Clonal Diversity of Blood-forming Stem Cells: Now What?

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February 12, 2014

Leukemia: Microscope, Autopsy, Rabbits and Cell Theory

Early data and interpretation theories of a newly recognized cancer ($\sim$ 170 yrs)

Leukemia History || Stem Cell History

1845	Rudolf Virchow *
1850's	Albert von Kölliker
1860's	Ernst Neumann **

* "White Blood" ($\Rightarrow \exists$ wh. & red bl.)
$\rightarrow$ *Leukemia* recognized as disease
** "unusual grey-green-yellow coloring of the marrow": "white marrow"
$\rightarrow$ birth of the "*Stem Cell*" concept
Ernst Neumann hypothesized that leukemia may originate from abnormal behaviors of certain cells, which he named "Ursprungszelle", "Mutterzelle" or "Stammzelle"

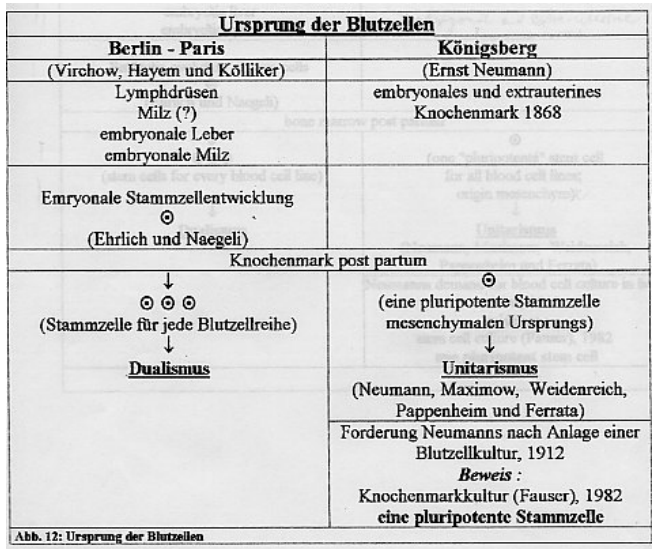


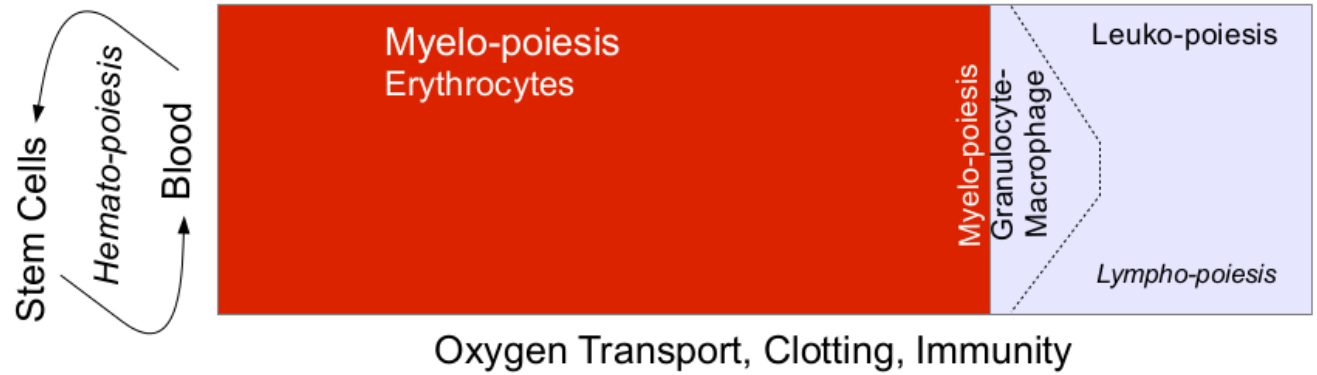
Abb. 12: Ursprung der Blutzellen

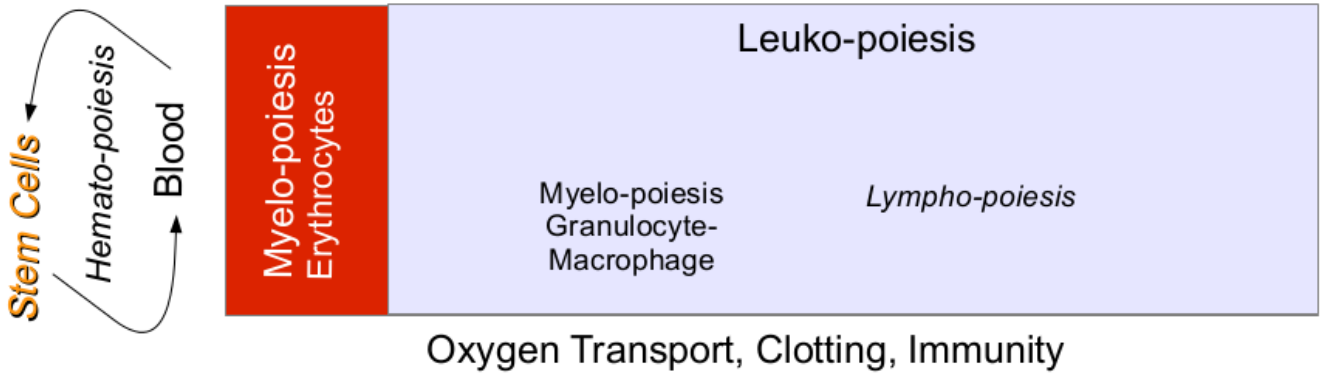
Revolutionary concept, as "Omnis cellula e cellula" not yet accepted

1825	Francois-Vincent Raspail
1837	Robert Remak
1858	Rudolf Virchow

Virchow-Kölliker-Neumann Data Legacy: A Blood System Model and A Cancer Theory

"Reversal of normal blood composition" & "Causation hypothesis"



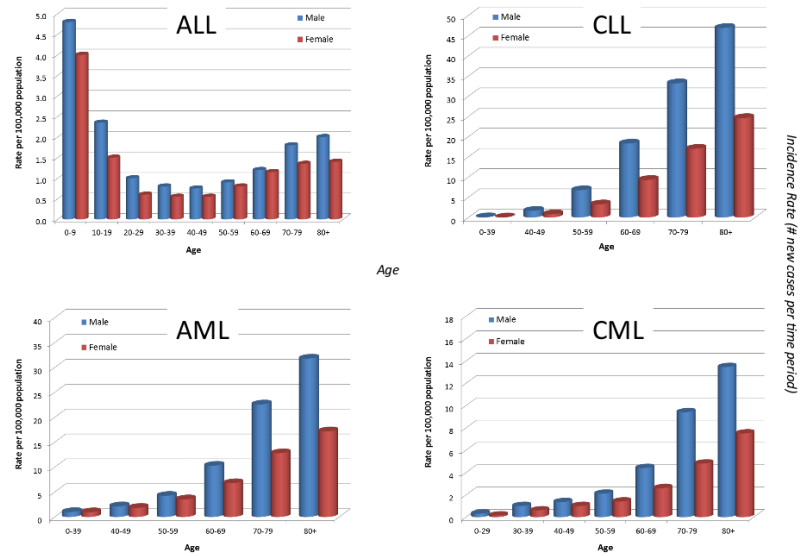


"White Blood" Cancer: A Cluster of Cancers

Modern Classification Schema:

	Acute	Chronic
Lymphoid	Acute lymphoid leukemia (ALL)	Chronic lymphoid leukemia (CLL)
Myeloid	Acute myeloid leukemia (AML)	Chronic myeloid leukemia (CML)

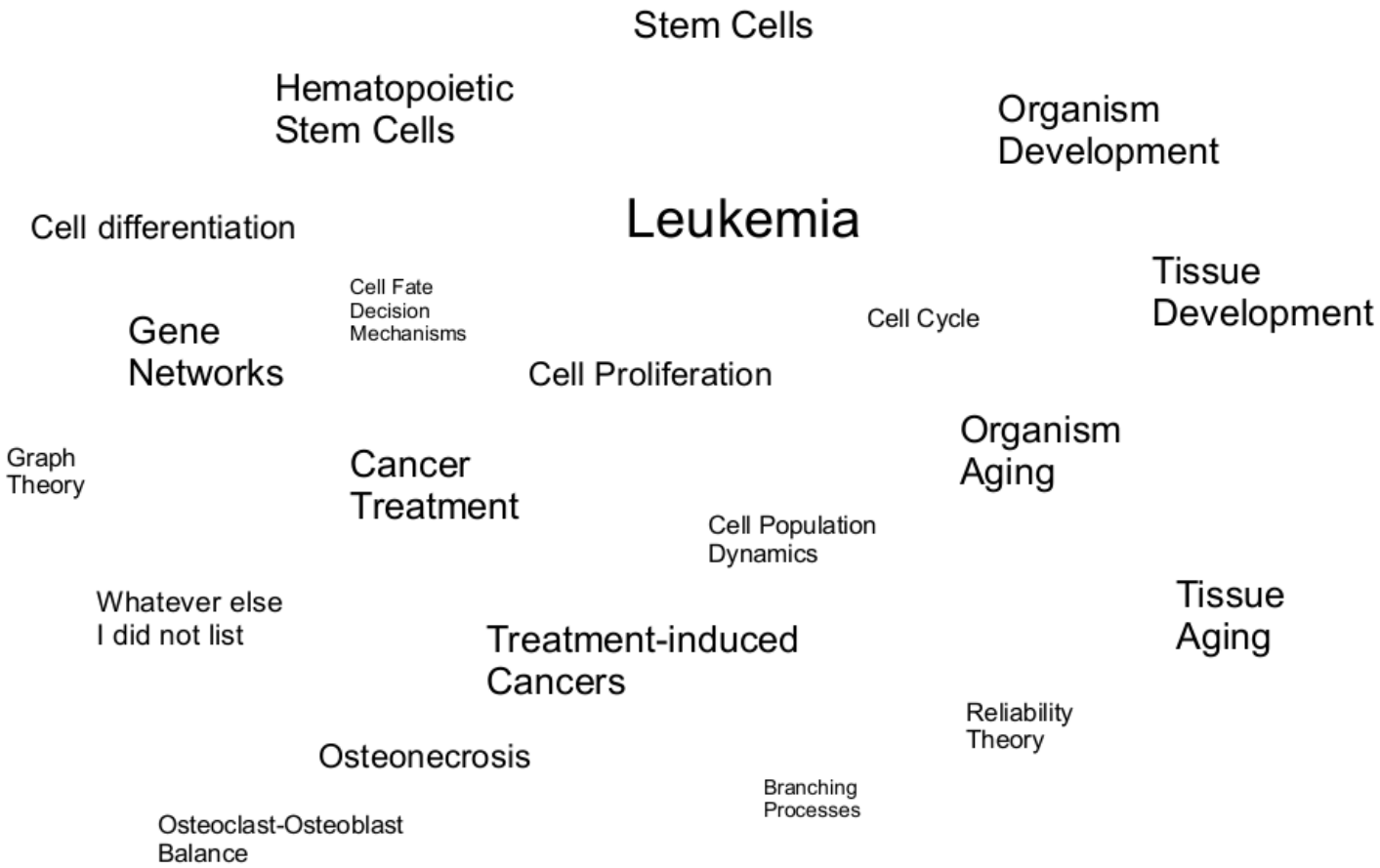
Current Incidence Rates by Age (US data; NCI)



Data Source: NCI; Graphics Source: Medinfographics (http://www.medinfographics.com)

Generally considered as among the class of "rare diseases" ($< 1\%$ population). ≈ 90 % adult. Increase with age. Gender divergence.

Leukemia: Still the Unsolved Problem under Wider Lens



How are Stem Cells Distinguished from Other Cells?

Definition (*Functional Definition*)

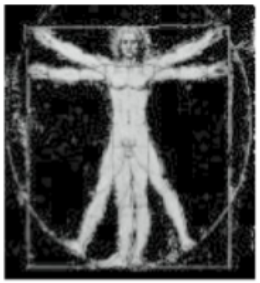
Any stem cell is defined by $\exists\, N = N_{\max}$ ("max" entity specific):

- N-Potency (N = Toti > Pluri > Multi > Oligo > Null)
  - All cell types of potency $< N$ (of an organism or tissue) can be derived by the process of differentiation ($=: \Uparrow: N \to N-1$)
- Self-renewal Capacity ($=: \circlearrowleft$; $N \to N$)
  - "N-potent cell can divide into N-potent daughter cells"

$N - m$, $m > 2$: oligo-potent ($\supset$ blast cells). $N = 1$: "uni-potent" (blast cells). $N = 0$: "null-potent" effector cells (may be able to divide, but are terminally differentiated).

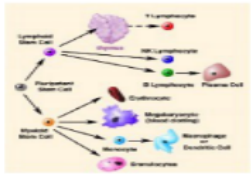
$$
\begin{array}{lllll}
N & S \to S + S & \vee & S \to S + D & \vee \quad S \to D + D \\
N-1 & D \to D + D & \vee & D \to D + E & \vee \quad D \to E + E \\
\vdots & \dots & & \dots & \vdots
\end{array}
$$

Nature-Made and Human-Made Stem Cells



Adult

Adult Tissue Stem Cells

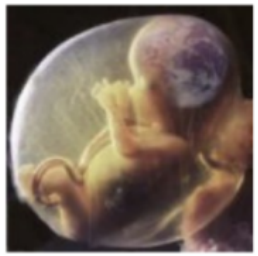


Hematopoietic

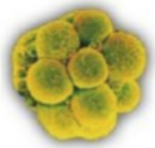
Mesenchymal

Intestinal

Neural



Fetal



Embryonic

Ex Machina Stem Cells

Induced Pluri-potent
Stem Cell

Conceptual Stem Cells

Cancer
Stem Cell

Why do we Need Hematopoietic Stem Cells (HSCs)?

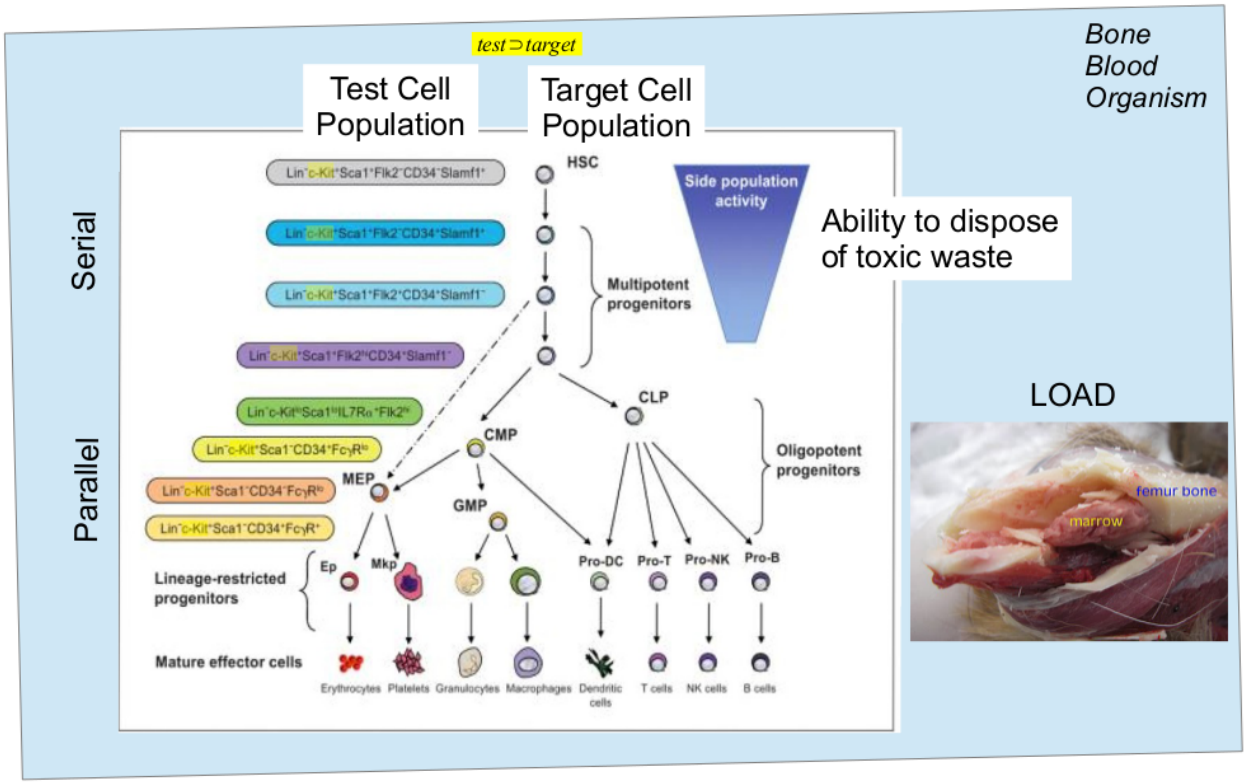
- Healthy: (Daily Turn-over) + Diseases =: "System Load"
  - Differentiation → Resupply effector cells
    - Red blood cells (Erythrocytes) for oxygen transport
    - White blood cells (Leukocytes) for immunity
  - Self-renewal → Maintain the HSC compartment
  - N-potency ∧ Self-renewal =: "System Strength"
- Treatment: Stem Cell ("bone marrow") Transplantation
  - Counteract "myelo-ablative"/"myelo-toxic" side-effects of chemotherapy and radiation therapy
  - Genetic or acquired leukopenia and anemia

Normal reliable system function:

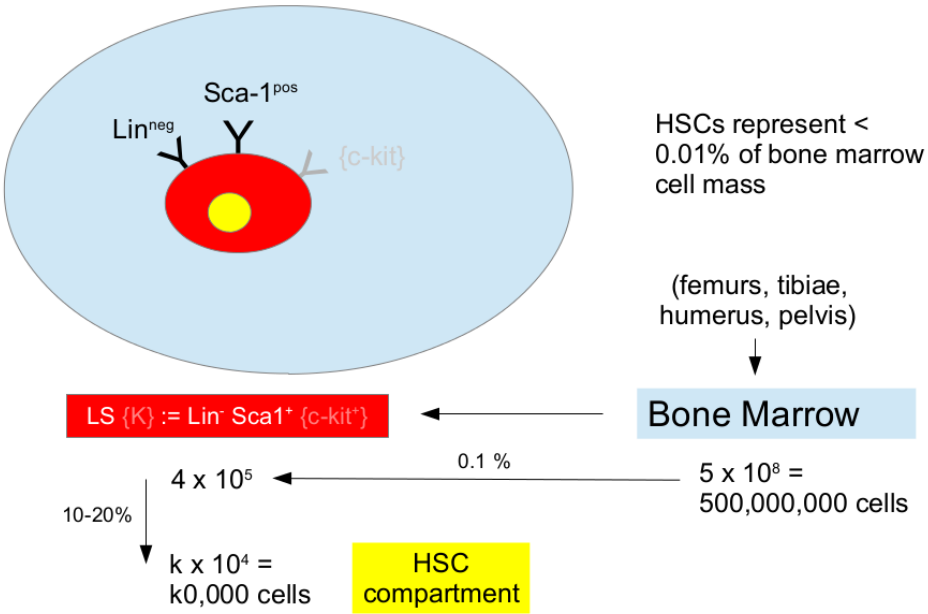
$$P\left[\,(\text{Strength} > \text{Load})_{t+\Delta t} \mid (\text{Strength} > \text{Load})_t\,\right] \gg 0$$

Our Theory of the System Drives our Inferences Drawn from the Evidence - which is in Flux

Beware of marker-defined "HSCs" (test populations e.g. Lin- Sca1+ ckit+ Cd34- CD150+ $\supset$ HSCs, not "$=$ HSC". Always need in vivo assay to verify.
Beware of "multi-potent progenitors" (Nakaushi group, Cell 2013))



(Hematopoietic) Stem Cell - Rare & Invisible "Was-there"



Lin → Lineage markers (various)

Sca-1 → stem cell antigen

c-kit (CD117) → tyrosine kinase receptor

(Numbers for Leukocyte Population)

$0 \leq k = k(t) \leq 1.7$ (murine system)

Need to use limiting dilution* + in vivo reconstitution to verify stem cell status ⇔ All lineages reconstituted! Granulocytes good quantitative measure of "was-there". Do not proliferate, need HSCs to replenish. 2-3 d lifespan.

* Rare events statistics

What is our Theory of In Vivo Hematopoietic Stem Cell Population Dynamics?

- Theory A:
  - All HSC are the same
  - They live forever (as a population*)
- Theory B:
  - HSCs are individually different
  - They have finite lifespans (as populations*)

Theory B provides a different basis for explaining the leukemia mechanism than Theory A.

* By definition, a hematopoietic stem cell will cease to be a hematopoietic stem cell as soon as it differentiates.

Also: Apoptosis or Senescence.

Jager's Theorem (1992) about Branching Processes: Connection to Cell Population Dynamics?

J. Appl. Prob. **29**, 770–780 (1992)
Printed in Israel

STABILITIES AND INSTABILITIES IN POPULATION DYNAMICS

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Abstract

Stability in population *size* is illusory: populations left to themselves either grow beyond all bounds or die out. But if they do not die out their *composition* stabilizes. These problems are discussed in terms of general abstract, multitype branching processes. The life and descent of a typical individual is described.

BRANCHING PROCESSES; KIN STRUCTURE; STABLE POPULATIONS; POPULATION EXTINCTION

AMS 1991 SUBJECT CLASSIFICATION: PRIMARY 60J80
SECONDARY 92A15

Connection lies in raising the Question: Do stem cells live forever??

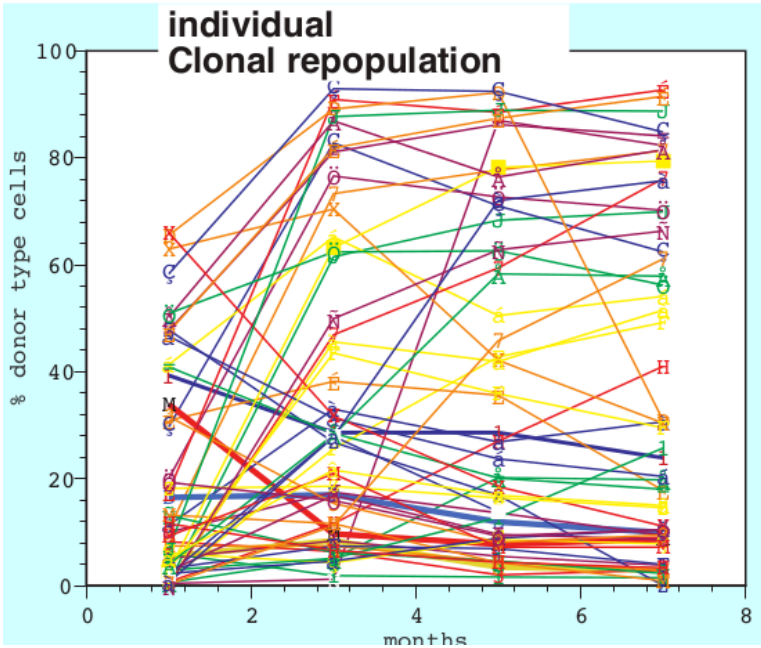
Hematopoietic Stem Cells Are Not Created Equal

In Vivo Data ($\geq$ *Muller-Sieburg et al, Blood 2002, Blood 2004*)

The repopulation kinetics of individual HSCs are wildly different.*

Proof.

$\rightarrow$ Transplant single HSCs into lethally irradiated recipients
$\rightarrow$ Obtain repopulation kinetics of clonal donor-derived mature cell populations (vert. axis) over time (hor. axis)
$\rightarrow$ Determine contribution of environment (0.2) vs HSC intrinsic behaviors (0.8) □



* By now sampled 8-10% of HSC Bio-mass ($\approx$ 1400 HSC clones)

Clonal Kinetics Heterogeneity: Order behind the Chaos?

In Vivo Data (*Muller-Sieburg, Blood 2006; Sieburg, In Silico Biology 2006*)

The HSC population is heterogeneous, but heterogeneity is limited

Proof.

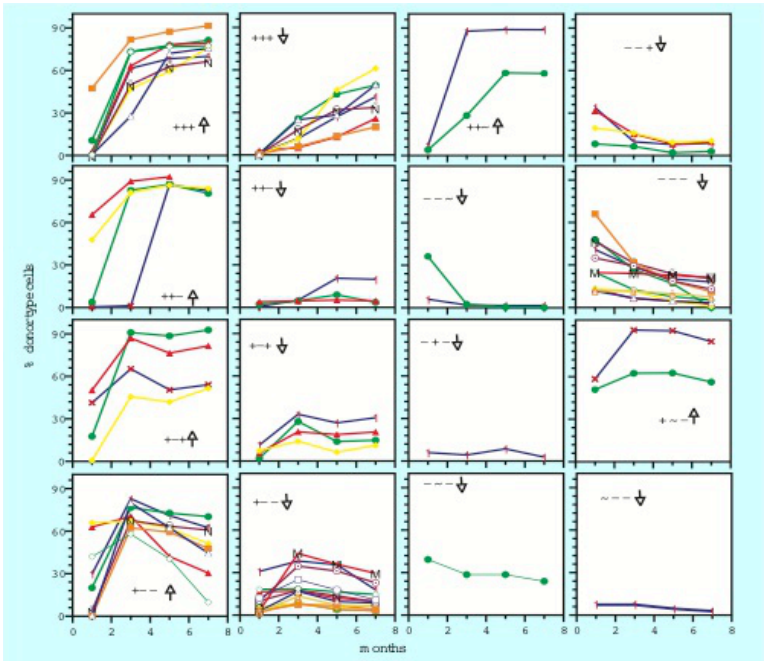
$\rightarrow$ Clonal transplants

$\rightarrow$ Symbolic Dynamics on alphabet $\{+, -, \sim\}$ ($|r_{t+1} - r_t| > B \vee \leq B$)

$\rightsquigarrow$ Found 16/81 possible shapes

$\rightarrow$ $P[\text{new groups}] \sim 10^{-72}$

(Laplace Add-one Estimator) □



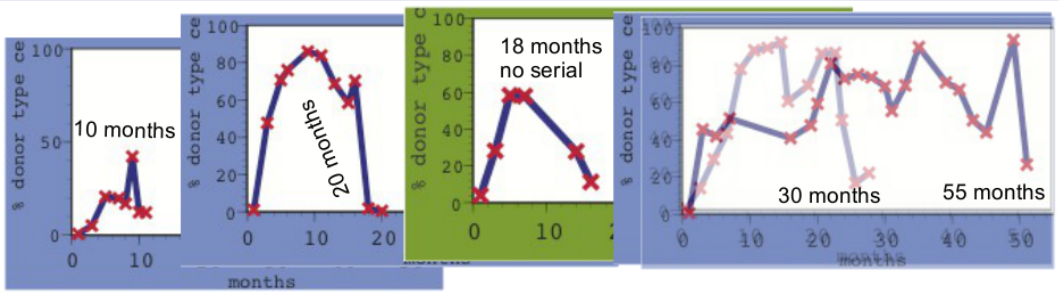
Clonal Diversity: Self-renewal Capacity Differs between Clones

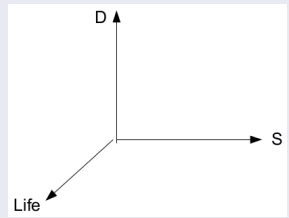
In Vivo Data (*Muller-Sieburg, Blood 2006*)

HSCs differ with respect to self-renewal capacity. Self-renewal capacity is limited ("healthy clones die out") and HSC specific.

Proof.

Follow repopulation kinetics of singly transplanted HSCs in lethally irradiated mice over time (for up to 6 years!) until 1 effector cell population is no longer reconstituted (=: clonal lifespan *).





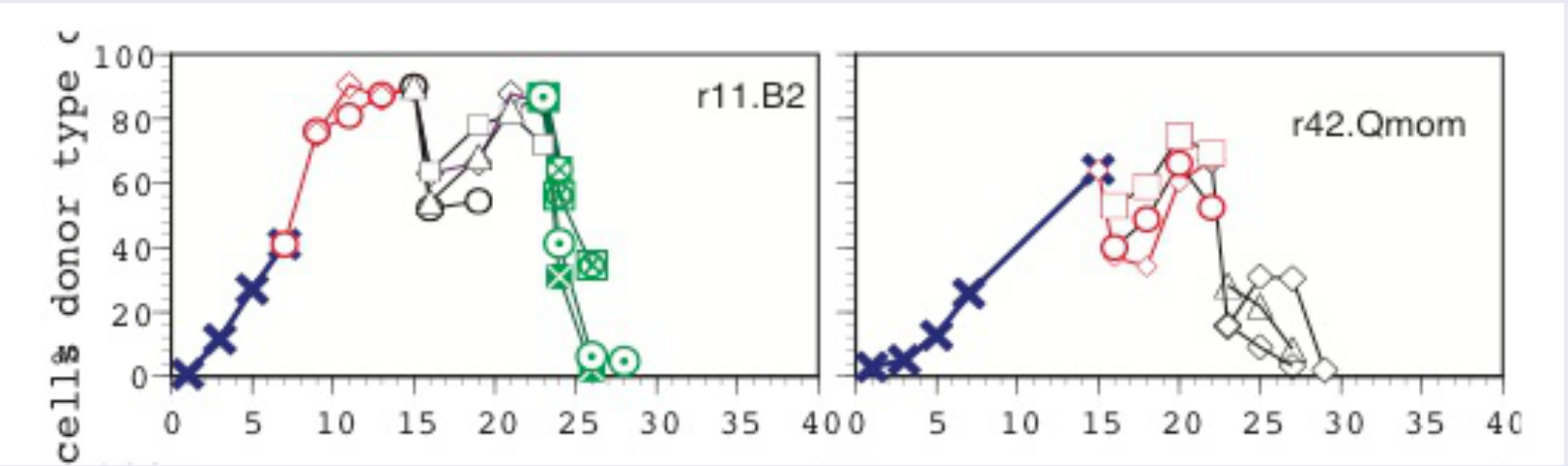
* Self-renewal "is" a time dimension (hor axis) and differentiation a "height" dimension ([%]; vert axis). □

Self-renewal Capacity: Clonal Lifespan Unique within Clones

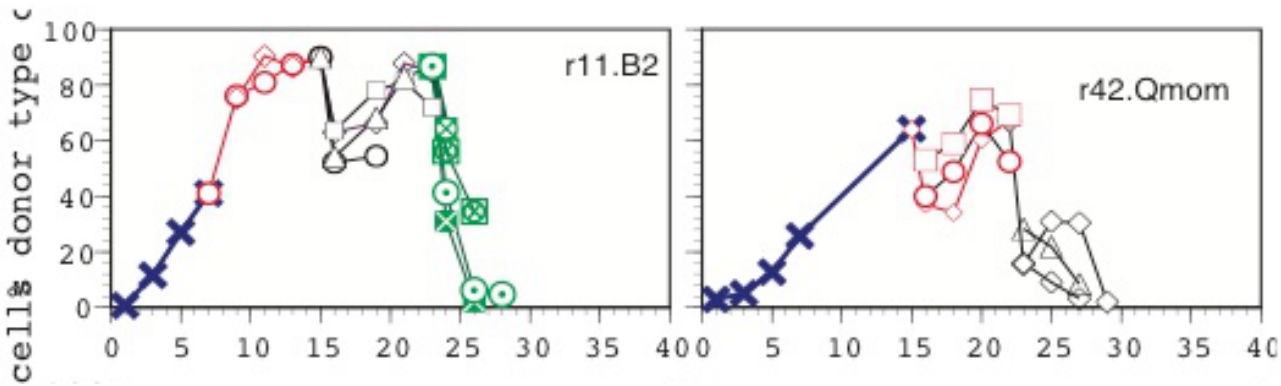
In Vivo Data (*Muller-Sieburg, Blood 2006*)

The lifespans of a partition of an HSC clone C into $m > 1$ parts $C_1, \ldots, C_m$ satisfy $T_{C_1} = T_{C_2} = \cdots = T_{C_m}$. The repopulation kinetics have similar shapes ("Programmed Lifespan").

Proof.



Lifespan (Self-renewal) Predictable Clonal System Variable



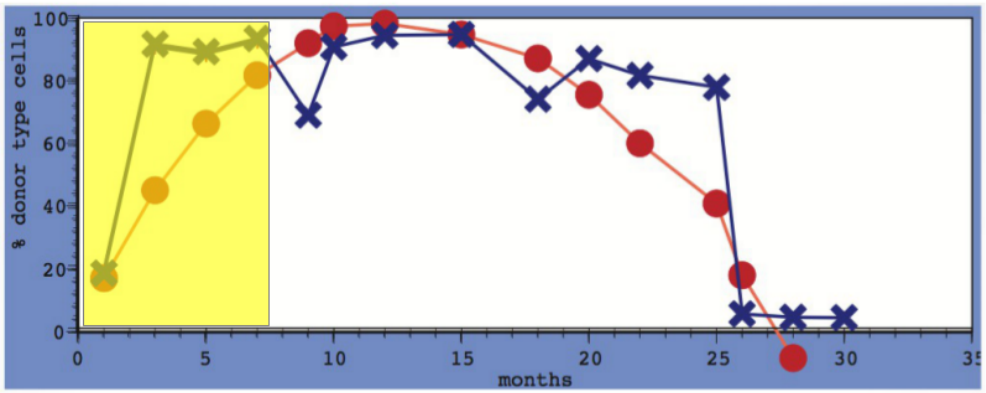
In Vivo Data (*Sieburg et al, PNAS 2011; PLOS Comp Biol 2013*)

*For every HSC with lifespan T there exist HSC specific parameters $(b, a, \alpha) : 0 < a < b \wedge \alpha > 1$ which depend on few initial conditions such that $R(t) := b\,t - a\,t^{\alpha}$ * satisfies $R(T) = 0$ ($\Leftrightarrow T = (b/a)^{1/(\alpha-1)}$). Moreover $\langle\alpha\rangle = 2 - \langle H\rangle$, $\langle H\rangle \approx 0.36$ and R minimizes $|\int_0^T R(u)du - \sum_{k=1}^{n} s_{[0,T]}(E_k)|$, E_k effector cell-types.*

* Strong Deterministic Component.

Lifespan (Self-renewal) Predictable Clonal System Variable

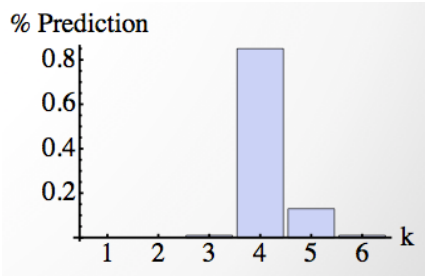
From data, most informative quantity is: [%-donor-type (time)] / time



Parameter Estim:

$b \approx 19.2$ [%/mth]
$a \approx 4.5$ [%/mth$^{\alpha-1}$]
$\alpha \approx 2 - H$, $H \approx 0.35$

Computational prediction method uses Bayesian learning algorithm from samples of complete kinetics. Data analysis shows that clonal expansion history matters. $k \geq 4$* data points needed to predict lifespan for 82% of kinetics. Further: $k \leq 5$.

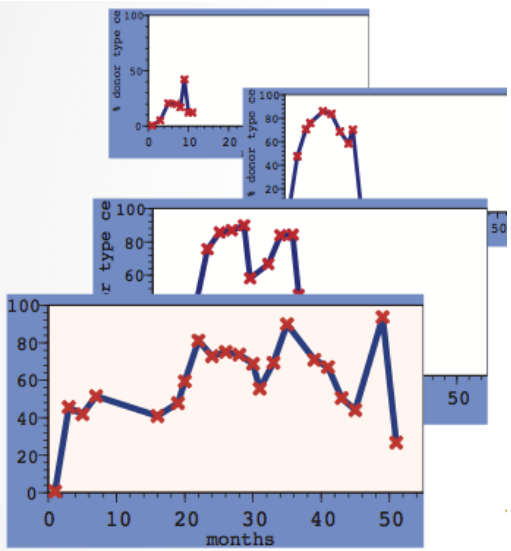


* By corollary, the findings for k allow a quantitatively precise estimate of the dreaded term "long-term repopulating HSC". "long-term" ≥ 7 [mth]

Curious Empirical Form for Self-renewal Probability

HSCs walk a thin red line between graft failure and cancer. Computer simulations to determine the conditions under which finite clonal lifespans could occur, identify a specific class of functions $\models$ self-renewal over clonal life.

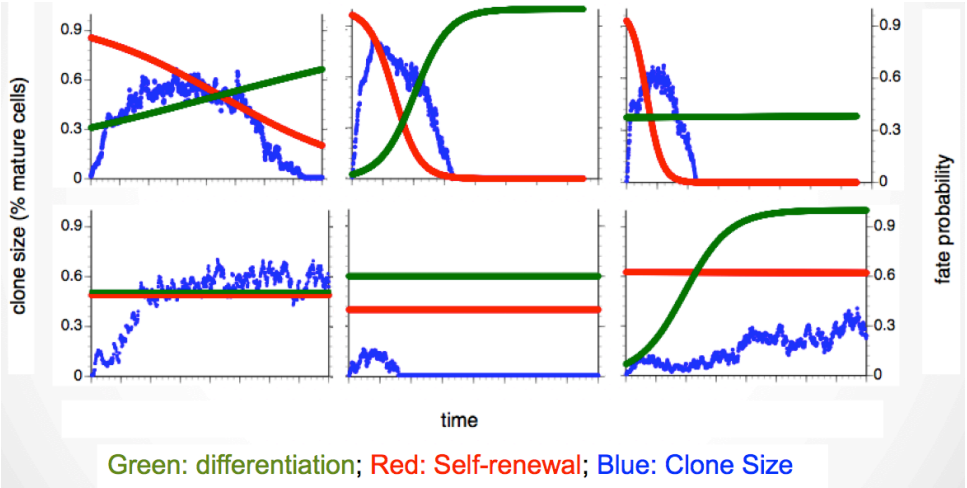
In Vivo Data



$$P_{\text{self}}(\tau, \Theta) = \frac{1}{1 + \omega \ \exp(\Theta/\tau)}$$

Fermi-Dirac Statistics and HSCs??

Simulation Data



Θ: Self-renewal history ("energy")
ω: Odds against self-renewal ($e^{-\Theta_0/\tau}$)
τ: Resistance to differentiation ("Temp")
Open Problem: $\Theta \leftrightarrow t$?

Clonal Diversity: Differentiation Capacity Differs

In Vivo Data (*Muller-Sieburg, Blood 2004*)

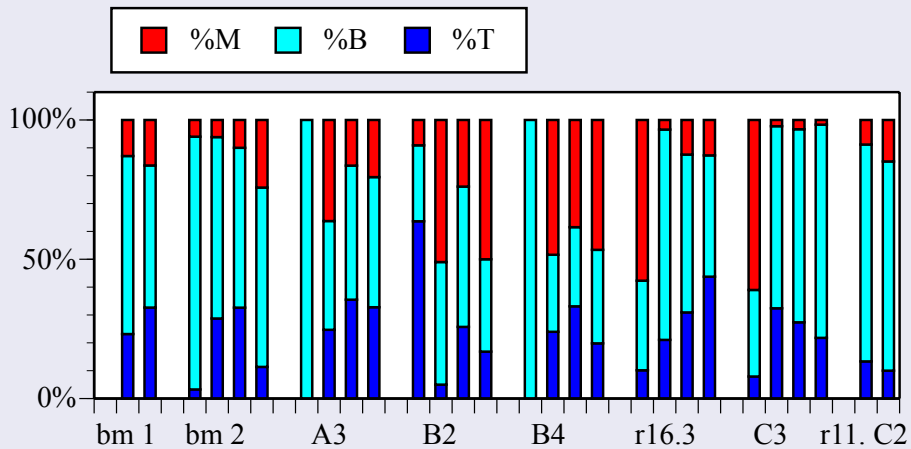
HSC population shows 3 significantly different patterns of lineage regeneration in adult. Stability: No conversion between patterns.

Proof.

Trace ratio $L(t)/M(t)$ of lymphoid to myeloid leukocytes over time:

HSC Type	Ratio
myeloid-biased*	$> 0, \leq 3$
balanced	$> 3, \leq 10$
lymphoid-biased**	$> 10, < \infty$

*: IL-7 defective Lymphocytes
**: IL-7r Δ-expressed



Conclusion: Reject Theory A; Consequences of Theory B

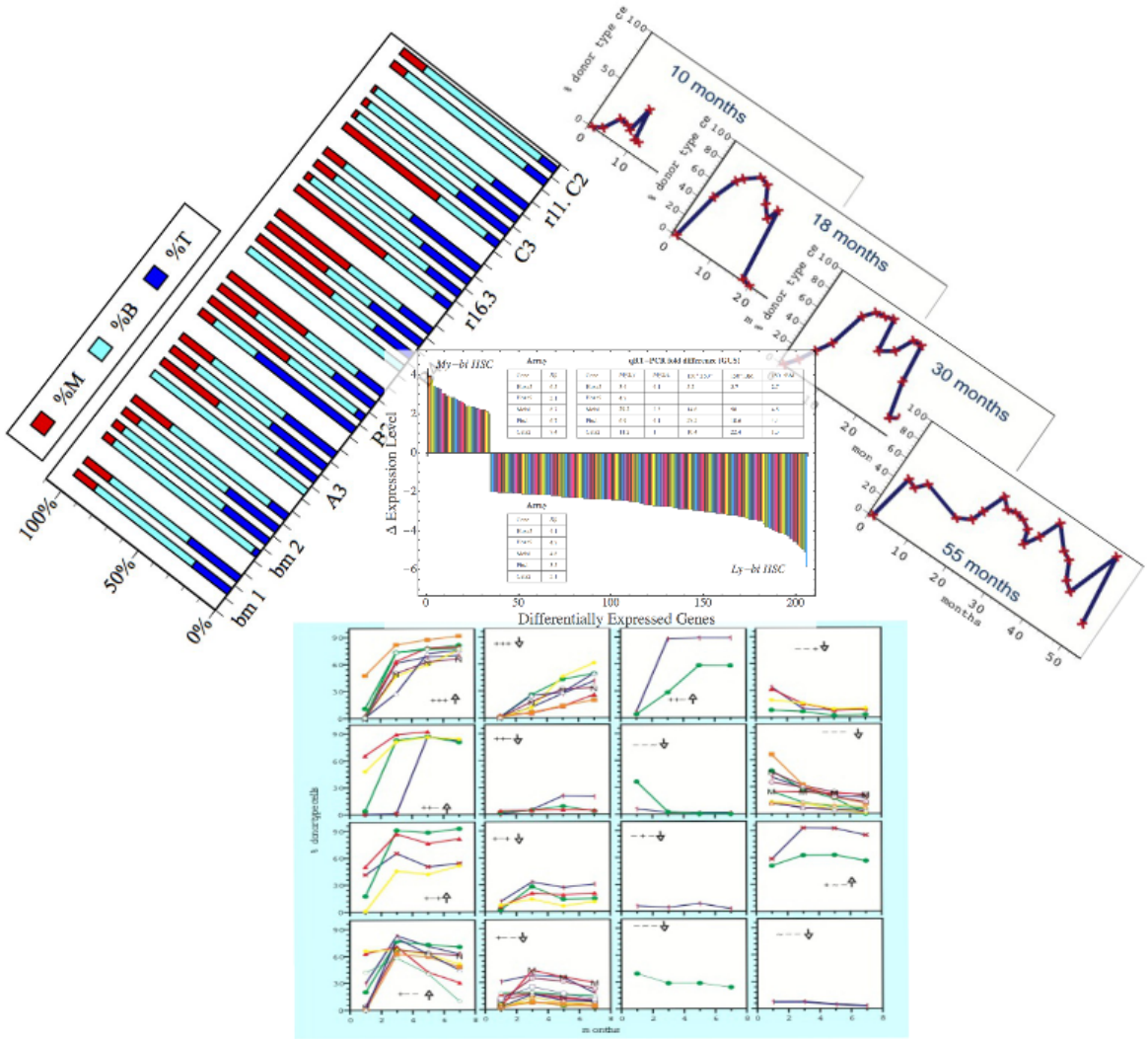
Theory A was long-time favorite, since long-term viability of transplants could be assumed.

... but reports from blood banks indicated that transplants failed in the long-run (Fred Hutchison, 1996).

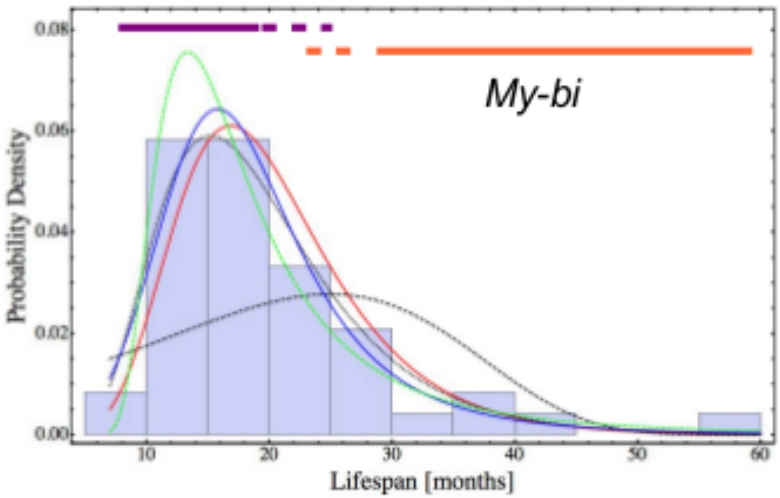
Diversity explains why.

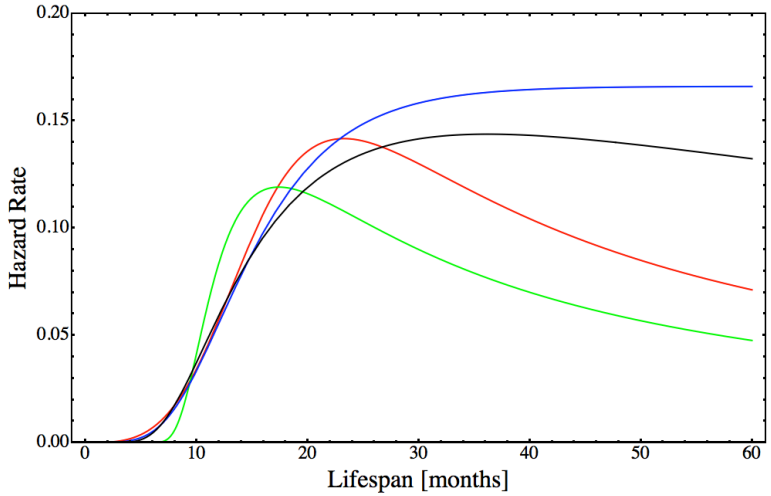
Gene expression analysis*

* Data shown from 7 [mth] Muller-Sieburg (2005), GEO # GSE41702



(Lifespan) Diversity as a Compartment Variable





Large in vivo dataset from ∼1400 clones identifies discrete distribution of lifespans. Fit continuous model to get bigger picture. Frêchet best distribution fit *.

Compartment Failure Expectation ↓
Young and adult supported by "good" differentiators (Ly-bi HSCs).
Aged supported by "good" self-renewers (My-bi HSCs) !!?

* Open Problem: What is the role of extreme value statistics in hematopoiesis?

Theory B: How come that Clones Ultimately Fail?

Definition (*"Stem Cell Reliability"*)

The reliability of a stem cell population is defined as the probability of self-renewal at time $t + \Delta t$ given self-renewal up to time t.

Theorem (*Sieburg et al, PLOS Comp Biol 2013*)

Lifespan differences in hematopoietic stem cells are due to imperfect repair and unstable mean-reversion.

"Daughter HSCs Not as Good as Old".

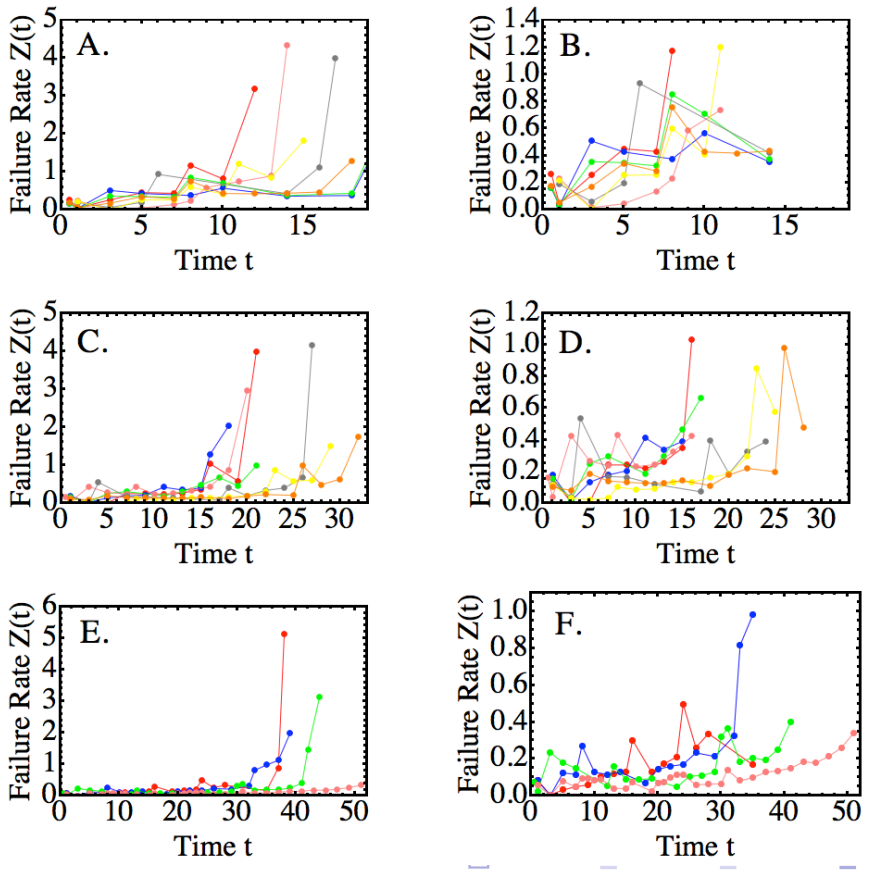
Data $\Rightarrow$ repair is needed to reestablish N-potency in new generations. Reliability: $S_k := \sum_{j=k+1}^{N} R(t_j)/t_j \;/\; \sum_{j=0}^{N} R(t_j)/t_j$, $k \geq 0$. Failure rates slowly incline with unstable mean reversion (Hurst exponent). Phase transition occurs indicated by sharp increase. Marks "point of no return" of clonal extinction. □

Δ(Repair Capacity) $\rightsquigarrow$ Δ(Clonal Reliability)

$$S = E_{\text{unrealized}}[\text{effector}|HSC] \;/\; E_{\text{total}}[\text{effector}|HSC]$$

"Reliability" motivated by demographic aging studies (Gavrilov et al, J Theor Biol 2001; Sci Aging, 2003; Handbook of Aging, 2006)

Approach: "Dissipation of failure" $\models$ Repair. "Daughter HSC not as good as old" ($:\Leftrightarrow \downarrow$ preserve N-potency) $\models$ Failure $\rightsquigarrow$ H_0: d_t(clonal failure rate) process $\sim$ Ornstein- Uhlenbeck process (baseline). Data diverge from OU near point of no return. Reject H_0



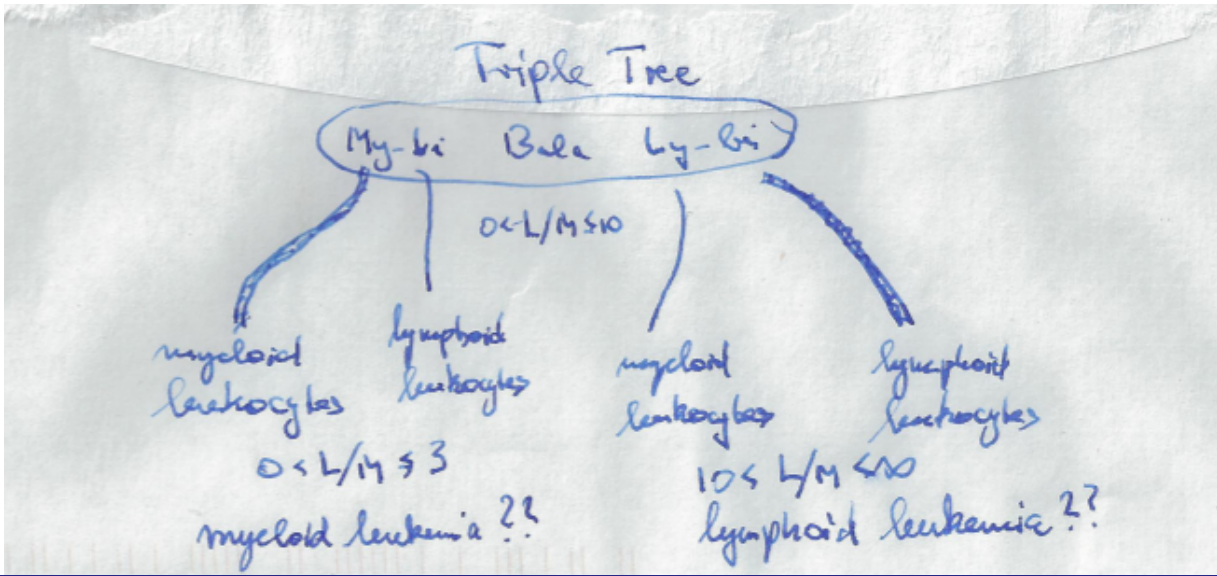
Theory B "is" our (Biological & Clinical) Theory of In Vivo Hematopoietic Stem Cell Population Dynamics

With diversity (Theory B) accepted, we can now go about drawing conclusions about cancer by comparing normal vs cancer cell population dynamics.

- Theory B (*"Stem Cells are Diverse"*):
  - HSCs are individually different
  - They have finite lifespans ($\neq$ organism time)

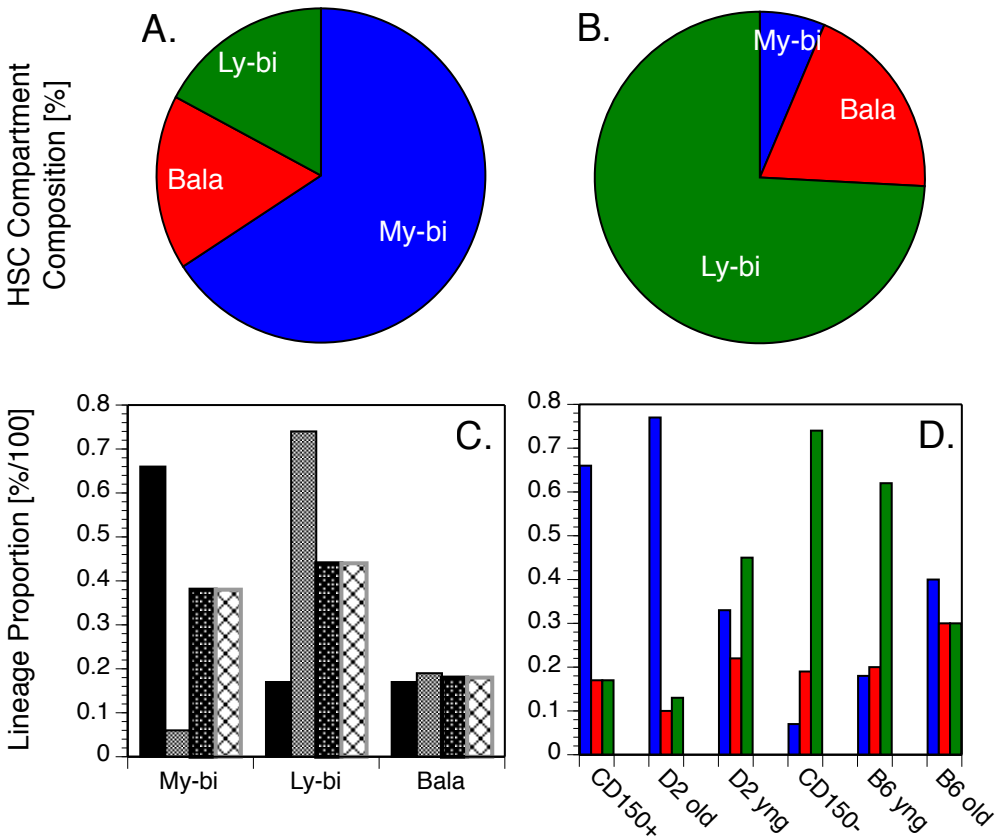
Qsts: (a) HSC Type $\models$ Leukemia Type?; (b) Why Age Increase?

In murine (Muller-Sieburg et al (2002); Dykstra et al (2009); Callen et al (2010)) and human systems (Weissman et al (2011))



Distribution of My-bi, Ly-bi, Bala HSCs Age-dependent

$$\{HSC\} = w_{\text{mybi}}(t)\ \{\text{HSC}_{\text{mybi}}\} + w_{\text{bala}}(t)\ \{\text{HSC}_{\text{bala}}\} + w_{\text{lybi}}(t)\ \{\text{HSC}_{\text{lybi}}\}$$



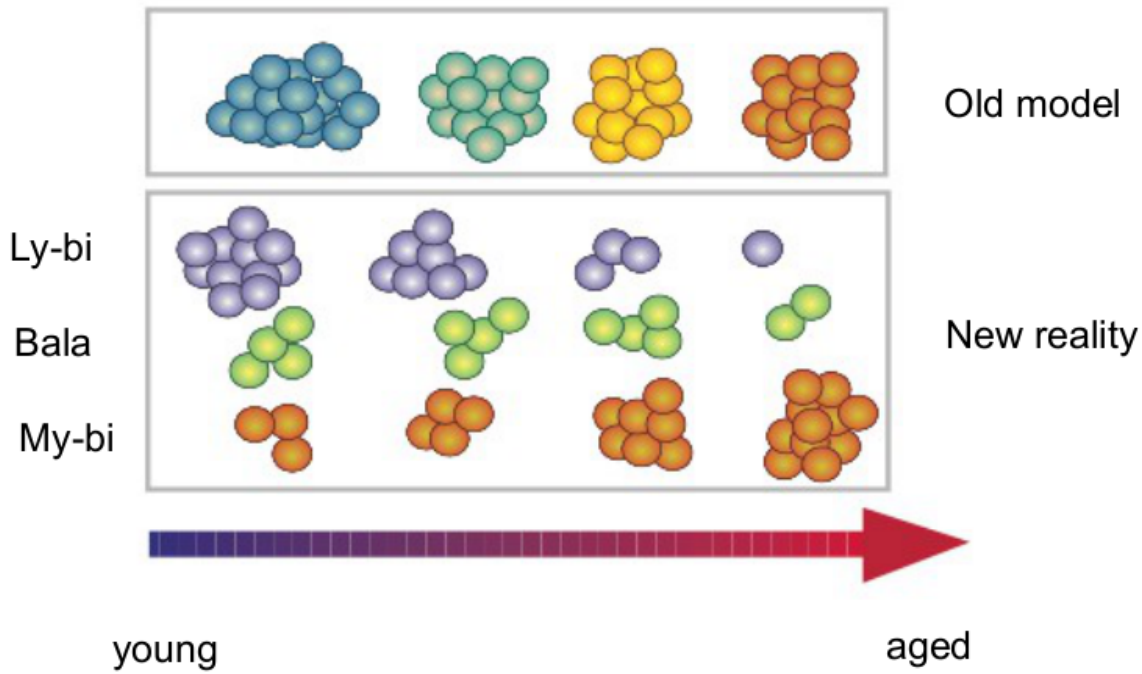
A, B: Weights for $CD150^+$, $CD150^-$ derived HSCs

C: $CD150^+$, $CD150^-$ HSC content significantly different

D: Age-, and strain differences for limiting dilution derived Lin^-Sca1^+ HSCs

Aging of Hematopoiesis "is" Shift in System Composition

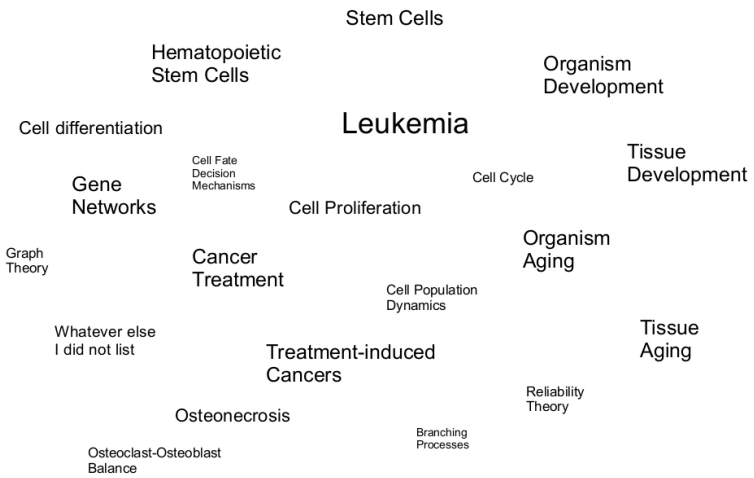
Hematopoietic aging is characterized by a shift in the composition of the HSC compartment (Muller-Sieburg et al, Blood 2008; Challen, Cell 2011) from Ly-bi HSC dominated in young to My-bi HSC dominated in aged.

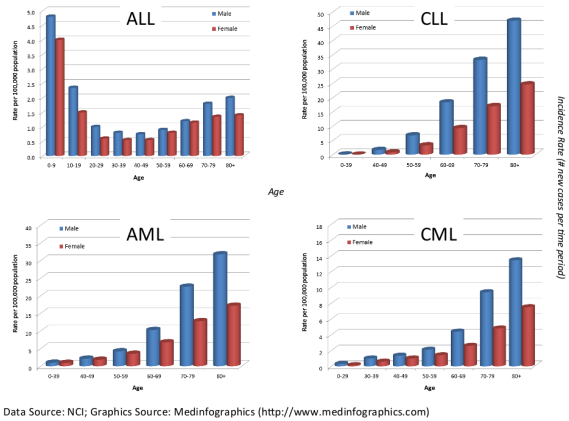


$\{$HSC$\}$ Aging Shift $\models$ Age Incidence of Leukemia?

Working Hypothesis

(a) Shift in composition to high myeloid-biased (My-bi) HSC content of HSC pool explains high incidence of myeloid leukemias in the aged.
(b) High incidence of acute lymphoid leukemia (ALL) in the young due to high lymphoid-biased (Ly-bi) HSC content. Lower ALL incidence in aged due to shift towards higher My-bi HSC content. Together, these account for the curious "bathtub" shape of ALL incidence rate.



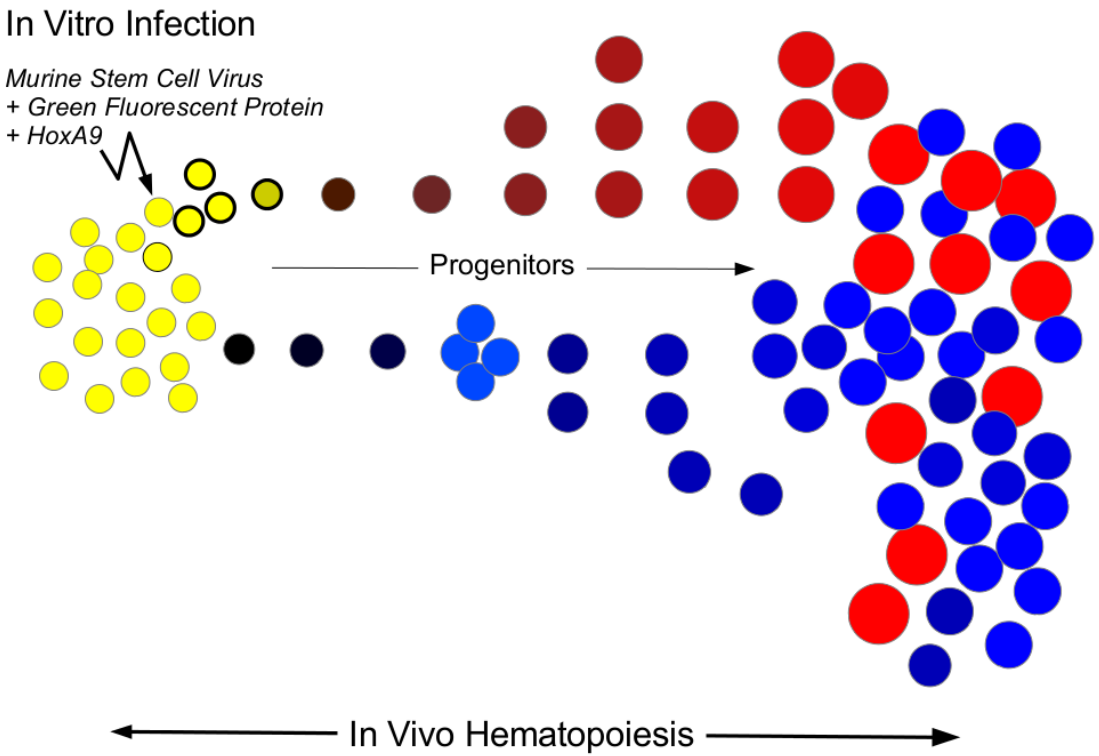


Data Source: NCI; Graphics Source: Medinfographics (http://www.medinfographics.com)

Epigenetic Program of HSC $\models$ Origin of Leukemia Type?

A Leukemia Model based on Over-Expressing HoxA9 in HSC

The Homeobox Cluster A 9 (HoxA9) gene is a body plan gene. HoxA9 $\Uparrow \rightsquigarrow$ HSC $\circlearrowleft\uparrow \wedge$ HSC $\pitchfork\approx$ ($\uparrow$ self-renewal $\leftrightarrow \downarrow$ lifespan). HoxA9 alone drives leukemia only slowly. Co-factors Meis1 & Pbx1 $\nearrow$ progression.



HoxA9$\Uparrow$ = designed failure event

Generates chimerism:

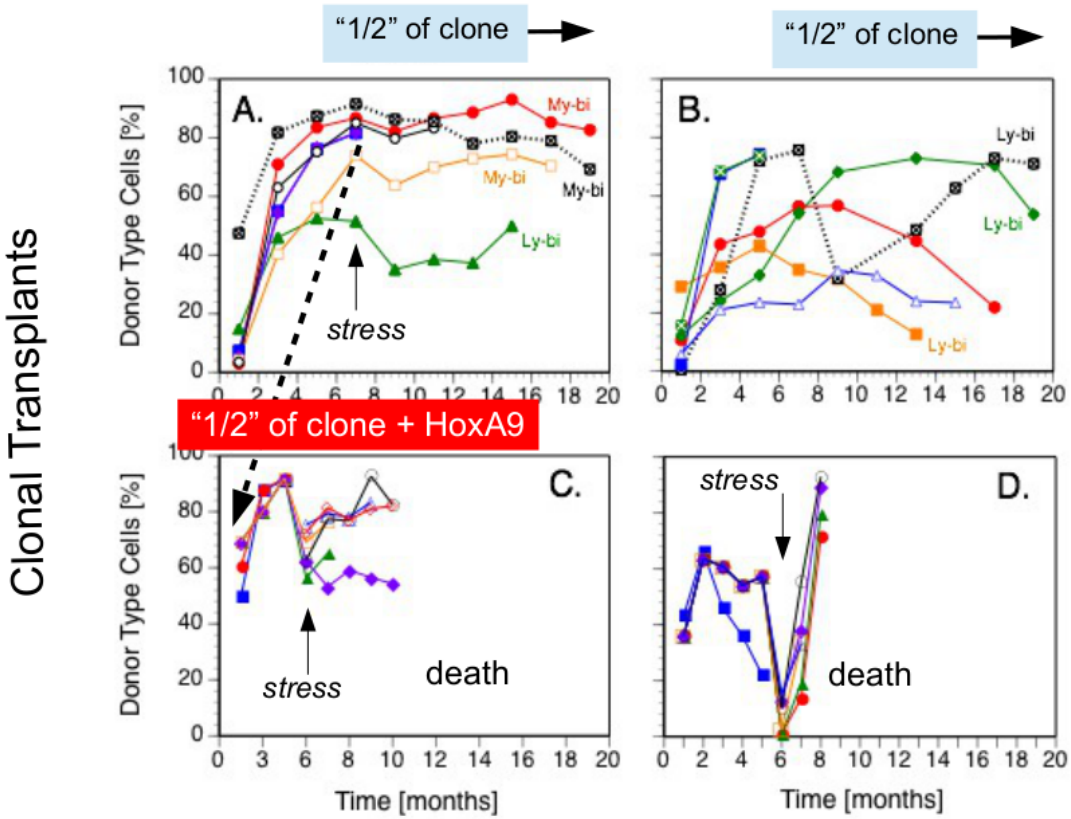
$$(\text{HSC population})_t = = (\text{HSC}^{\text{hoxa9}\sim})_t + (\text{HSC}^{\text{hoxa9}\Uparrow})_t$$

& two time scales

$$\frac{(\text{HSC}^{\text{hoxa9}\sim})_t}{(\text{HSC}^{\text{hoxa9}\Uparrow})_t} \rightarrow 0 \ ?$$

Repopulation Kinetics Differences HSCs vs HSCs-HoxA9⇑

Chimeric transplants regenerate systems normally (lifespan shortening suggested for Ly-bi). 1 [mth] post stress, Ly-bi regen capacity low.



A, B: Normal.
C: My-bi HSCs HoxA9⇑
D: Ly-bi HSCs HoxA9⇑

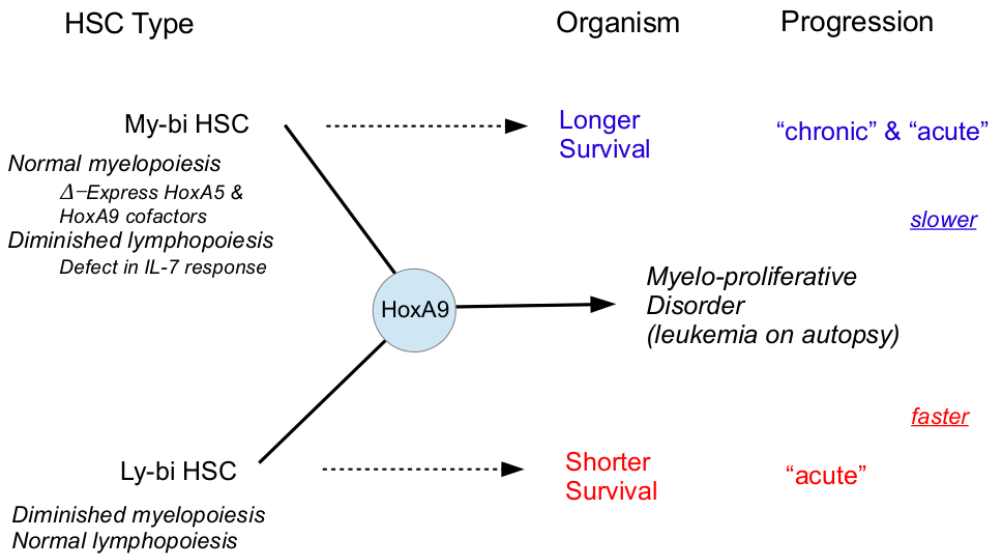
Colors: Repopulation kinetics of different clones

Dashed arrow: Normal HSCs transfected with HoxA9 & transplanted into new recipients.

HoxA9 Over-Expression: System Effects Found

In Vivo Data (*unpublished*)

Myeloid proliferative disorder in both. Faster progression in recipients reconstituted with Ly-bi HSCs. "Chronic" phase My-bi only.



Interpretation (*Using Diversity*)

$(HSC\ population)_t =$
$= (HSC^{hoxa9\sim})_t + (HSC^{hoxa9\Uparrow})_t$

Hoxa9 $\Uparrow \rightsquigarrow$ *self-renewal* $\uparrow$ *& differentiation unaffected*

$\rightsquigarrow$ *HoxA9* $\Uparrow$ *shortens lifespan*

$\Rightarrow$ *More efficient self-renewers (My-bi HSCs) last longer* ✓

Accelerated clonal aging is a key effect of HoxA9$\Uparrow$. Faster aging can also be achieved via the Wnt inhibitor Dkk, but w/o white blood and large spleens.

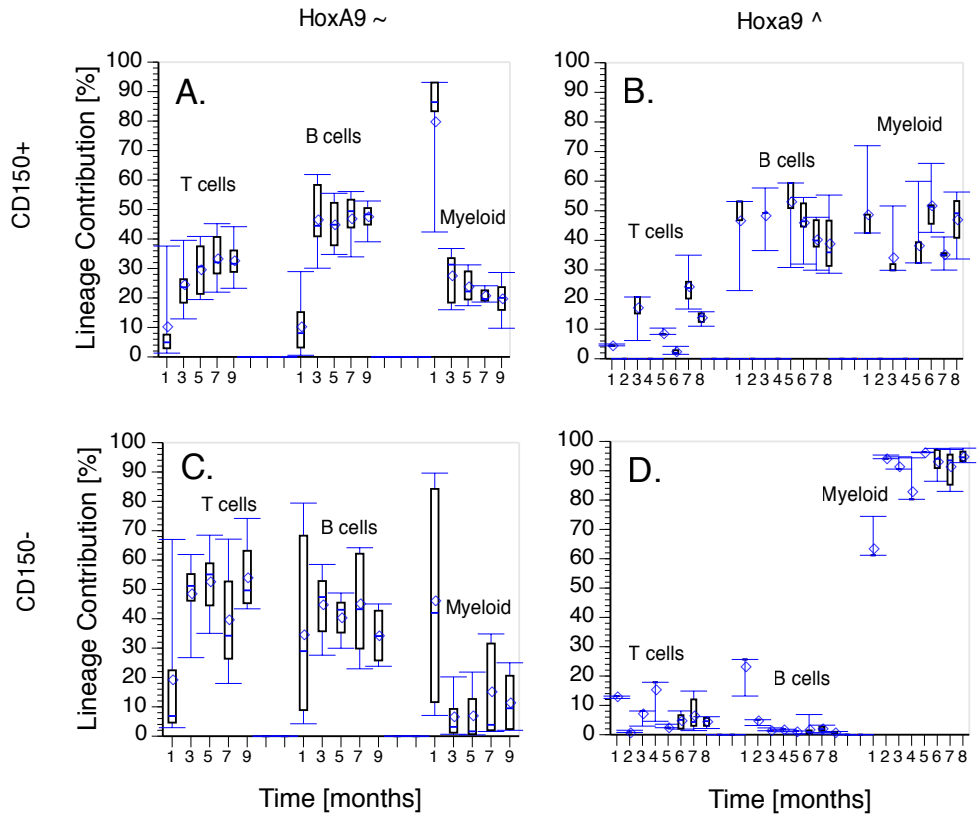
Differentiation Pattern Differences HSCs vs HSCs-HoxA9⇑

Kinetics of T and B lymphocyte (L), and macrophage granulocyte myeloid cell (M) populations. In the limit: $M(t) \uparrow\uparrow$, $L(t) \ll 1$.

	HSC	HoxA9⇑
A:	My-bi	no
B:	My-bi	yes
C:	Ly-bi	no
D:	Ly-bi	yes

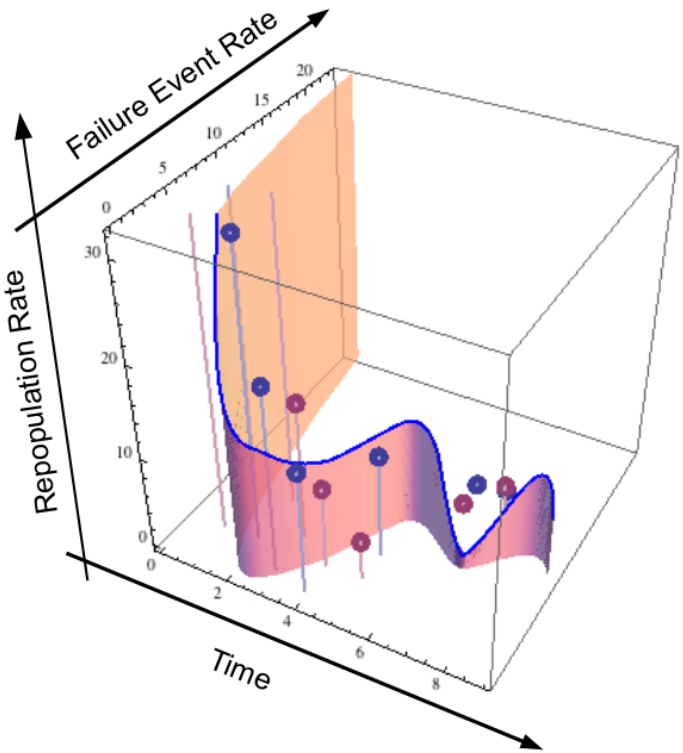
My-bi := myeloid-biased HSC
Ly-bi := lymphoid-biased HSC

Data for multiple clones (box-whisker plots over time [mth])

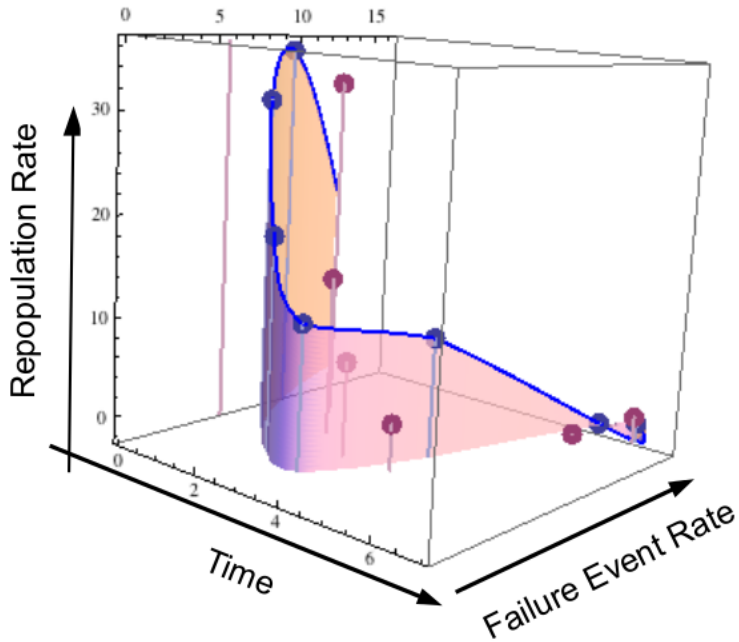


Disease Progression Dynamics: $F(t, g(t)/t, r(t)/t)$

Used raw data of % donor-type cells $(r_1, \ldots, r_n)$ and % GFP positive cells $(g_1, \ldots, g_n)$ at time points $t_1, \ldots, t_n$ to define rates $\rho_i := r_i/t_i$ and $\gamma_i := g_i/t_i$. Plotted (t_i, ρ_i, γ_i) as ball-and-stick and interpolation function (blue curve).



Myeloid-biased HSCs HoxA9⇑

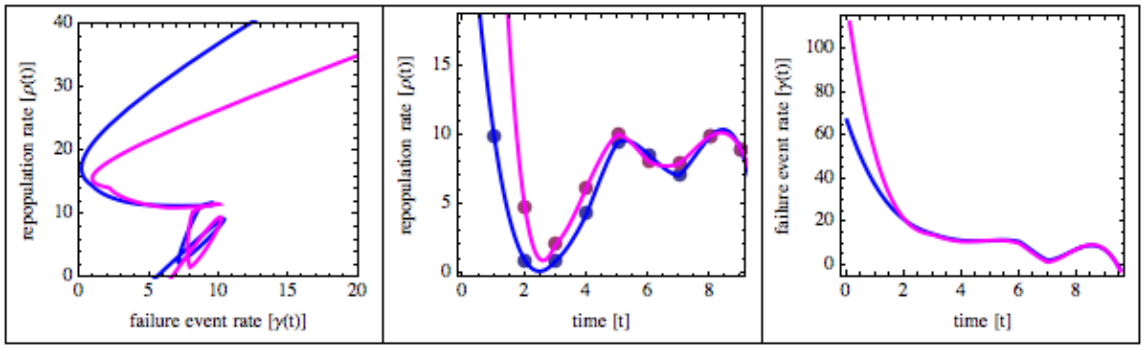


Lymphoid-biased HSCs HoxA9⇑

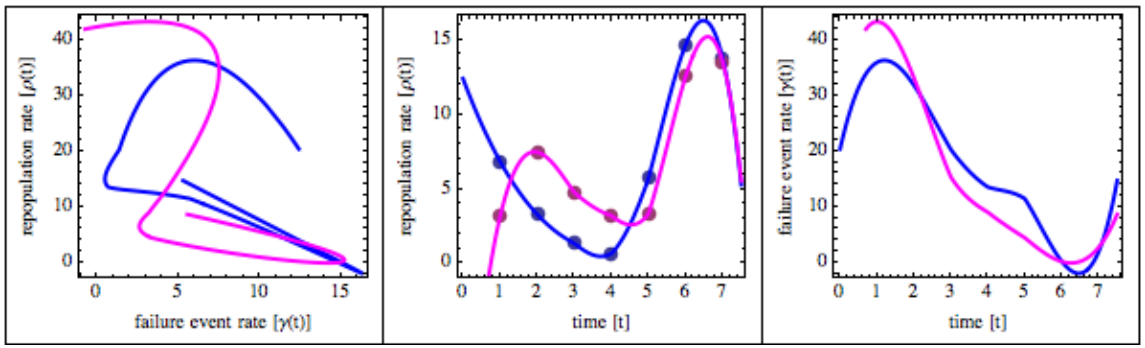
Decipher Dynamics Information: Planar Projections

Used raw data of % donor-type cells $(r_1, \ldots, r_n)$ and % GFP positive cells $(g_1, \ldots, g_n)$ at time points $t_1, \ldots, t_n$ to define rates $\rho_i := r_i/t_i$ and $\gamma_i := g_i/t_i$. Plots (ρ_i, γ_i), (t_i, ρ_i), (t_i, γ_i) for 2 sample experiments (blue, magenta curves).

Myeloid-biased HSCs HoxA9⇑

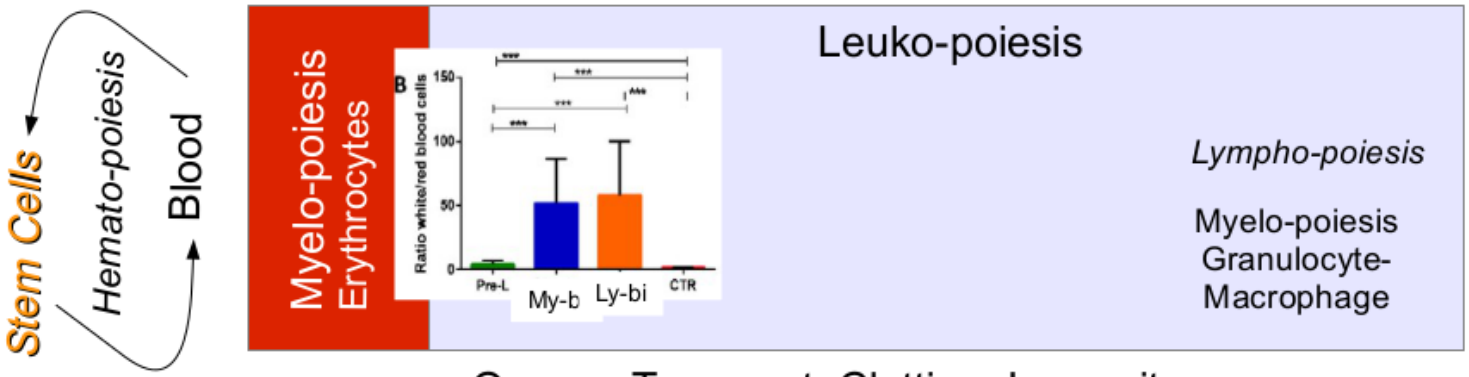


Lymphoid-biased HSCs HoxA9⇑

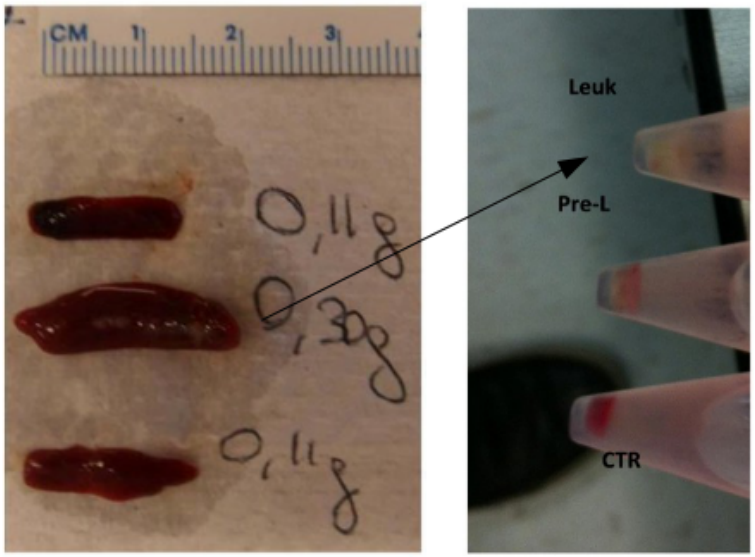


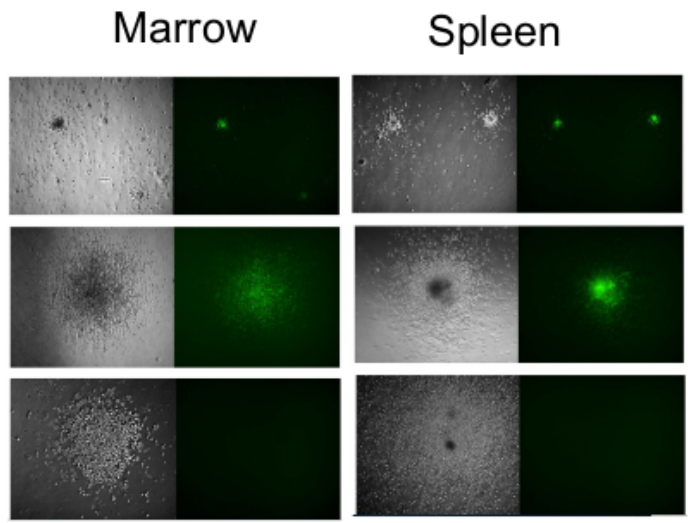
Saturation attractor. Coupling of $d_t(r(t)/t)$ and $d_t(g(t)/t)$?

Virchow-Kölliker-Neumann Type Evidence? Yes.



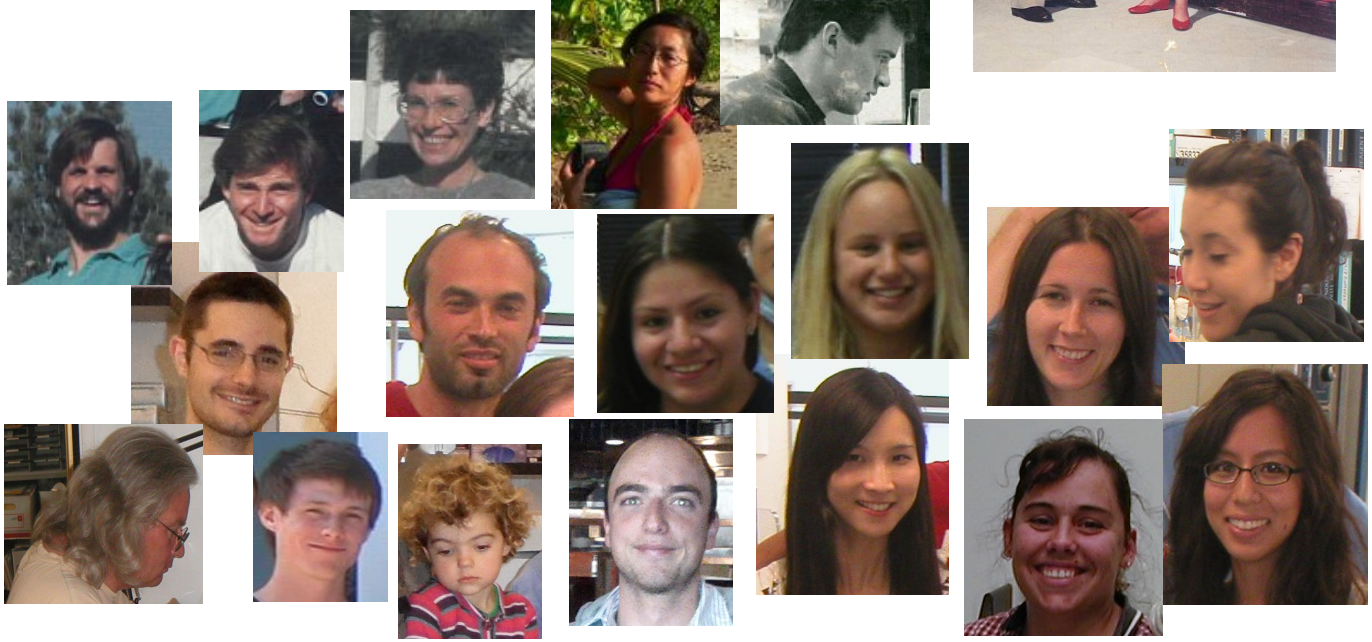
Oxygen Transport, Clotting, Immunity





Thank you to Christa and the Christa Muller-Sieburg Laboratory

- Leukemia Lymphoma Society
- National Institute for Diabetes, Digestive & Kidney Disease
- National Institute for Aging
- National Heart, Lung and Blood Institute
- Department of Defense
- Eli Lilly Pharmaceuticals



List of Notations Used.

$\exists$	"there exists"
$\Rightarrow$	"implies
$\Leftrightarrow$	"equivalent"
$A := B$	A "defined by" B
$\rightsquigarrow$	"leads to"
$\ll$	"much less than"
$\gg$	"much larger than"

$\circlearrowleft$	self-renewal
$\pitchfork$	differentiation
$X \Uparrow$	X "over-expressed"
$X \nearrow$	X increasing
$X \searrow$	X decreasing
$X(\uparrow) \uparrow$	X (highly) increased
$X(\downarrow) \downarrow$	X (highly) decreased
$X \approx$	X "normal"
$X \rightarrow Y$	X transforms to Y