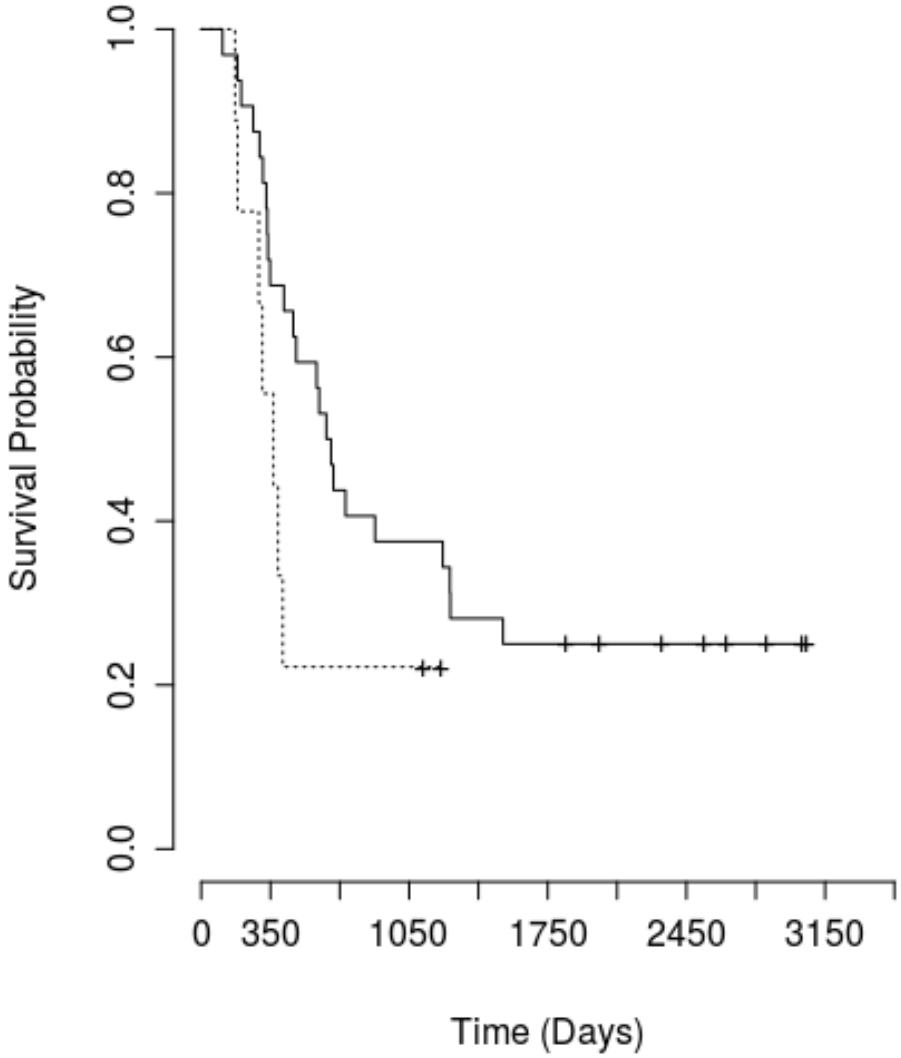
Data from Duval et al.

- Data not compatible with Model 2.

- Cytokine treatment may stimulate cancer growth (Duval et al 2014).
- Patient with 2 relapses
- Comparable situation after the first and the second chemotherapy
- Cytokine administration only after the second chemotherapy
- Cytokine administration leads to a rapid expansion of leukemic cells

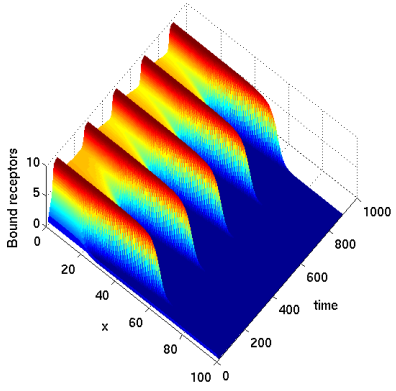
Cytokine sensitivity vs. patient prognosis

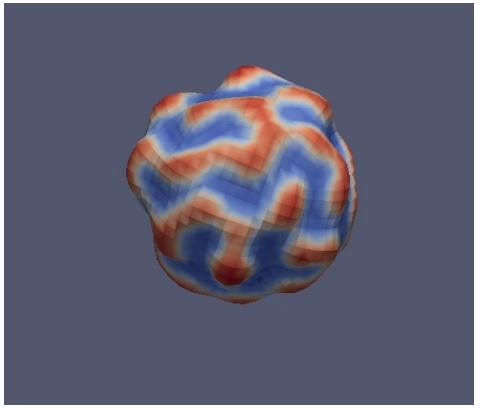
- The models may help to distinguish in a given patient which mechanism (cytokine sensitive vs insensitive blast expansion) is more relevant.

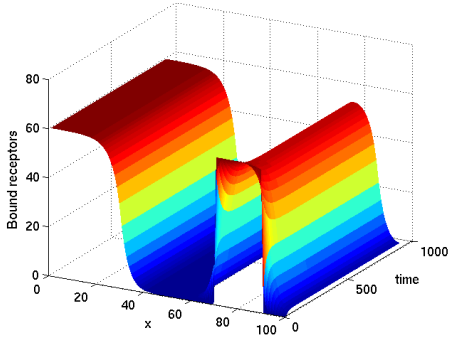


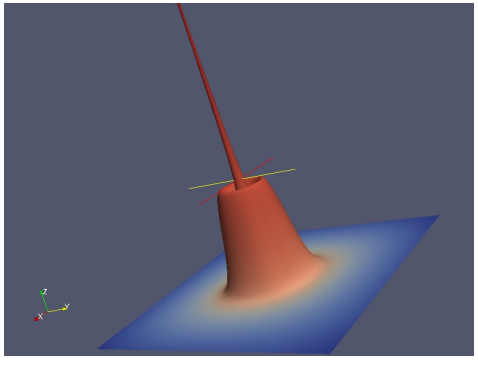
Conclusions

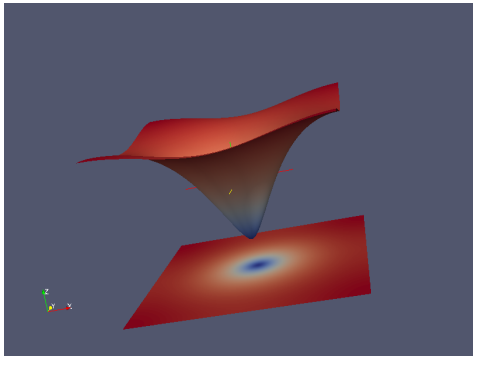
- Mathematical model provides a possible explanation of the clonal selection observed in experimental data.
- Clonal selection may be a dynamic property reducing the number of relevantly contributing leukemic clones.
- Therapy may lead to a selection of more aggressive clones.
- LSC properties can be estimated using mathematical modelling:
  - Estimated cell properties differ between different individuals.
  - Estimated cell properties differ between different relapses in the same individual.
  - Estimated cell properties correlate with patient survival.

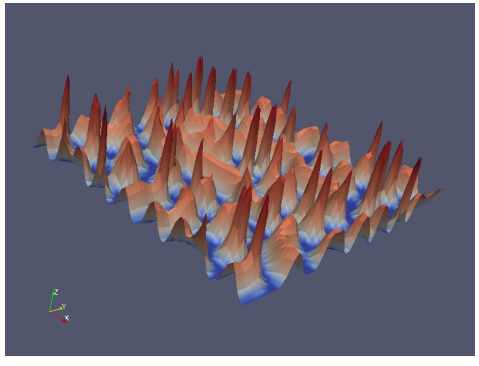

Bound receptors
10
5
0
0
20
40
x
60
80
100
0
200
400
time
600
800
1000




Bound receptors
80
60
40
20
0
0
20
40
x
60
80
100
0
500
time
1000







Thank you!

Sketch of the proof. Boundedness of masses

- Equations for the total mass

$$\begin{aligned}\frac{d}{dt}\rho_1(t) &= \int_\Omega \left(\frac{2a(x)}{1+K\rho_2(t)} - 1\right) p u_1(t,x)\mathrm{d}x,\\ \frac{d}{dt}\rho_2(t) &= 2\int_\Omega \left(1 - \frac{a(x)}{1+K\rho_2(t)}\right) p u_1(t,x)\mathrm{d}x - d\int_\Omega u_2(t,x)\mathrm{d}x.\end{aligned}$$

- Estimates using $\bar{a} = \max_{x\in\overline{\Omega}} a(x)$ and $\underline{a} = \min_{x\in\overline{\Omega}} a(x)$.

Lemma

Both ρ_1 and ρ_2 are uniformly bounded and strictly positive.

- We need an estimate $\rho_1(t) \leq M_1\rho_2(t)$
- It results from uniform boundedness of $U(t,x) = \frac{u_1(t,x)}{u_2(t,x)}$

Sketch of the proof. Positivity of masses

Lemma

There exists a constant $M_2 > 0$ *and* $0 < \gamma < 1$ *such that* $\rho_2(t) \leq M_2 \rho_1^\gamma(t)$ *for all* $t \geq 0$.

- $$\frac{d}{dt}\frac{\rho_2(t)}{\rho_1^\gamma(t)} \leq 2pM_2^{1-\gamma} + \frac{\rho_2(t)}{\rho_1^\gamma(t)}(\gamma p - d).$$
- Taking $\gamma p - d < 0$ leads to the desired estimate
- The equation for masses yields positivity of ρ_1

$$\frac{d}{dt}\rho_1(t) \geq \left(\frac{2\underline{a}}{1 + KM_4\rho_1(t)^\gamma} - 1\right) p\rho_1(t),$$

Sketch of the proof. Exponential extinction of solutions in $x \notin \Omega_a$

Lemma

Let $x_1, x_2 \in \Omega$ such that $a(x_1) - a(x_2) < 0$. Then,

$$\frac{u_1(t, x_1)}{u_1(t, x_2)} \leq \frac{u_1^0(x_1)}{u_1^0(x_2)} e^{p \frac{2\left(a(x_1) - a(x_2)\right)}{1 + KM_3} t} \xrightarrow{t \to \infty} 0.$$

- The Lemma implies that the solution decays exponentially to zero in all points x except those with maximal value of $a(x)$.
- Strict positivity of masses excludes extinction of the solution
- Together with boundedness of mass, it leads to the conclusion that the model solutions converge to Dirac measures localised in points corresponding to the maximum of function a.

Sketch of the proof. Convergence of solutions

Theorem

It holds $(\rho_1(t), \rho_2(t)) \to (\bar{\rho}_1, \bar{\rho}_2)$, *as* $t \to \infty$, *where* $(\bar{\rho}_1, \bar{\rho}_2)$ *are stationary solutions of the corresponding ordinary differential equations model with the maximal value of the self-renewal parameter*

$$\begin{aligned} 0 &= \left(\frac{2\bar{a}}{1+K\bar{\rho}_2} - 1\right) p\bar{\rho}_1, \\ 0 &= 2\left(1 - \frac{\bar{a}}{1+K\bar{\rho}_2}\right) p\bar{\rho}_1 - d\bar{\rho}_2. \end{aligned}$$

- Proof is based on the Lyapunov function for the discrete model

Getto, M-C, Nakata and dM Vivanco, Math. Biosci., 2013

Sketch of the proof. Comparison result

- Our system can be rewritten as

$$\begin{aligned}\frac{d}{dt}\rho_1 &= \left(\frac{2\bar{a}}{1+K\rho_2}-1\right)p\rho_1+\frac{2p}{1+K\rho_2}\int_\Omega (a(x)-\bar{a})\,u_1\mathrm{d}x,\\ \frac{d}{dt}\rho_2 &= 2\left(1-\frac{\bar{a}}{1+K\rho_2}\right)p\rho_1+\frac{2p}{1+K\rho_2}\int_\Omega (\bar{a}-a(x))\,u_1\mathrm{d}x-d\rho_2\end{aligned}$$

Lemma

Let u be a solution of $\frac{du}{dt}=F(u)$ with a globally stable stationary solution $\bar{u}$ and let $V(u)$ be a Lyapunov function for this equation with compact level sets and the minimum δ achieved at the stationary solution $\bar{u}$. If $\tilde{u}$ is a solution of $\frac{d\tilde{u}}{dt}=F(\tilde{u})+f$, where $f\in L^1(\mathbb{R}^+)$, then $\tilde{u}\to\bar{u}$ for $t\to\infty$.

- $\int_\Omega (a(x)-\bar{a})\,u_1(t,x)\mathrm{d}x \overset{t\to\infty}{\longrightarrow} 0$, since

$$\int_\Omega (a(x)-\bar{a})\,u_1\mathrm{d}x=\int_{\Omega_a}(a(x)-\bar{a})\,u_1\mathrm{d}x+\int_{\Omega\setminus\Omega_a}(a(x)-\bar{a})\,u_1\mathrm{d}x.$$

Convergence result in flat metric

- For $\mu, \nu \in \mathcal{M}^+(\mathbb{R}^+)$ the flat metric ρ is defined by

$$\rho_F(\mu,\, \nu) \quad := \quad \sup \left\{ \int_{\mathbb{R}^+} \psi \, d(\mu - \nu) \;\middle|\; \|\psi\|_{W^{1,\infty}} \leq 1 \right\}.$$

- To estimate the distance between a solution $u(t,x)$ and the stationary measure $c\delta_{\bar{x}}$, we use the following inequality for the distance of two measures μ_1 and μ_2

$$\rho_F(\mu_1, \mu_2) \leq min\{\rho_1, \rho_2\} W_1\left(\frac{\mu_1}{\rho_1}, \frac{\mu_2}{\rho_2}\right) + |\rho_1 - \rho_2|,$$

  where W_1 is the Wasserstein metric

- Convergence results from the exponential estimates and convergence of masses.

Model calibration

Available data

- Initial conditions
- Proliferation rates in a steady state
- Steady state population sizes
- Clearance of leukocytes from blood stream

Initial conditions

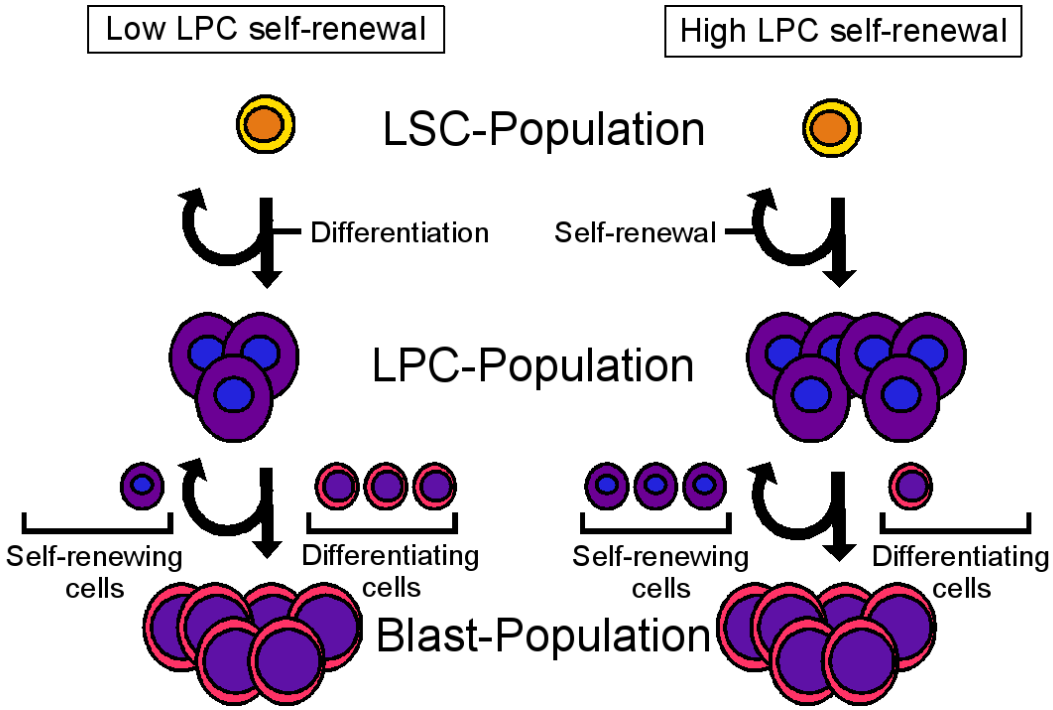
Cell Type	number of transplanted cells per kg body weight
prim HSC [1]	$\approx 3 \cdot 10^3$
LTC-IC	$\approx 36 \cdot 10^3$
CFU-GM	$\approx 155 \cdot 10^3$
CFU-G	$\approx 54 \cdot 10^4$
Myeloblast	0
Promyelocyte	0
Myelocyte	0
Mature neutrophil	0

Parameter sets

Parameter	Value	Parameter	Value	Parameter	Value	Parameter	Value
a_1	0.5	$a_{1,max}$	0.77	p_1	$2.15 \cdot 10^{-3} \frac{1}{\text{day}}$	$p_{1,max}$	$7.6 \cdot 10^{-3} \frac{1}{\text{day}}$
a_2	0.4993	$a_{2,max}$	0.7689	p_2	$11.21 \cdot 10^{-3} \frac{1}{\text{day}}$	$p_{2,max}$	$39.6 \cdot 10^{-3} \frac{1}{\text{day}}$
a_3	0.4779	$a_{3,max}$	0.7359	p_3	$5.66 \cdot 10^{-2} \frac{1}{\text{day}}$	$p_{3,max}$	$0.2 \frac{1}{\text{day}}$
a_4	0.4986	$a_{4,max}$	0.7678	p_4	$0.1586 \frac{1}{\text{day}}$	$p_{4,max}$	$0.56 \frac{1}{\text{day}}$
a_5	0.1	$a_{5,max}$	0.154	p_5	$0.32 \frac{1}{\text{day}}$	$p_{5,max}$	$0.32 \frac{1}{\text{day}}$
a_6	0.0714	$a_{6,max}$	0.11	p_6	$0.7 \frac{1}{\text{day}}$	$p_{6,max}$	$0.7 \frac{1}{\text{day}}$
a_7	0.3929	$a_{7,max}$	0.605	p_7	$1 \frac{1}{\text{day}}$	$p_{7,max}$	$1 \frac{1}{\text{day}}$

Parameter	Value	Parameter	Value	Parameter	Value	Parameter	Value
a_1	0.5	$a_{1,max}$	0.77	p_1	$2.15 \cdot 10^{-3} \frac{1}{\text{day}}$	$p_{1,max}$	$7.6 \cdot 10^{-3} \frac{1}{\text{day}}$
a_2	0.4994	$a_{2,max}$	0.769	p_2	$11.21 \cdot 10^{-3} \frac{1}{\text{day}}$	$p_{2,max}$	$39.6 \cdot 10^{-3} \frac{1}{\text{day}}$
a_3	0.4743	$a_{3,max}$	0.7304	p_3	$5.66 \cdot 10^{-2} \frac{1}{\text{day}}$	$p_{3,max}$	$0.2 \frac{1}{\text{day}}$
a_4	0.4982	$a_{4,max}$	0.7673	p_4	$0.1586 \frac{1}{\text{day}}$	$p_{4,max}$	$0.56 \frac{1}{\text{day}}$
a_5	0.4286	$a_{5,max}$	0.66	p_5	$0.32 \frac{1}{\text{day}}$	$p_{5,max}$	$0.32 \frac{1}{\text{day}}$
a_6	0.0714	$a_{6,max}$	0.11	p_6	$0.7 \frac{1}{\text{day}}$	$p_{6,max}$	$0.7 \frac{1}{\text{day}}$
a_7	0.0357	$a_{7,max}$	0.055	p_7	$1 \frac{1}{\text{day}}$	$p_{7,max}$	$1 \frac{1}{\text{day}}$

Is this reasonable?



- low self-renewal of non-LSC $\Rightarrow$ small intermediate population but high percentage differentiates to blast stages
- high self-renewal of non-LSC $\Rightarrow$ large intermediate population but low percentage differentiates to blast stages.