Mathematical modeling of heterogeneity and clonal selection in acute leukemias

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Interdisciplinary collaboration

- 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

Healthy blood cell formation (Hematopoiesis)

- Stem cells: Slow division
- Progenitor cells: Fast division
- Mature cells: No division

Give rise to all types of blood cells
Give rise to a limited number of cell types
Functional cells
Hematopoiesis and Leukemia

Healthy blood cell formation (Hematopoiesis)

Germ cell

Give rise to all types of blood cells

HSC

Give rise to a limited number of cell types

HPC

Functional cells „mature cells“

Stem cells

Slow division

Progenitor cells

Fast division

Mature cells

No division

Leukemia

Give rise to all types of malign cells

LSC

Give rise to a limited number of malign cell types

LPC

No function „blasts“
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

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

M-C, Stiehl, Jäger, Ho, Wagner, SC Dev 18, 2009
Structured population model: continuous structure

\[ \partial_t u(x, t) + \partial_x [g(x, \nu(t))u(x, t)] = p(x)u(x, t) \]

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)

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

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

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: \((0, \ldots, 0, \bar{u}_k, \ldots, \bar{u}_n)\) - linearly unstable iff there exists a steady state with more positive components
- Unique positive steady state: \((\bar{u}_1, \ldots, \bar{u}_n)\) - 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 bifurcation and oscillations in the 3-compartment model and in the structured population model.

Stiehl and Marciniak-Czochra, Math. Comp. Models., 2010
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...)

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
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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Clonal selection

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- Only clones contributing at least 1% to total cell mass are depicted

Therapy: Toxicity increases with increasing proliferation:
\[-k \cdot p_i \cdot l_i\]
Clonal selection

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

- What are cell properties at diagnosis and relapse?

**Answer:**
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- 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:
  - $x \in \{x_1, \ldots, x_N\}$ (a discrete structure)
  - $x \in \overline{\Omega}$ (a continuous structure)

\[
\frac{\partial}{\partial t} u(t, x) = \left( \frac{2a(x)}{1 + K\rho_2(t)} - 1 \right) p(x)u(t, x),
\]
\[
\frac{\partial}{\partial t} v(t, x) = 2 \left( 1 - \frac{a(x)}{1 + K\rho_2(t)} \right) p(x)v(t, x) - dv(t, x),
\]

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

\[
\Omega_a = \arg \max_{x \in \Omega} a(x) = \left\{ \bar{x} \in \bar{\Omega} \mid a(\bar{x}) = \max_{x \in \bar{\Omega}} a(x) \right\}
\]

as \( t \) tends to infinity.

(ii) If \( \Omega_a \) 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 \( \Omega_a \) is a set with positive measure, then the solution converges to a discontinuous bounded function.

Dynamics of the clones with hethoregenity in \((a, p)\)

- Dynamically changing maximal growth rate:
  \[
  \max \{ (\frac{2a(x)}{1+k_2(t)} - 1)p(x) \}, \text{ but the fitness corresponds to } \max a(x)
  \]
Application to therapy and cancer relapse
Cellular Properties at Relapse

- (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**

<table>
<thead>
<tr>
<th></th>
<th>Diagnosis t=0</th>
<th>Control t=150</th>
<th>Relapse t=200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clone 1 (FLT3-ITD, 39 bp)</td>
<td>present</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Clone 2 (FLT3-ITD, 42 bp)</td>
<td>0</td>
<td>present</td>
<td>present</td>
</tr>
<tr>
<td>Clone 3 (FLT3-ITD, 63 bp)</td>
<td>0</td>
<td>0</td>
<td>present</td>
</tr>
</tbody>
</table>

**Marrow Blast Fraction**

- % Blasts
- Time [days]

**Clonal Contribution**

- Mitotic
- Post-Mitotic
- Time [days]

= Marrow aspiration data
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

- 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

Data from Duval et al.

- Data not compatible with Model 2.
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.
Thank you!
Sketch of the proof. Boundedness of masses

• Equations for the total mass

\[
\frac{d}{dt} \rho_1(t) = \int_{\Omega} \left( \frac{2a(x)}{1 + K\rho_2(t)} - 1 \right) pu_1(t, x) dx,
\]

\[
\frac{d}{dt} \rho_2(t) = 2 \int_{\Omega} \left( 1 - \frac{a(x)}{1 + K\rho_2(t)} \right) pu_1(t, x) dx - d \int_{\Omega} u_2(t, x) dx.
\]

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

Lemma

Both \( \rho_1 \) and \( \rho_2 \) 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 $\rho_1$

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

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

- 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 $(\rho_1(t), \rho_2(t)) \rightarrow (\bar{\rho}_1, \bar{\rho}_2)$, as $t \rightarrow \infty$, 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

\[
0 = \left( \frac{2\bar{a}}{1 + K\bar{\rho}_2} - 1 \right) p\bar{\rho}_1,
\]

\[
0 = 2 \left( 1 - \frac{\bar{a}}{1 + K\bar{\rho}_2} \right) p\bar{\rho}_1 - d\bar{\rho}_2.
\]

- 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

\[
\frac{d}{dt}\rho_1 = \left(\frac{2\bar{a}}{1 + K\rho_2} - 1\right) p\rho_1 + \frac{2p}{1 + K\rho_2} \int_{\Omega} (a(x) - \bar{a}) u_1 dx, \\
\frac{d}{dt}\rho_2 = 2 \left(1 - \frac{\bar{a}}{1 + K\rho_2}\right) p\rho_1 + \frac{2p}{1 + K\rho_2} \int_{\Omega} (\bar{a} - a(x)) u_1 dx - d\rho_2
\]

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 \( \delta \) achieved at the stationary solution \( \bar{u} \). If \( \tilde{u} \) is a solution of \( \frac{d\tilde{u}}{dt} = F(\bar{u}) + f \), where \( f \in L^1(\mathbb{R}^+) \), then \( \tilde{u} \to \bar{u} \) for \( t \to \infty \).

- \( \int_{\Omega} (a(x) - \bar{a}) u_1(t, x) dx \xrightarrow{t \to \infty} 0 \), since

\[
\int_{\Omega} (a(x) - \bar{a}) u_1 dx = \int_{\Omega_a} (a(x) - \bar{a}) u_1 dx + \int_{\Omega \setminus \Omega_a} (a(x) - \bar{a}) u_1 dx.
\]
Convergence result in flat metric

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

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

• 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 $\mu_1$ and $\mu_2$

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

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

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>number of transplanted cells per kg body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>prim HSC</td>
<td>$\approx 3 \cdot 10^3$</td>
</tr>
<tr>
<td>LTC-IC</td>
<td>$\approx 36 \cdot 10^3$</td>
</tr>
<tr>
<td>CFU-GM</td>
<td>$\approx 155 \cdot 10^3$</td>
</tr>
<tr>
<td>CFU-G</td>
<td>$\approx 54 \cdot 10^4$</td>
</tr>
<tr>
<td>Myeloblast</td>
<td>0</td>
</tr>
<tr>
<td>Promyelocyte</td>
<td>0</td>
</tr>
<tr>
<td>Myelocyte</td>
<td>0</td>
</tr>
<tr>
<td>Mature neutrophil</td>
<td>0</td>
</tr>
</tbody>
</table>
### Parameter sets

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