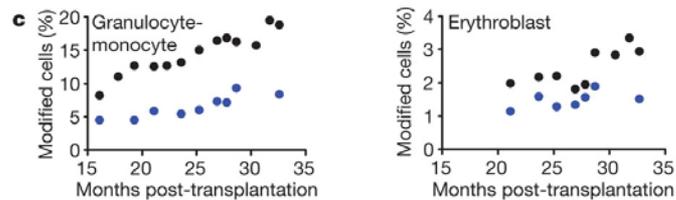
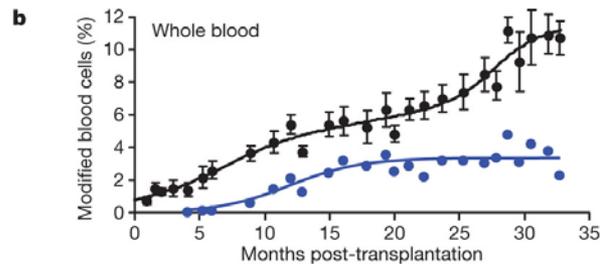
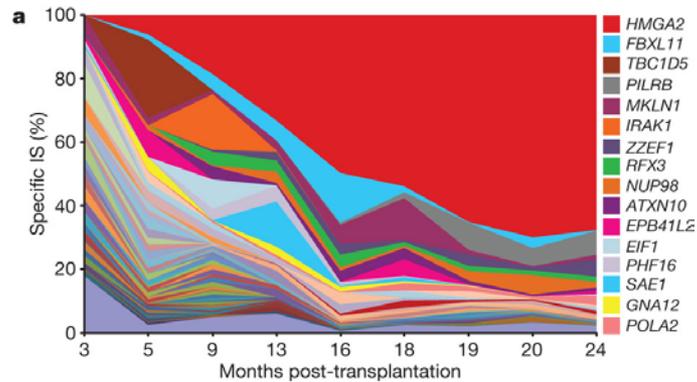


**What has been learned from
mathematical modeling of
hematopoietic stem cell repopulation
and studies of the development of
clonal dominance that can be applied
to gene transfer research?**

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The issue



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BM subset	Months	
	20	24
BM BFU-E/ <i>HMGA2</i> IS (%)	16.7	8.2
BM CFU-GM/ <i>HMGA2</i> IS (%)	15.6	8.7
BM LTC-IC/ <i>HMGA2</i> IS (%)		5.6

Can clonal dominance be a stochastic event?

Does clonal dominance imply neoplasia?

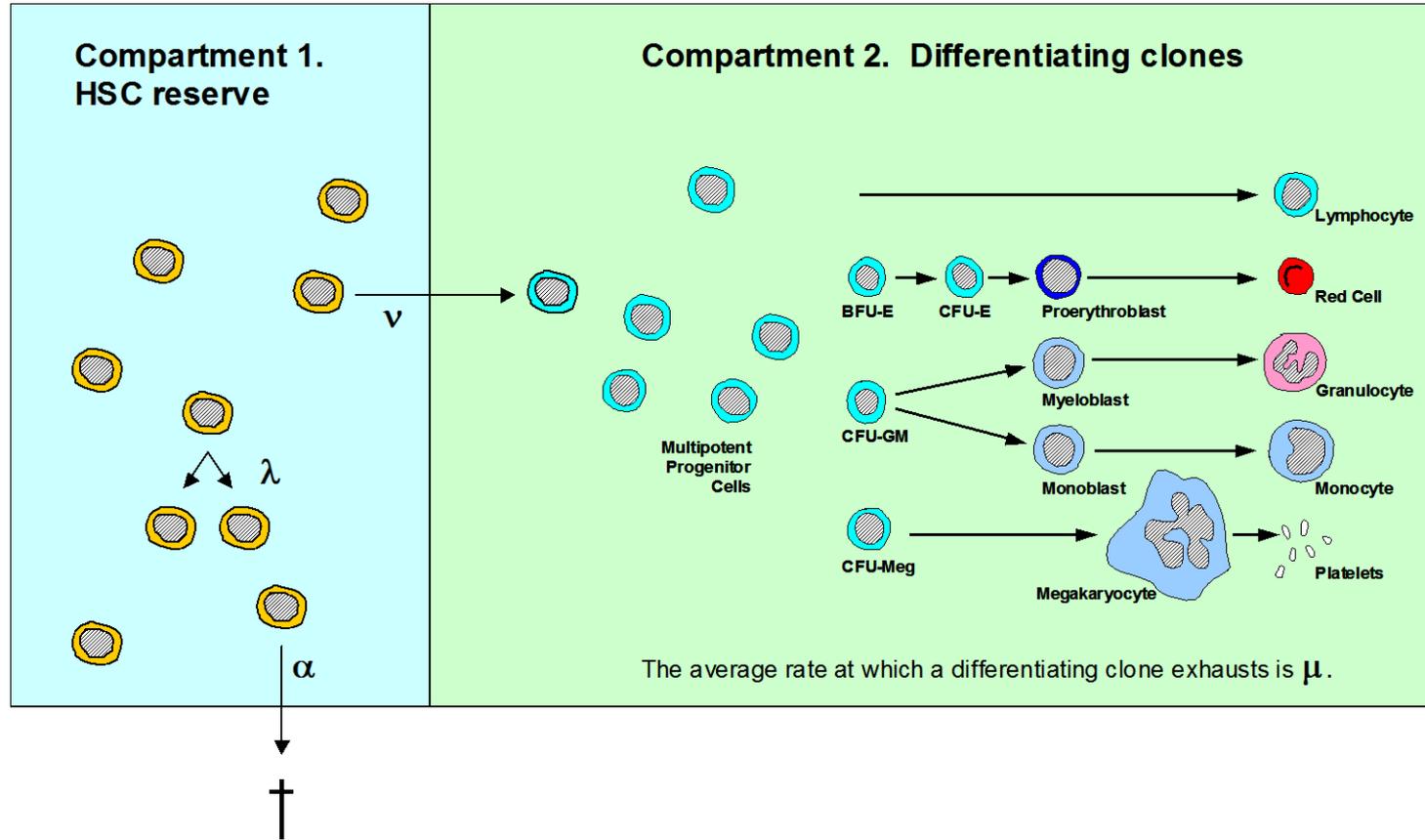
Stochastic modeling as an approach to heterogeneity

Traffic as a system



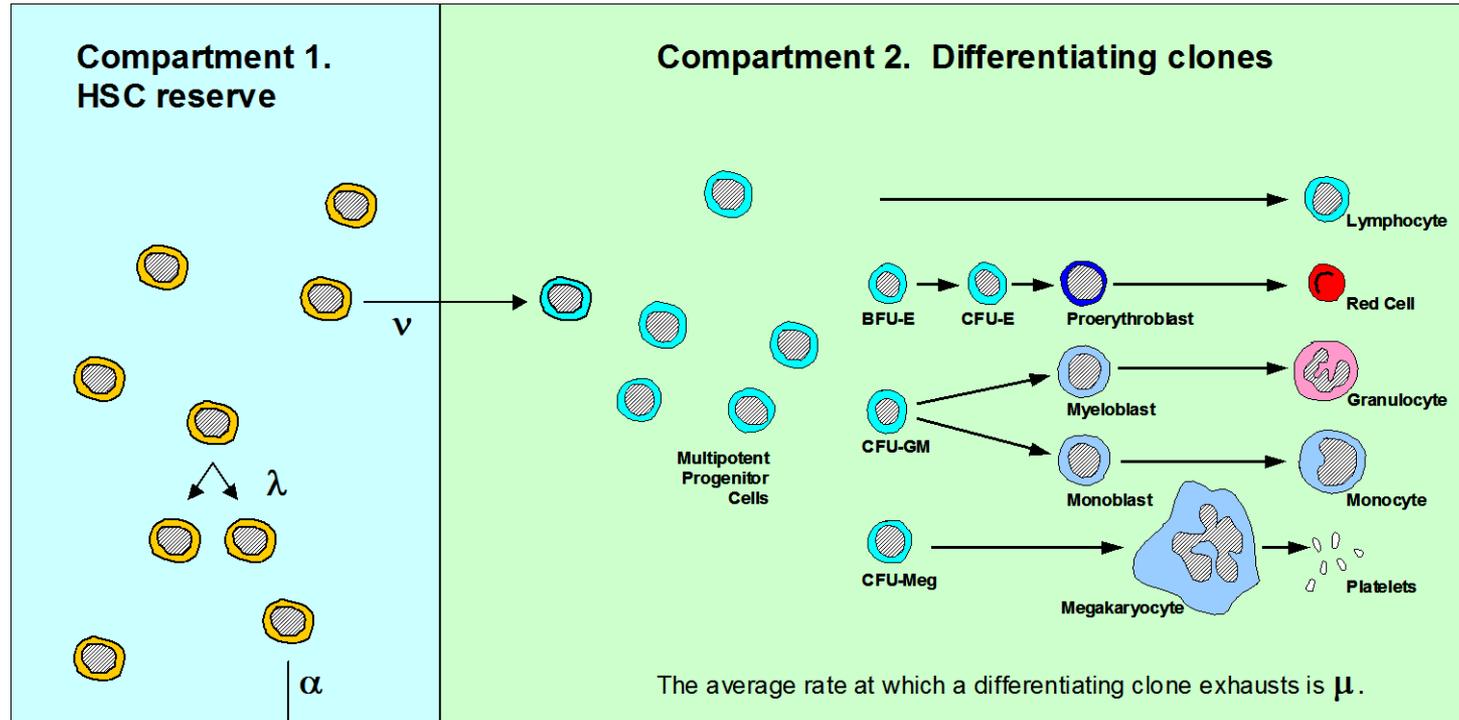
- Persons, akin to HSCs, live in homes, make independent and unique decisions, and mobilize.
- One could query decision-making with surveys.
- Alternatively, one could view traffic patterns (system outputs) and deduce average behaviors and predict system perturbation.

Hematopoiesis as a system



- There is a number of HSCs (N) and a steady state number of HSCs (K).
- HSCs can replicate, differentiate or die. Mean rates are termed λ , ν , and α , respectively.
- Once a HSC differentiates there is a mean time over which its progeny contribute to blood cell production (the rate of exhaustion is termed μ).
- Thus we can describe hematopoiesis with **5 facts**: N is $\leq K$ and λ , ν , α , and μ exist.

Hematopoiesis as a system



†

2 assumptions.

- HSCs do not act based on history (do not remember prior decisions), but rather act depending on immediate input, such as interactions with the environment (the Markovian assumption).
- On average, all contributing clones contribute equally.

Questions

- How frequent are hematopoietic stem cells in the marrow? What is N ?
- How do they support hematopoiesis over a lifetime? What are λ , ν , α , and μ ?

Methods

- Analysis of limiting dilution competitive transplantation studies in mouse and female Safari (G6PD heterozygous) cat.
- Simulated outcomes with arbitrary parameter values and asked if the observed data could be a random draw from the simulated data. If yes, the values were retained; if no, the values were discarded. Explored 5 dimensional space and determined plausible values.
- Showed that the results were concordant with experiments of others using unrelated methods, such as BrdU labeling and single cell transplantation in mouse.

Results

	<u>Cat</u>	<u>Mouse</u>
Frequency of HSCs	6 per 10^7 NMCs	4-8 per 10^5 NMCs*
λ (HSC replication rate)	1 per 8.3-10 wks	1 per 2.5 wks*
ν (HSC differentiation rate)	1 per 12.5 wks	1 per 3.4 wks
α (HSC apoptosis rate)	0-1 per 50 wks	1 per 20 wks
$1/\mu$ (Time clones contribute to hematopoiesis)	6.7 wks	6.9 wks*

Nature Med 2:190,1996; Blood 96:3399,2000.

* Confirmed by others with flow cytometry, BrdU labeling and single cell transplantation studies.

What about human HSCs?

- Competitive transplantation studies were not feasible.
- Use surrogate assays.
 - Telomere shortening with aging in granulocytes (Peter Lansdorp, Vancouver, Canada).
 - Skewing of X-chromosome inactivation pattern in blood cells from women (Lambert Busque, Montreal, Canada; Rosemary Gale, London UK).
- Validate the analysis methods in cat, then apply this to man.

λ for human HSCs = once per 45 weeks (range 23-67 weeks)(telomere data)¹

λ for human HSCs = once per 40 weeks (range 25-50 weeks)(XCI data)²

¹ Exp Hematol 32:1080, 2004; ²submitted, in revision.

Does this make sense?

Simulations of human hematopoiesis

Virtual marrow transplantations (70 kg donor, 70 kg recipient)

- Transplantation of 2×10^8 marrow cells/kg = 100 HSCs. All virtual recipients engraft and maintain polyclonal hematopoiesis.
- Transplantation of 1×10^8 marrow cells/kg = 50 HSCs. Graft failure occurs in 15% of the simulations.
- Interestingly, this is not b/o a lack of HSCs, but a lack of differentiating clones.
- As an example, 4×10^6 CD34+ cells/kg is $\sim 4 \times 10^8$ marrow cells/kg or 200 HSCs in the autologous transplantation of a 70 kg donor/recipient.

**A simulation tool to visualize the
hematopoietic system and for
virtual experiments**



Application to gene therapy

- Simulation studies suggest one must transduce more than 6 HSCs (i.e. >3% of 200 HSCs) to continually detect marked granulocytes. In simulations in which 4 (2%) of 200 HSCs are transduced, granulocyte marking is intermittent, but T-lymphocytes are continuously marked and numerous. The excellent outcomes seen in SCID gene therapy likely results from the preferential expansion and persistence of corrected T-lymphocytes.
- When more than 20 HSCs (10% of 200 HSCs) are transduced, clonal dominance is rare (when assessed by integration site and not by a hemizygous marker, such as G6PD phenotype). For this to be a frequent event, fewer than 5-10 HSCs need to be transduced.
- A small selective advantage b/o insertional mutagenesis easily results in clonal dominance should the advantage occur at the HSC level, because HSC are long-lived. This is especially when true small numbers of HSCs (i.e. <50 HSCs) are transduced. A similar selective advantage is less relevant at the STRC level.

Conceptual issues to remember

- When a retrovirus labels a HSC and it then divides (self-renews), 2 HSCs have identical labels.
- Young children likely have a higher density of HSCs, i.e., more HSCs per marrow mononuclear cells, than adults.
- T cells are long lived and whole blood clonality assessments will have a mixture of granulocytes (reasonable surrogates for HSC marking) and T cells.
- Some cells have multiple insertions so that the number of insertions is not a perfect reflection of the number of clones. However, this should be discernable by following the patterns of contributions over time.
- Following clonal dynamics via insertion site analysis is a powerful experimental method and should allow direct testing of fundamental questions of HSC biology.

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Peter Lansdorp. Terry Fox.

Cindy Dunbar, NIH.

Hans Peter Kiem, FHCRC.

Lambert Busque, Montreal.

Rosemary Gale. London.

Insights into HSC behavior from stochastic modeling

- Number conserved through evolution. Relatively stable with aging. HSCs are more frequent in mouse than cat or man.
- Niches control HSC number. Mobilization is the physiological process that allows HSCs to distribute through the marrow space.
- HSCs are relatively quiescent. Mouse HSCs replicate once per ~2.5 wks. HSCs in larger animals and man replicate less frequently, perhaps once per ~40 week in man. HSCs divide ~80-200 times per animal lifetime. This allows HSCs to maintain genetic integrity, yet support blood cell production throughout an animal's lifetime.
- Differentiation must occur less frequently than replication. In larger animal and man, differentiating stem cell clones are especially important (and vulnerable) as each must contribute many cells to maintain blood cell numbers.