Dynamics of hematopoiesis and its disorders

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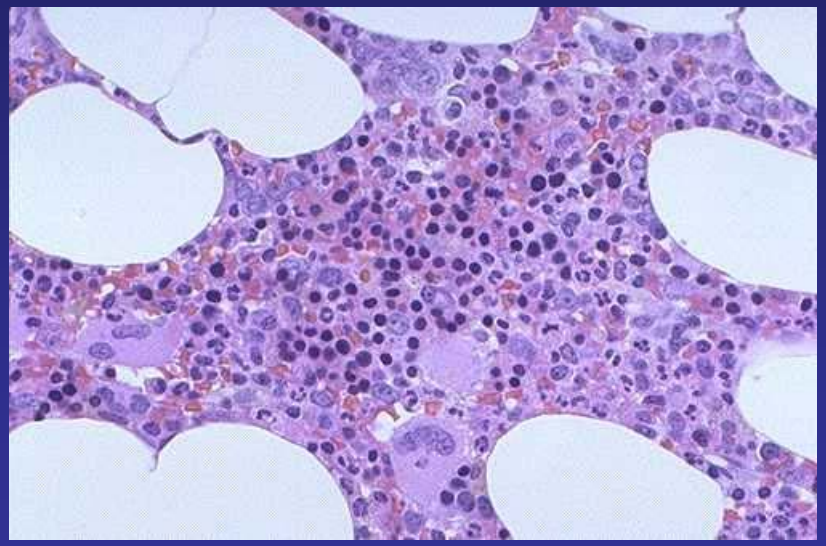
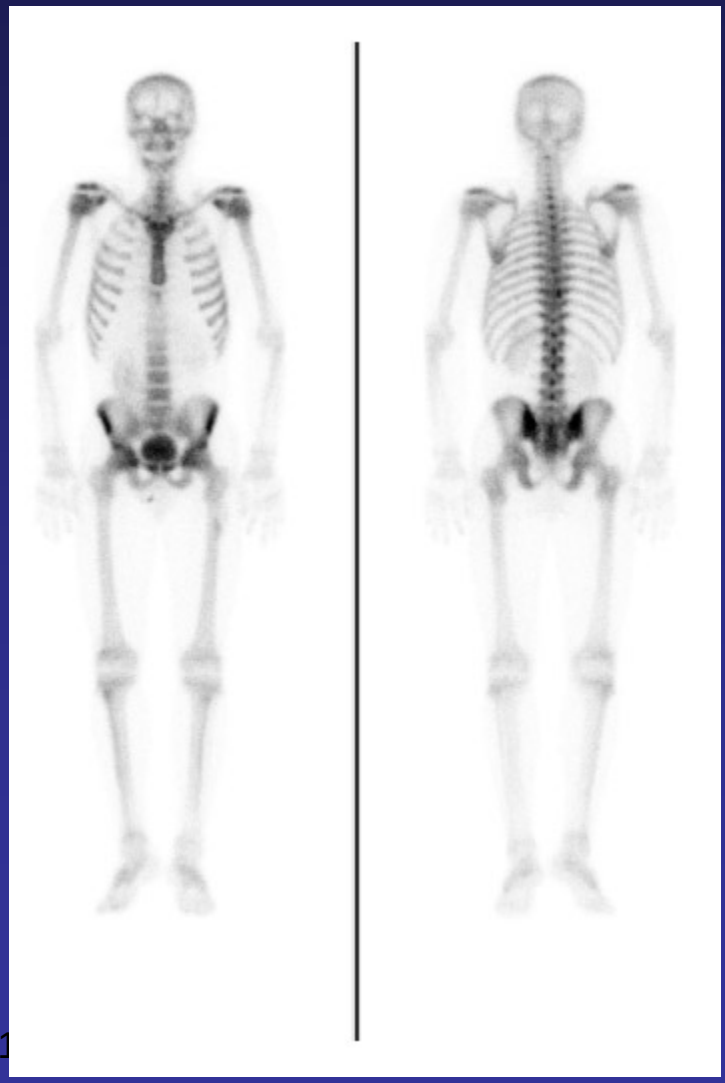
Outline

- Hematopoiesis
  - Stem cells
  - *"…… the rest of the story."*
- Chronic myeloid leukemia
  - Deterministic model
  - Stochastic model
- Reproductive fitness and oncogenes
  - Therapy and reproductive fitness
  - A tale on two drugs

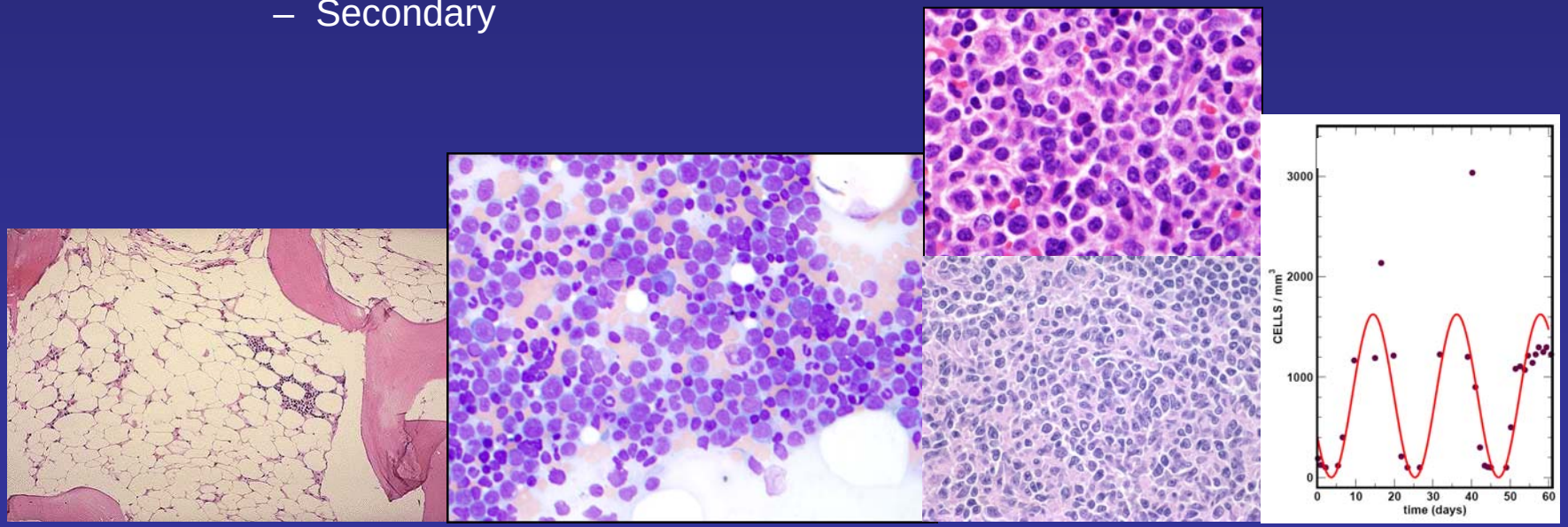
Hematopoiesis



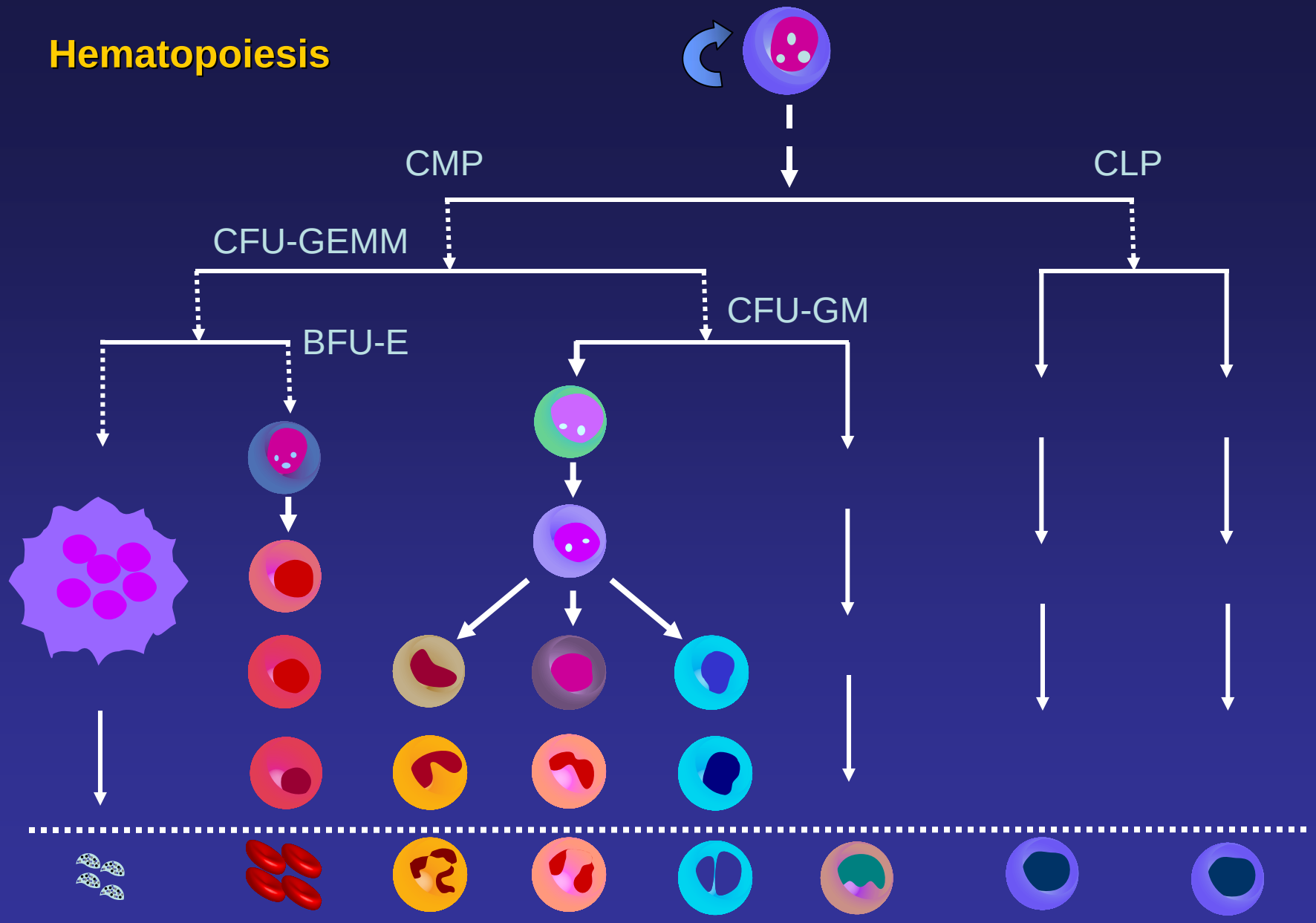


Disorders of hematopoiesis

- Clonal
  - Neoplastic
  - Non-neoplastic
- Non-clonal
  - Failure
    - Primary
    - Secondary



Hematopoiesis



Hematopoietic stem cells

- Self-renewal
  - For how long?
- Differentiate into various types of cells

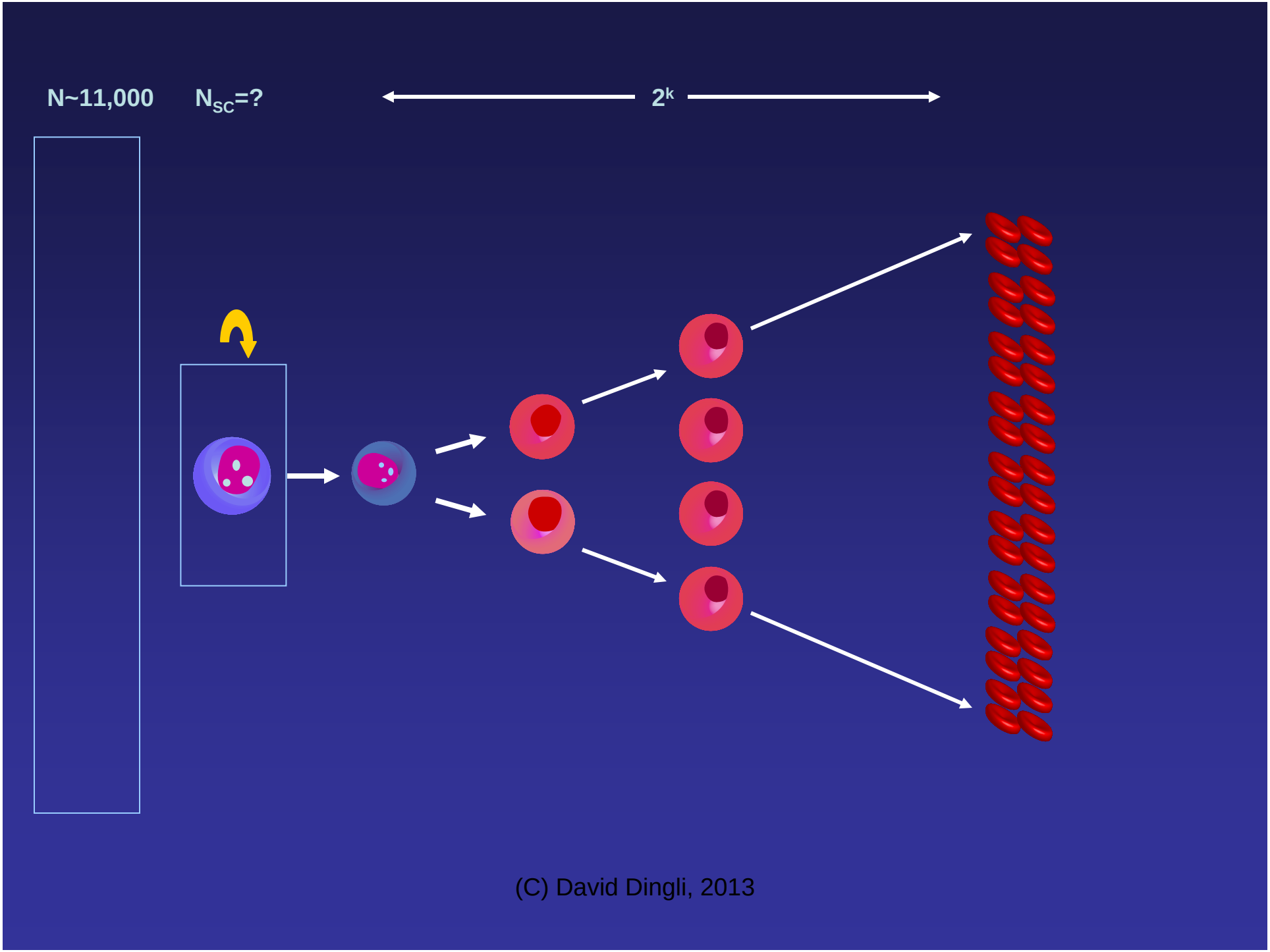
Hematopoietic stem cells

- Replicate slowly: ~1/year in humans
- Once selected to contribute to hematopoiesis they tend to do so for a long time
- Clonal succession?
- Stem cell niche
- Stochastic behavior?

How many stem cells?

- The number of HSC is conserved across mammals
- 11,000 – 22,000 cells
- Different animals have different demands on hematopoiesis
  - Mouse
  - Cat
  - Humans

Abkowitz *et al*, *Blood*, 2002



Allometry and the stem cell pool

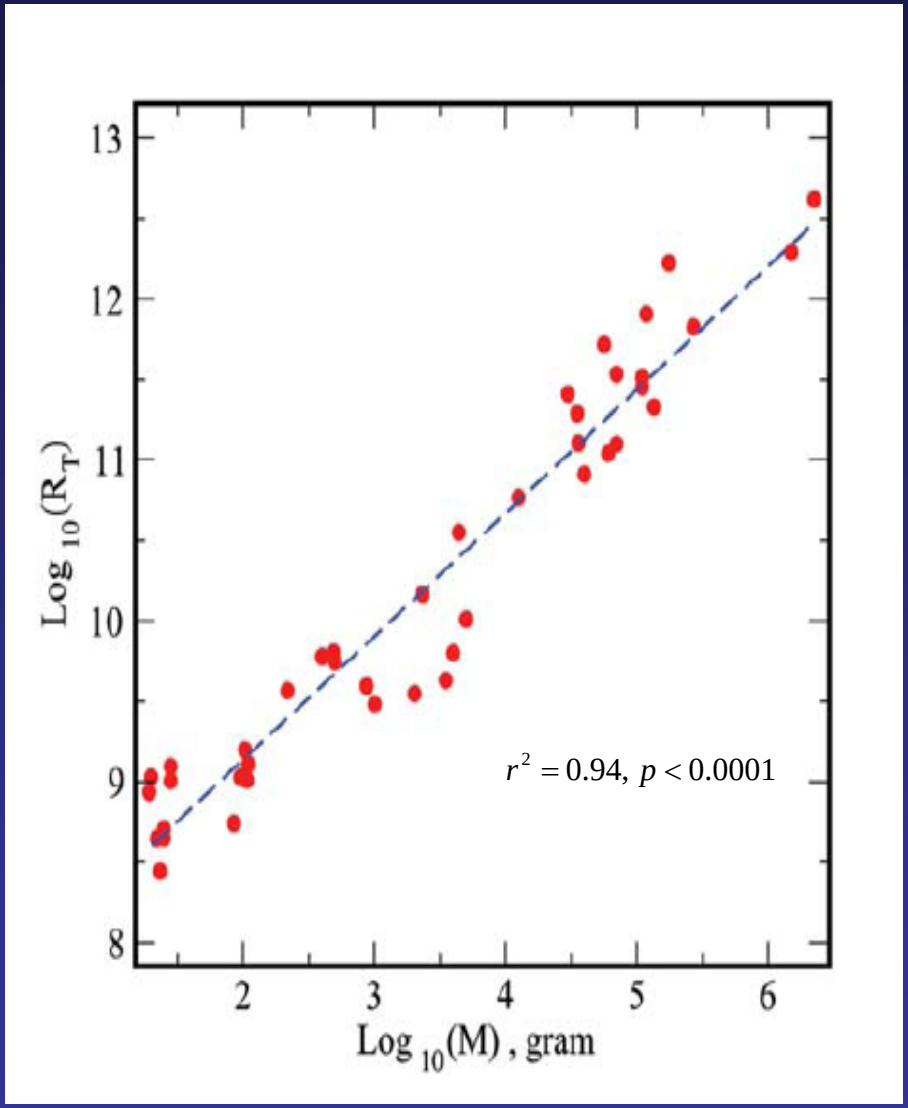
- We consider the active stem cell pool as an organ
- Hematopoiesis is similar across mammals

$$R_M \sim M^{-1/4}$$

$$N_{SC} \cdot R_M \sim R_{TD}$$

$$R_{TD} \sim R_T \tau^{-1} \sim R_T \cdot R_M$$

$$N_{SC} \sim R_T$$



$$N_{SC} \sim M^{3/4}$$

Dingli & Pacheco, *PLoS ONE*, 2006

Predictions (1)

- Use data from cats for calibration
- Under normal conditions, ≥40 cells
- *Prediction*: ~385 cells in adult humans
- *Experiment*: ~400 based on CGD

Buescher *et al, J Clin Invest,* 1985

- Feline stem cells divide every 8 – 10 weeks
- *Prediction*: Human HSC ~ 60 weeks
- *Experiment*: Every 1 – 2 years

Rufer, *et al, J Exp Med,* 1999

Dingli & Pacheco, *PLoS ONE*, 2006

Predictions (2)

- ~13 stem cells reconstitute hematopoiesis in the cat after BMT
- *Prediction*: 111 HSC after transplant in humans
- *Experiment*: 116 different clones in humans

Nash *et al, Blood,* 1988

Dingli & Pacheco, *PLoS ONE*, 2006

Predictions (3)

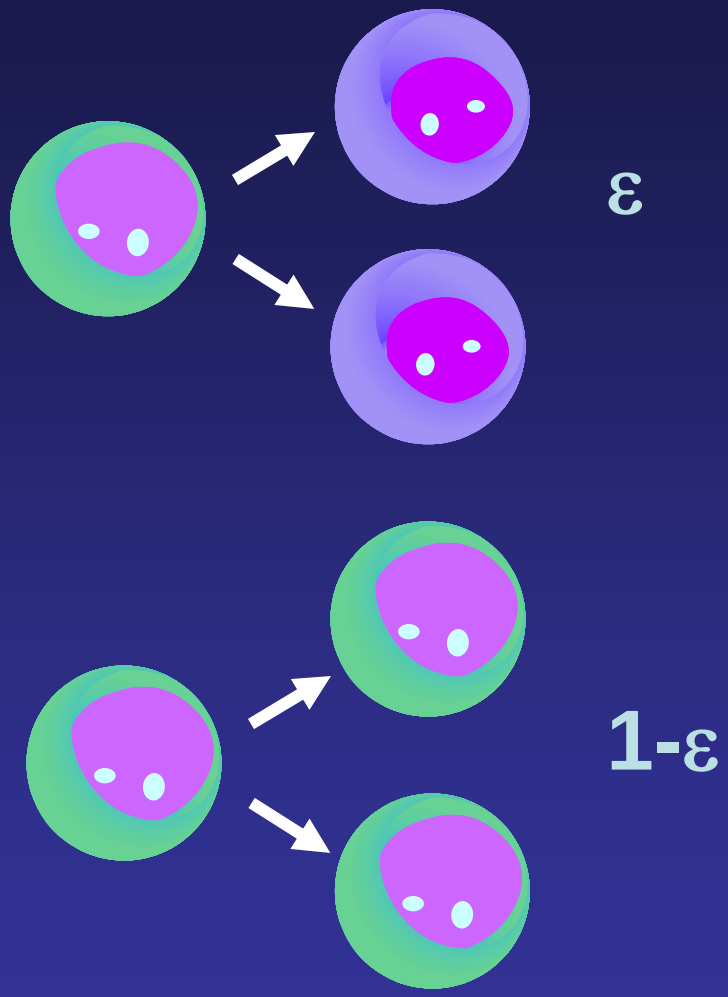
- *Prediction*: 1 SC maintains hematopoiesis in the mouse
- *Experiment*: 1 HSC can reconstitute a mouse for its lifetime and more
- *Prediction*: Pilot whale, HSC ~ 4690

  Elephant, HSC ~ 9640

Dingli & Pacheco, *PLoS ONE*, 2006

From stems cell to blood

- 400 SC replicate ~ 1/year
- Total daily marrow output ~ 3.5×10^{11} cells
- Consider
  - Replication (amplification)
  - Differentiation



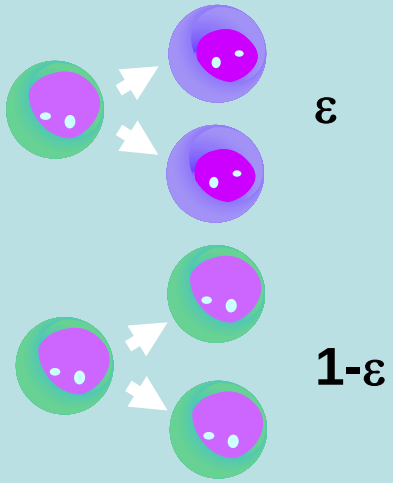
Consider a given compartment, *i* with N_i cells and there are *k* compartments

N_i on average changes as:

$$-1 \cdot \varepsilon \cdot N_i + 1 \cdot (1-\varepsilon) \cdot N_i = (1-2\varepsilon) \cdot N_i$$

We assume that ε is the same across compartments

$0.5 < \varepsilon < 1.0$



Cells move from compartment *i*-1 to replace cells lost from compartment *i* due to export.

Let r_i be the rate of replication in compartment *i.*

Then, per unit time step, compartment *i* loses:

$$(2\varepsilon - 1) \cdot N_i \cdot r_i$$

Rate of replication in comp *i*-1 is r_{i-1} and this compartment replaces cells lost in compartment *i.* Then

$$\frac{N_i}{N_{i-1}} \cdot \frac{r_i}{r_{i-1}} = \frac{2\varepsilon}{2\varepsilon - 1}$$

We assume that the ratio of replication rates between adjacent compartments is constant, *r.*

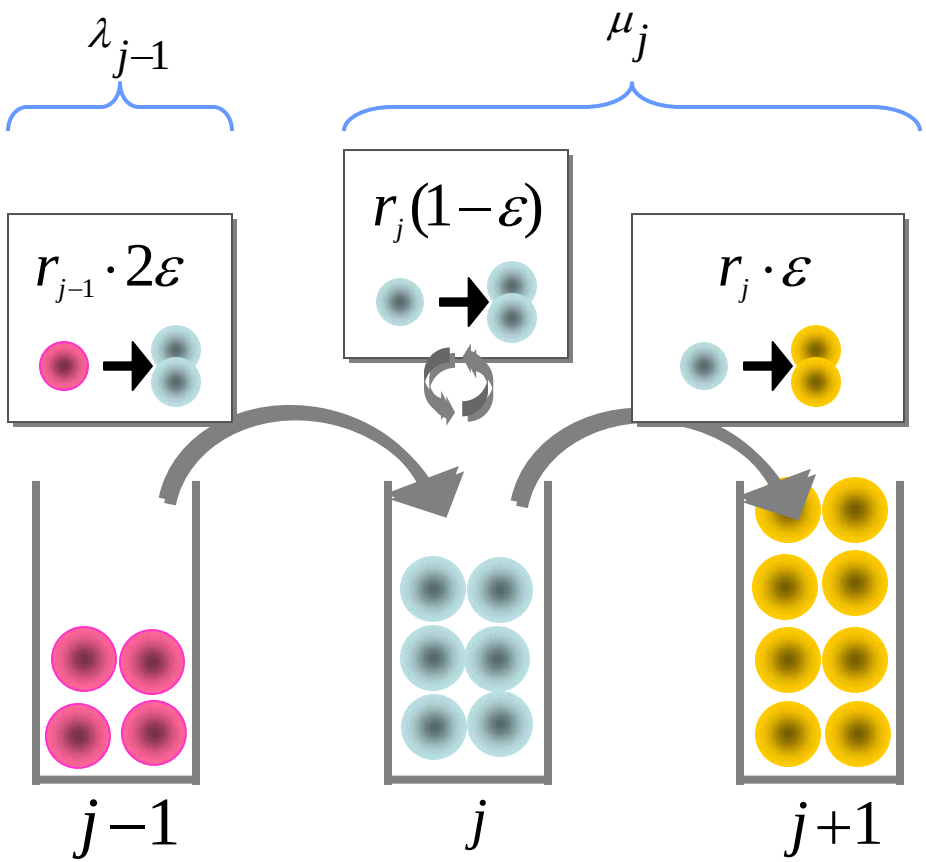
$$\frac{r_i}{r_{i-1}} = r$$

$$\frac{N_i}{N_{i-1}} = \gamma = \frac{2\varepsilon}{2\varepsilon - 1} \cdot \frac{1}{r}$$

$$\frac{2\varepsilon}{2\varepsilon - 1} > r, \quad \gamma > 1$$

Cell division processes

Compartments



Dingli *et al*, Cell Prol (2009)

Parameter estimates

During granulopoiesis, ~10^{10} myeloblasts give rise to ~1.4×10^{11} myelocytes in 4 replication steps.

Therefore:

$$\gamma \approx \left(\frac{1.4 \times 10^{11}}{10^{10}} \right)^{1/4} \approx 1.93$$

If we start with ~400 HSC and have a daily output of ~3.5×10^{11} cells/day, then

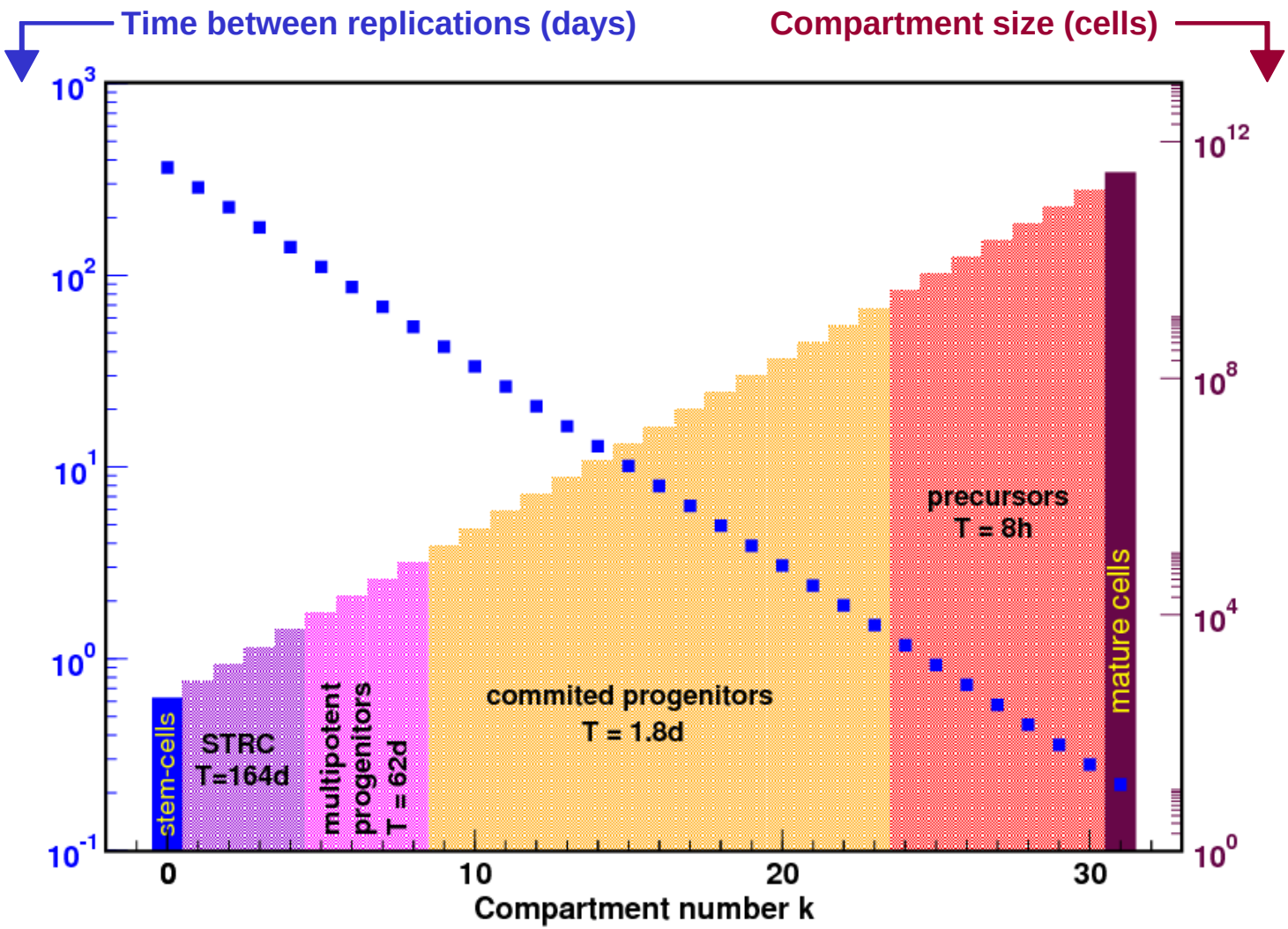
$$k = \frac{\log\left[\frac{3.5 \times 10^{11}}{400} \right]}{\log[1.93]} \approx 31$$

Parameter estimates

Granulocyte precursors can replicate up to ~5 times/day while HSC replicate ~1/year. Therefore:

$$r = \left(\frac{5}{\frac{1}{365}} \right)^{1/k} \approx 1.27$$

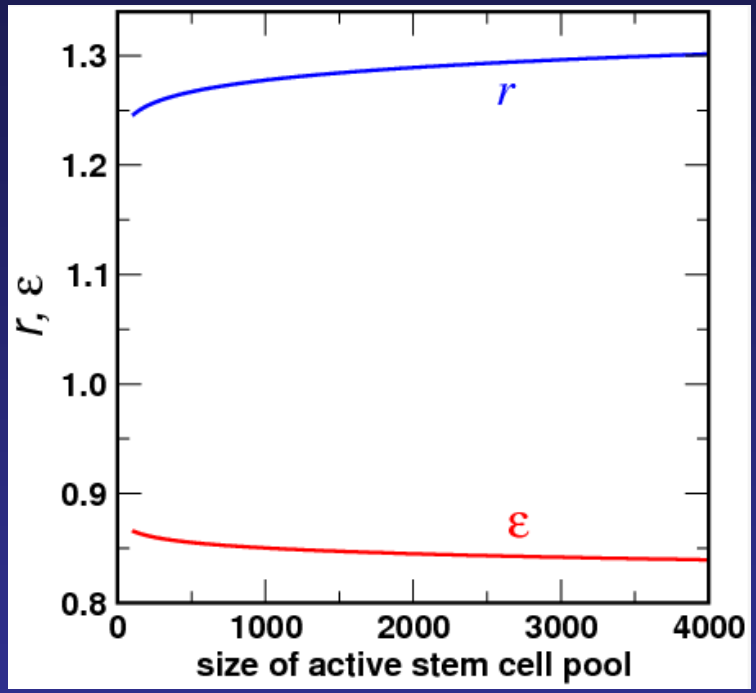
$$\varepsilon = \frac{r \cdot \gamma}{2(r \cdot \gamma - 1)} \approx 0.84$$



(C) David Dingli, 2013

Dingli *et al*, *PLoS ONE* 2007

Model robustness



Model predictions

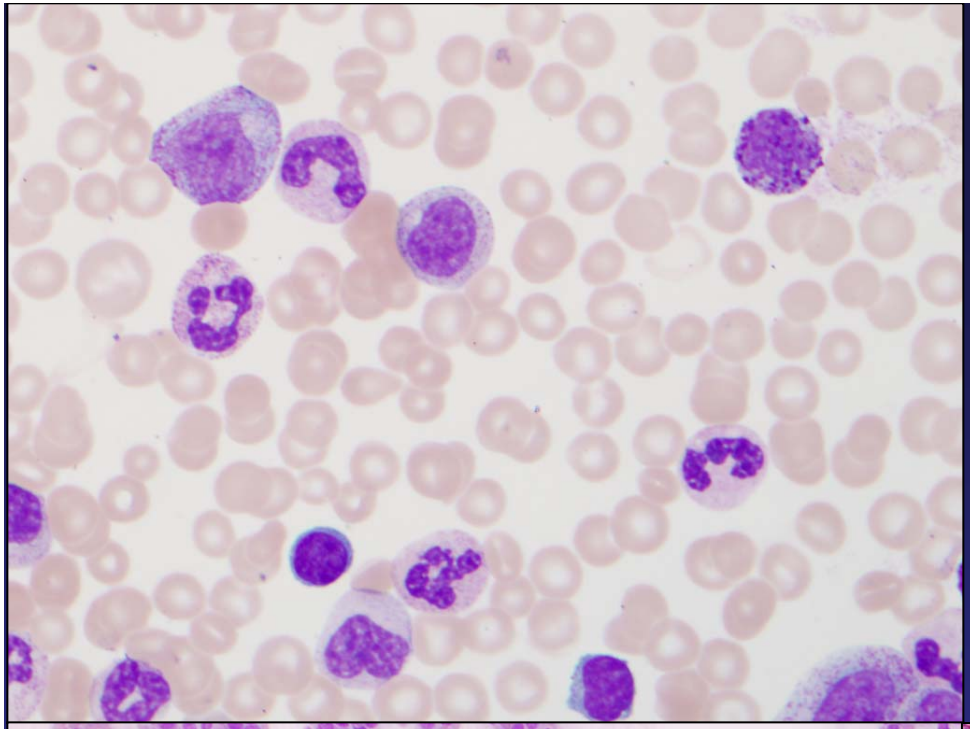
- Size and replication rate of each compartment
- Mitotic events ≥ 31
- Testing the model:
  - *PIG-A* mutations with loss of CD55 and CD59
  - Healthy adults have 11-51/10^6 mutant neutrophils
  - Adults have 20,000 to 100,000 CFU-GEMM
  - CFU-GEMM are in compartments 5 to 8
  - Model predicts that these cells contribute for 61 to 120 days (Araten *et al*, *PNAS*, 1999)

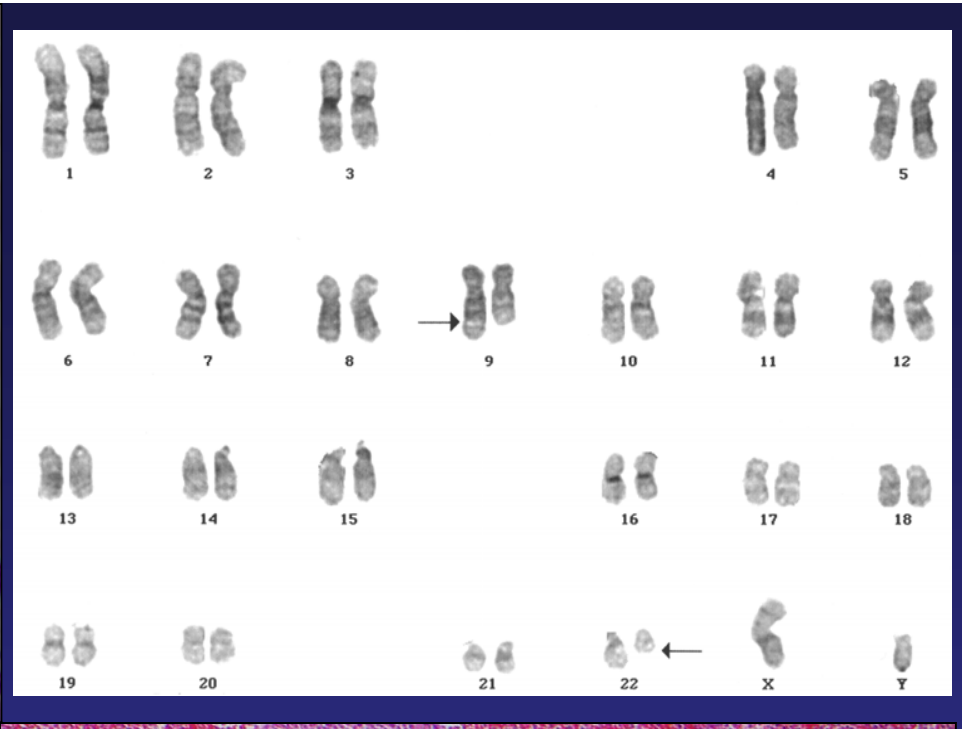
Conclusions

- Simple multi-compartment model of hematopoiesis
- Exponential expansion of cells
- Generally, cells divide and move to the downstream compartment
- Model fits well the limited data available

Chronic Myeloid Leukemia

- Hematopoietic stem cell disorder
- Initial event: Philadelphia chromosome
  - t(9;22): *bcr-abl*
- ? Enough to drive chronic phase
- Clonal expansion and myeloproliferation
- Stem cell derived but progenitor cell driven
- *Abl* kinase inhibitors very effective

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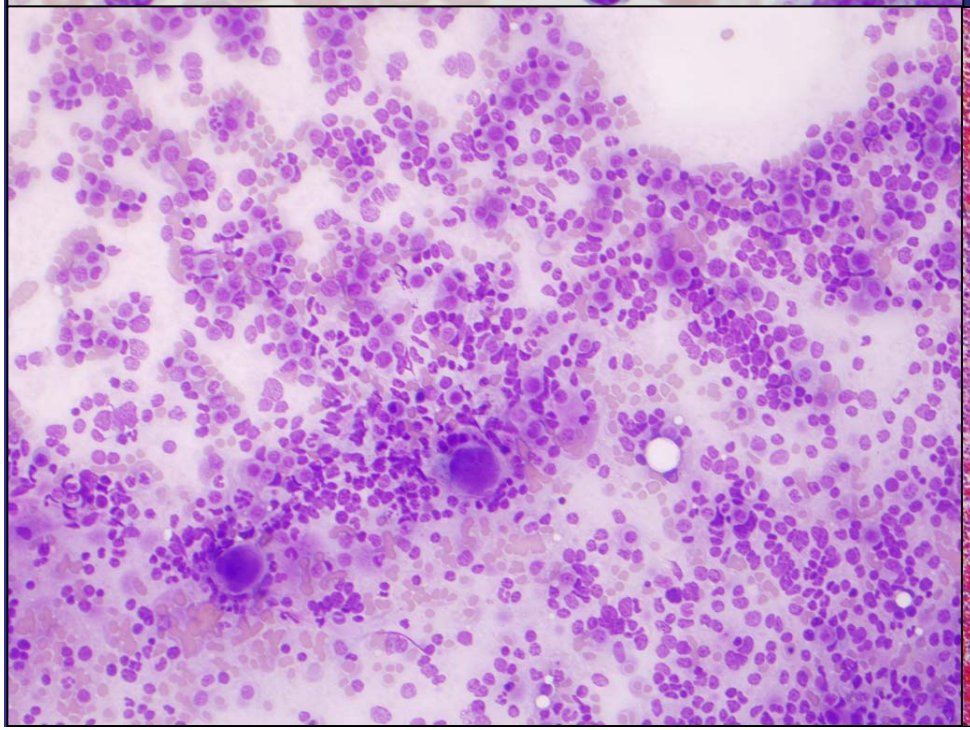
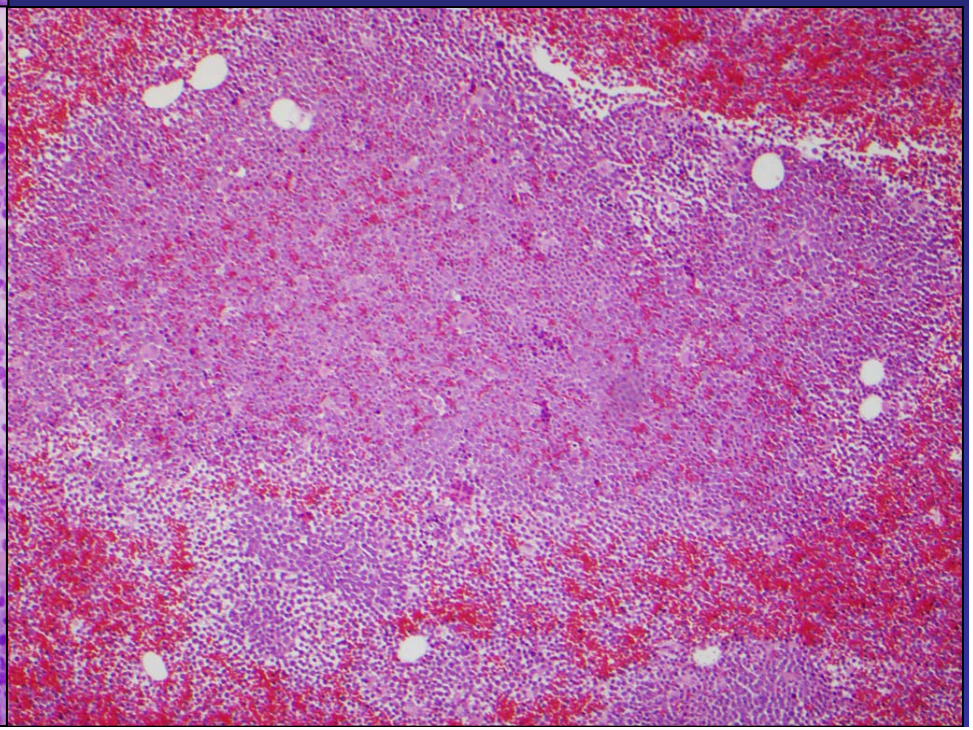
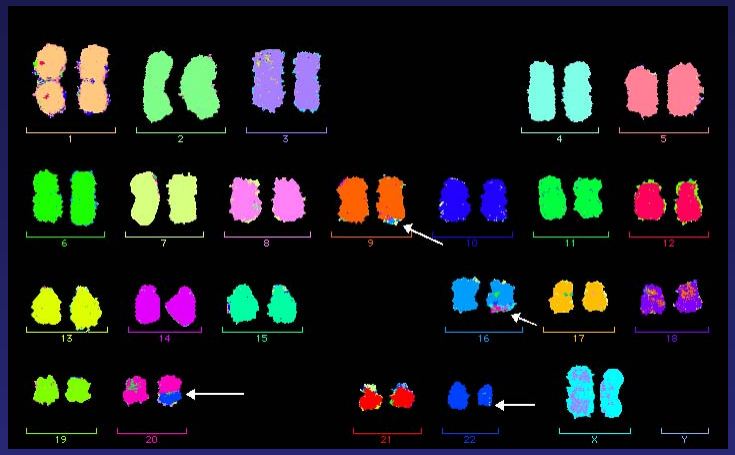
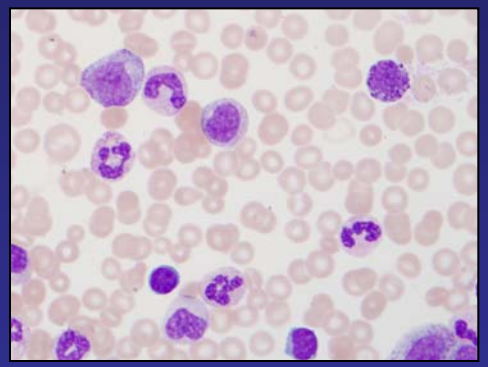
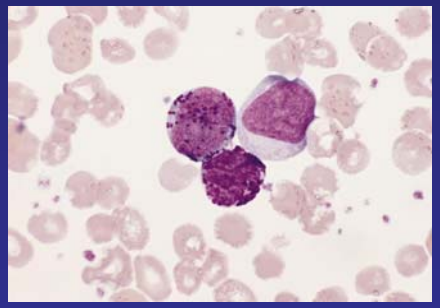


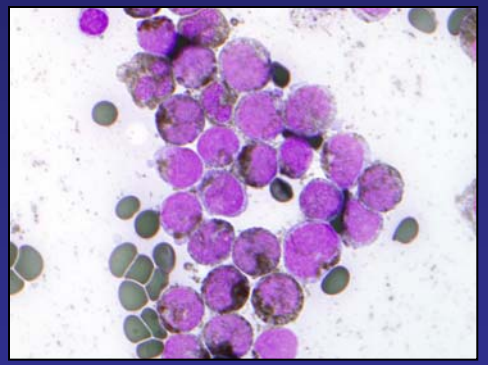


Natural history of CML





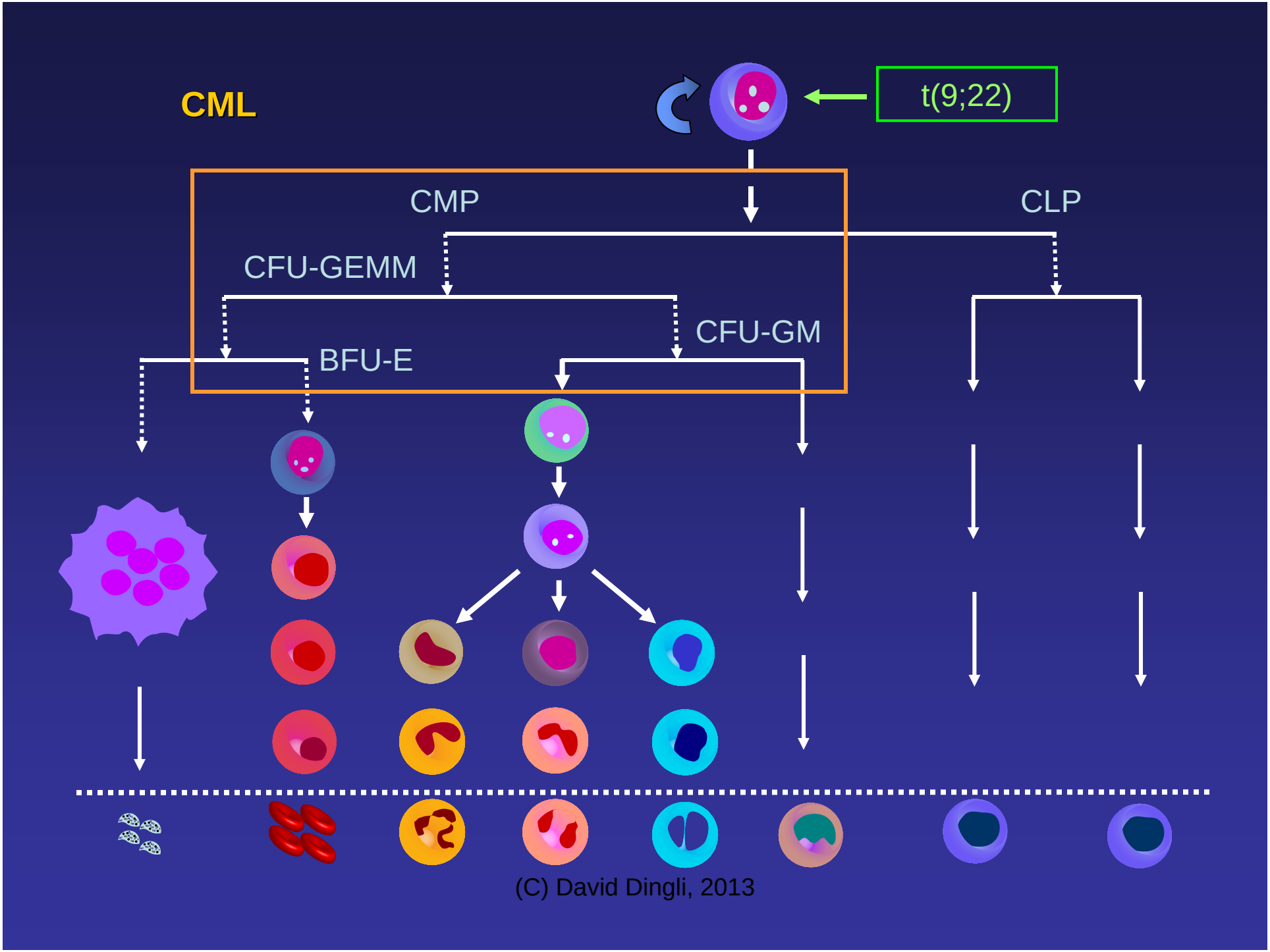




Chronic phase → Accelerated phase → Blast crisis

It all changed with imatinib….

- Median survival 3 – 5 years
- Curative therapy only BMT
- Some cured with Interferon


CML
t(9;22)
CMP
CLP
CFU-GEMM
CFU-GM
BFU-E
(C) David Dingli, 2013

CML dynamics

- Q-RT-PCR data from patients treated with imatinib
- 2 data sets available
  - Michor *et al, Nature*, 2005
  - Roeder *et al, Nature Medicine*, 2006
- Data fitting

Model features

- How many stem cells drive CML
- How is the disease progenitor driven given the stem cell origin
- How many CML progenitor cells are there
- Bone marrow expansion
- Bone marrow output
- How does imatinib work
  - Does imatinib induce cell death?
- How many cells are responding to imatinib

Model constraints

- Time from initial insult to diagnosis is 3.5 – 6 years
- Progenitor cell expansion >14%
- Total number of active HSC is *not* increased
- Daily bone marrow output is ~ 3 x normal

Bcr-abl and phenotype

- CML progenitors have enhanced self-renewal
- In our model: $\varepsilon_{CML} < \varepsilon_0$
- t(9;22) has no impact on LSC!
- How do they expand?

CML dynamics with imatinib

At any time, a fraction, *z* of CML cells in compartment *i* are responding to imatinib. We can define

$$d_i = (2\varepsilon - 1) r_i$$

$$b_{i-1} = 2 \cdot \varepsilon \cdot r_{i-1}$$

$$\dot{N}_i^{CML} = -(1-z) \cdot d_i^{CML} \cdot N_i^{CML} - z \cdot d_i^{IMAT} \cdot N_i^{CML} + (1-z) \cdot b_{i-1}^{CML} N_{i-1}^{CML} + z \cdot b_{i-1}^{IMAT} N_{i-1}^{CML}$$

Bcr-abl and phenotype

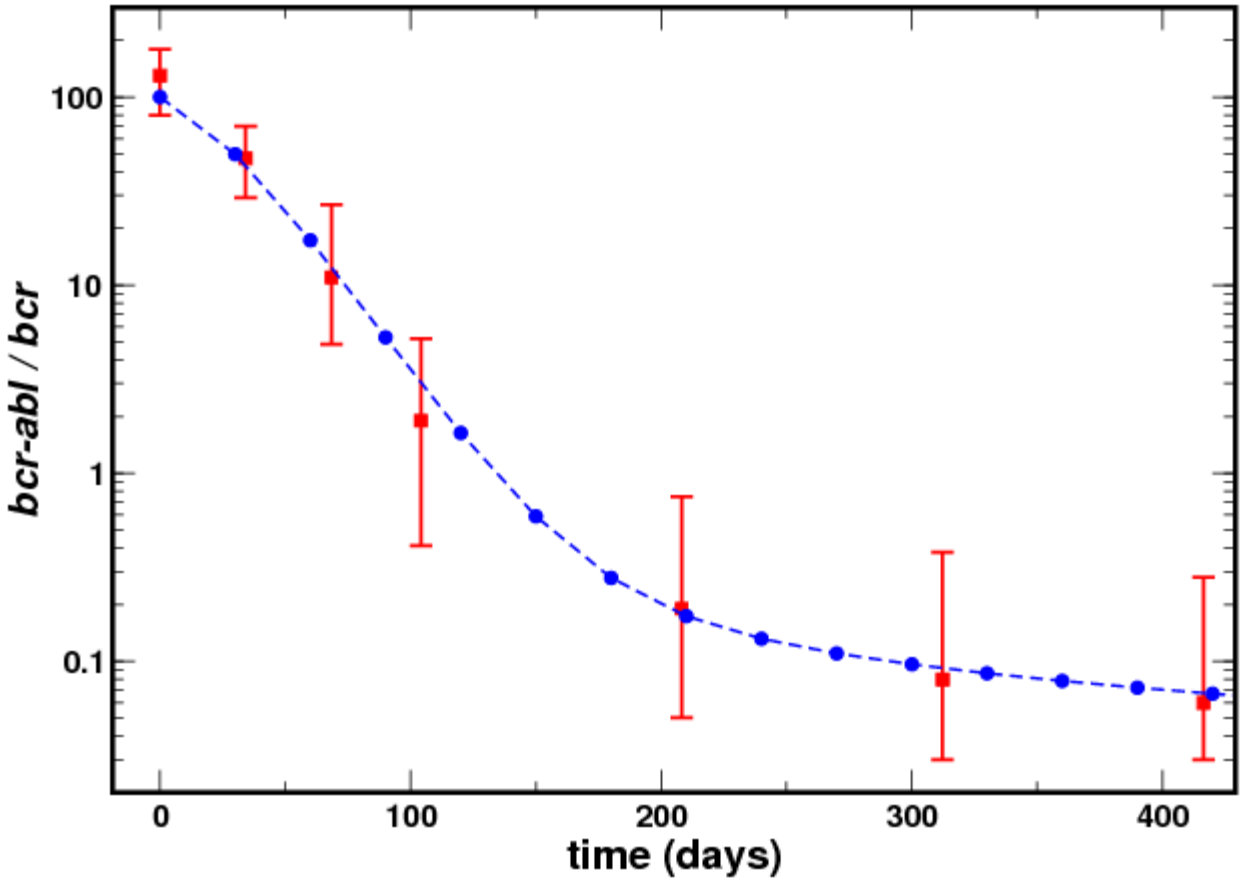
If $\varepsilon_{CML} < \varepsilon_0$, we can estimate the number of divisions *C*, that cells undergo before appearing in the circulation. If the minimum number of division is *K, D=C-K.*

The average number of divisions is given by a Poisson distribution *P(D)* where

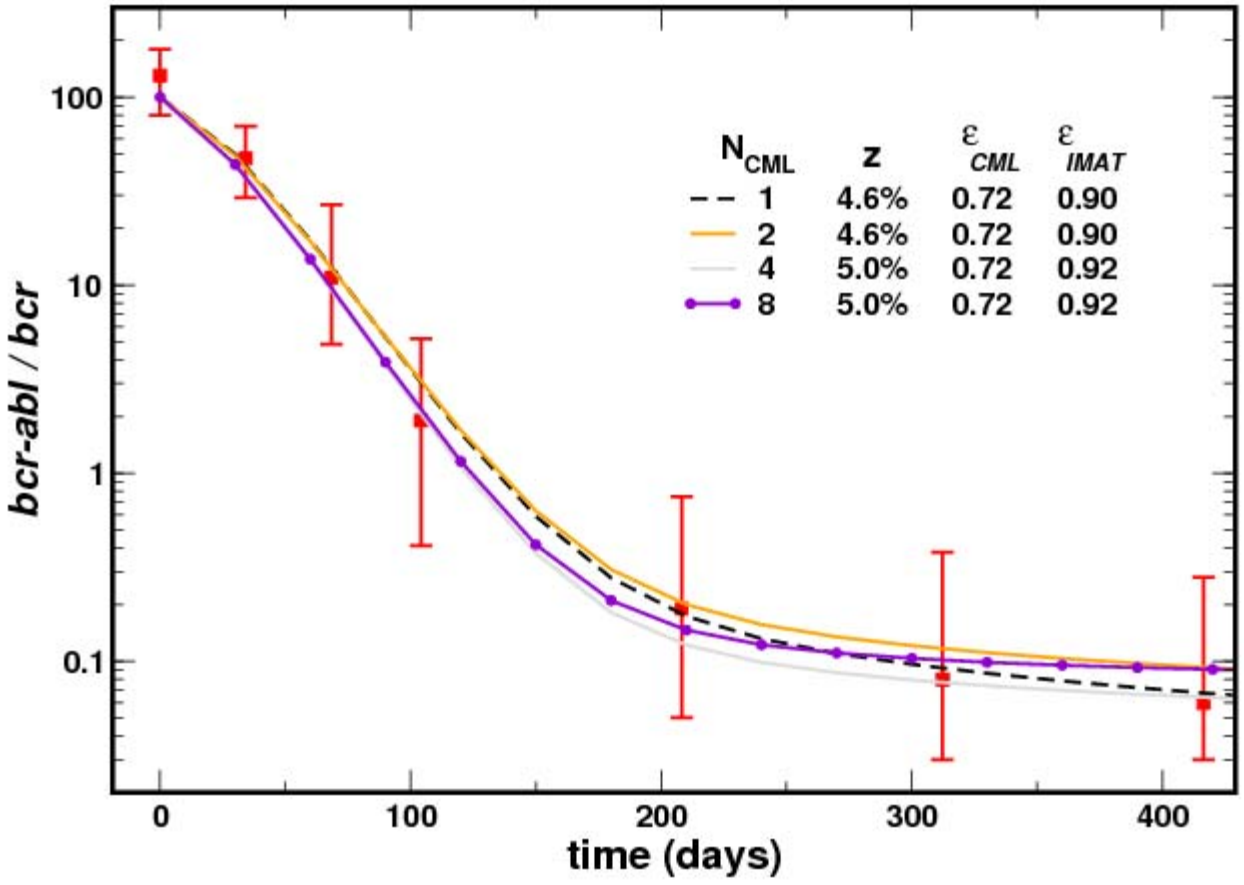
$$P(D) = \frac{\lambda^D}{D!} e^{-\lambda} \quad \text{with} \quad \lambda = K(1-\varepsilon)$$

Therefore

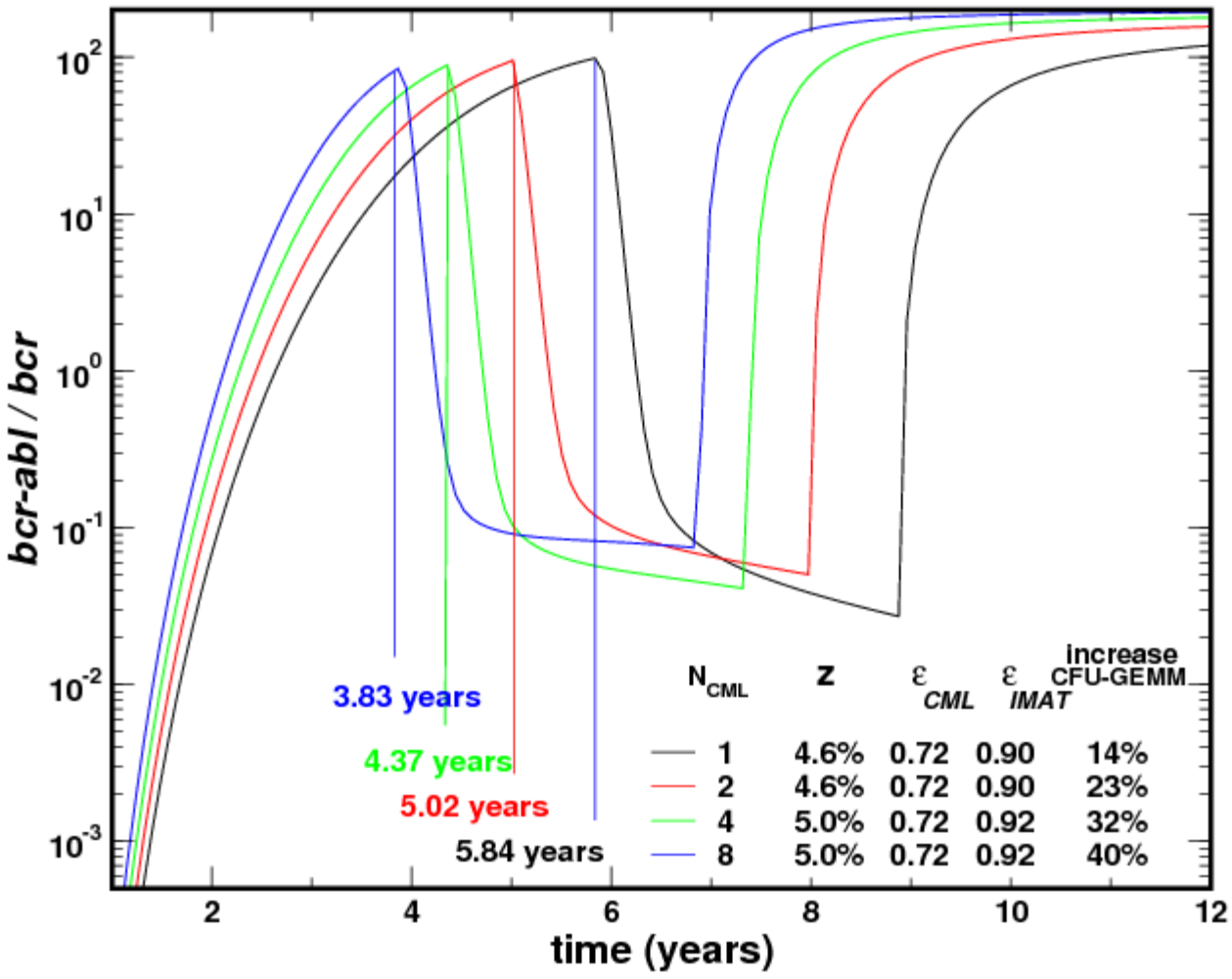
$$C = K + \langle P(D) \rangle = K + K(1-\varepsilon)$$



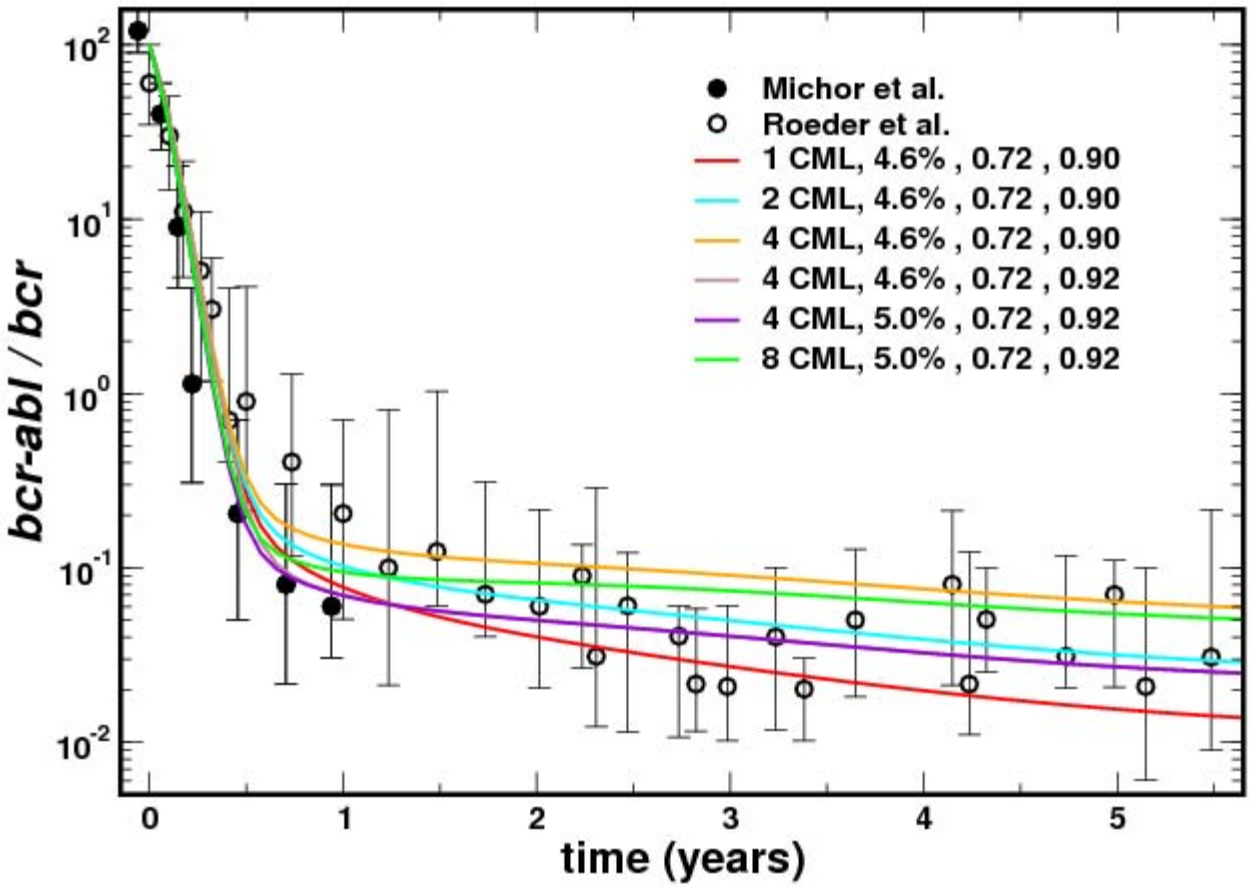
Dingli, *et al*, *Clin Leukemia*, 2008



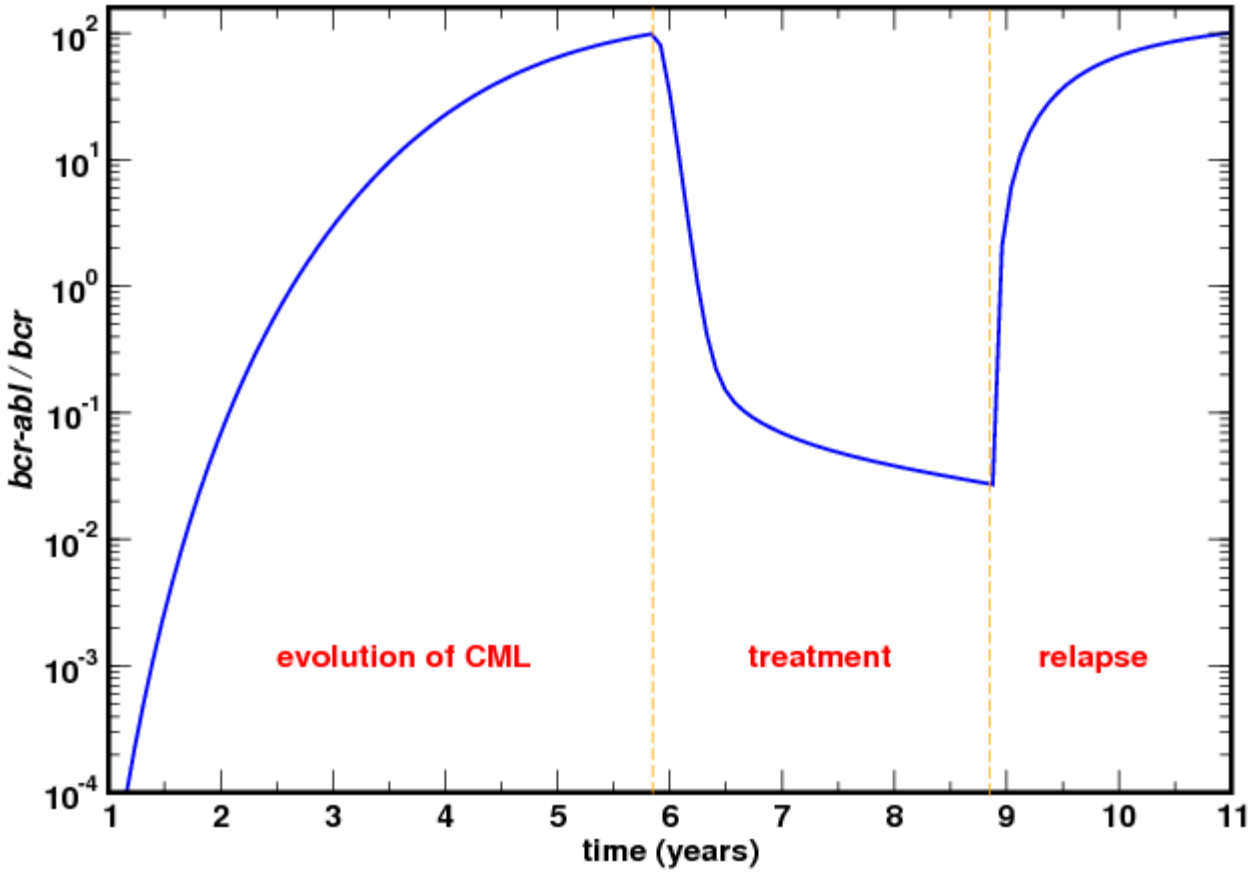
Dingli, *et al*, *Clin Leukemia*, 2008



Dingli, *et al*, *Clin Leukemia*, 2008



Dingli, *et al*, *Clin Leukemia*, 2008



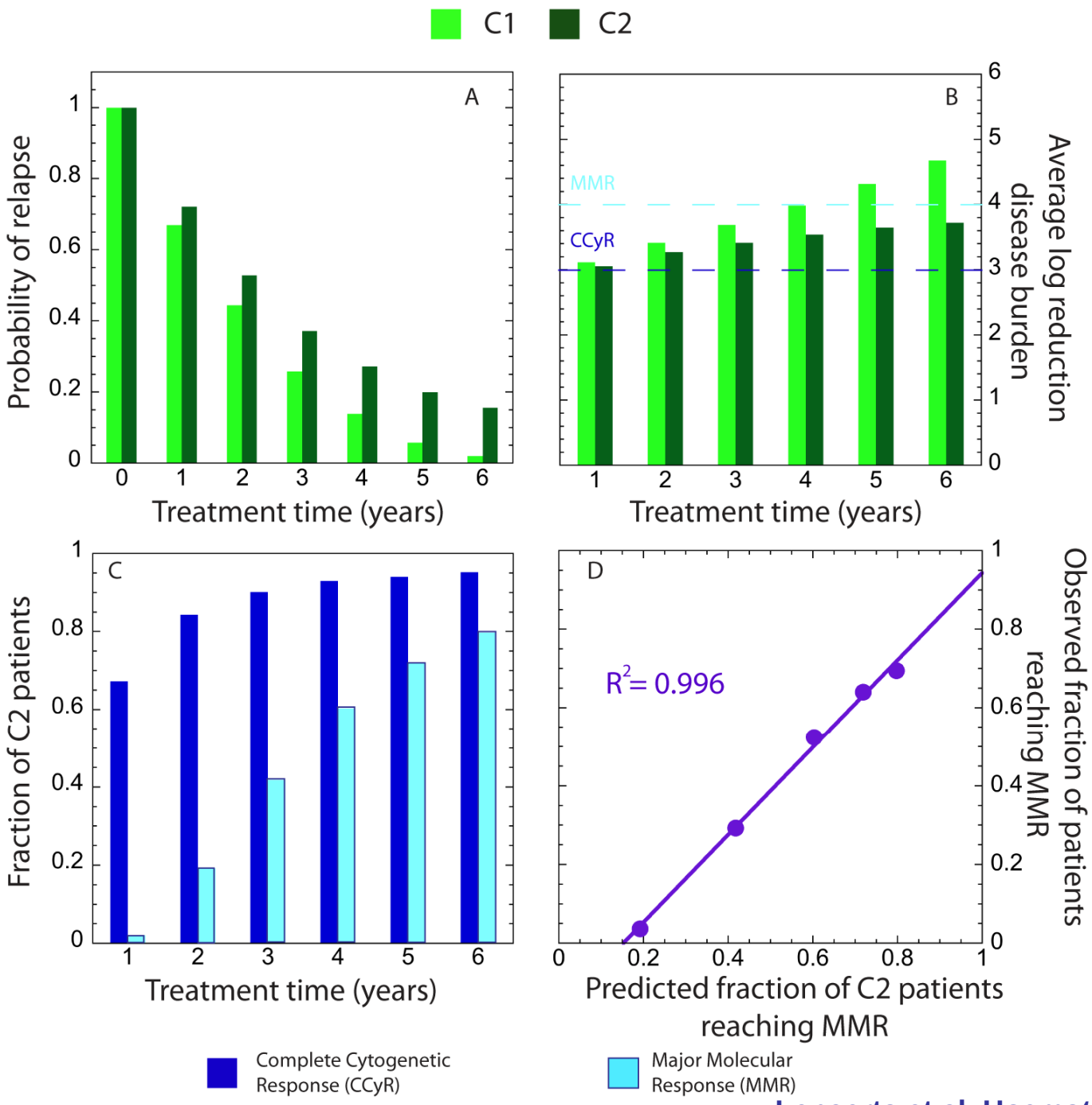
Dingli, *et al*, *Clin Leukemia*, 2008

Conclusions – Deterministic Dynamics

- CML is driven by a small number of neoplastic stem cells
- Many CML progenitors persist
- Only a fraction of CML cells are responding to therapy at any time
- Relapse is driven by CML progenitors not just stem cells

Stochastic → Deterministic Dynamics

- Small stem cell population
- *BCR-ABL* has no impact on LSC
- Stochastic effects important
- Where does the stochastic to deterministic transition occur?



Lenaerts et al, H*aematologica*, 2010

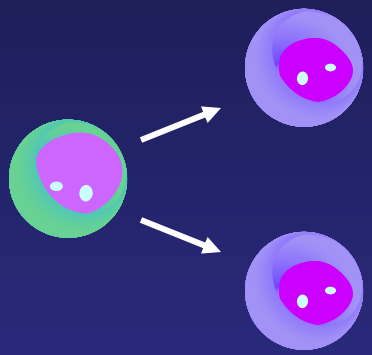
Conclusions (Stochastic dynamics)

- Imatinib can effectively cure the disease without affecting the LSC directly
- Many patients with CML will not have the LSC still present at diagnosis
- Therapy may have to be prolonged to ensure cure

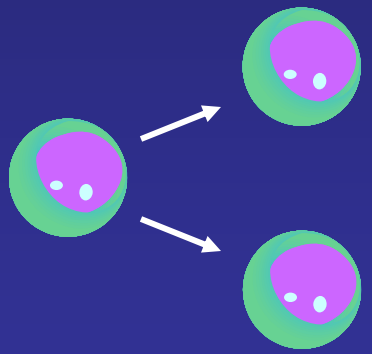
Reproductive fitness and oncogenes

- Evolution
  - Reproduction
  - Mutation
  - Selection
- Oncogenes
  - How big is the advantage?
- BCR-ABL

Modeling Hematopoiesis and CML



ε



1 - ε

$$\frac{d}{dt}N_k = -(2\varepsilon - 1)r_k N_k + 2\varepsilon r_{k-1} N_{k-1}$$

$$\varepsilon_0 = 0.85$$

$$\varepsilon_{CML} = 0.72$$

Dingli et al, *PLoS ONE*, 2007; Dingli et al, *Clin Leuk*, 2008; Lenaerts et al, *Haematologica*, 2010

$$T^{+}_{CML}(i) = (1-\varepsilon_{CML})\frac{i}{N_k}$$

$$T^{-}_{CML}(i) = \varepsilon_{CML}\frac{i}{N_k}$$

$$T^{+}_{0}(i) = (1-\varepsilon_{0})\frac{N_k - i}{N_k}; \qquad T^{-}_{0}(i) = \varepsilon_{0}\frac{N_k - i}{N_k}$$

$$\rho_{CML} = \frac{T^{+}_{CML}(i)}{T^{-}_{CML}(i)} = \frac{1-\varepsilon_{CML}}{\varepsilon_{CML}}; \quad \rho_{0} = \frac{1-\varepsilon_{0}}{\varepsilon_{0}}$$

$$\varepsilon_0 > 0.5; \qquad \rho_0 < 1$$

P(t) = Probability that a cell undergoes *t* divisions in a compartment is given by:

$$P(t) = (1-\varepsilon)^{t-1}\varepsilon^{1}$$

$$n = \sum_{t=1}^{\infty}(1-\varepsilon)^{t-1}\varepsilon \cdot t = \frac{1}{\varepsilon}$$

The number of offspring a cell of a given type leaves in a given compartment is given by *n-1*

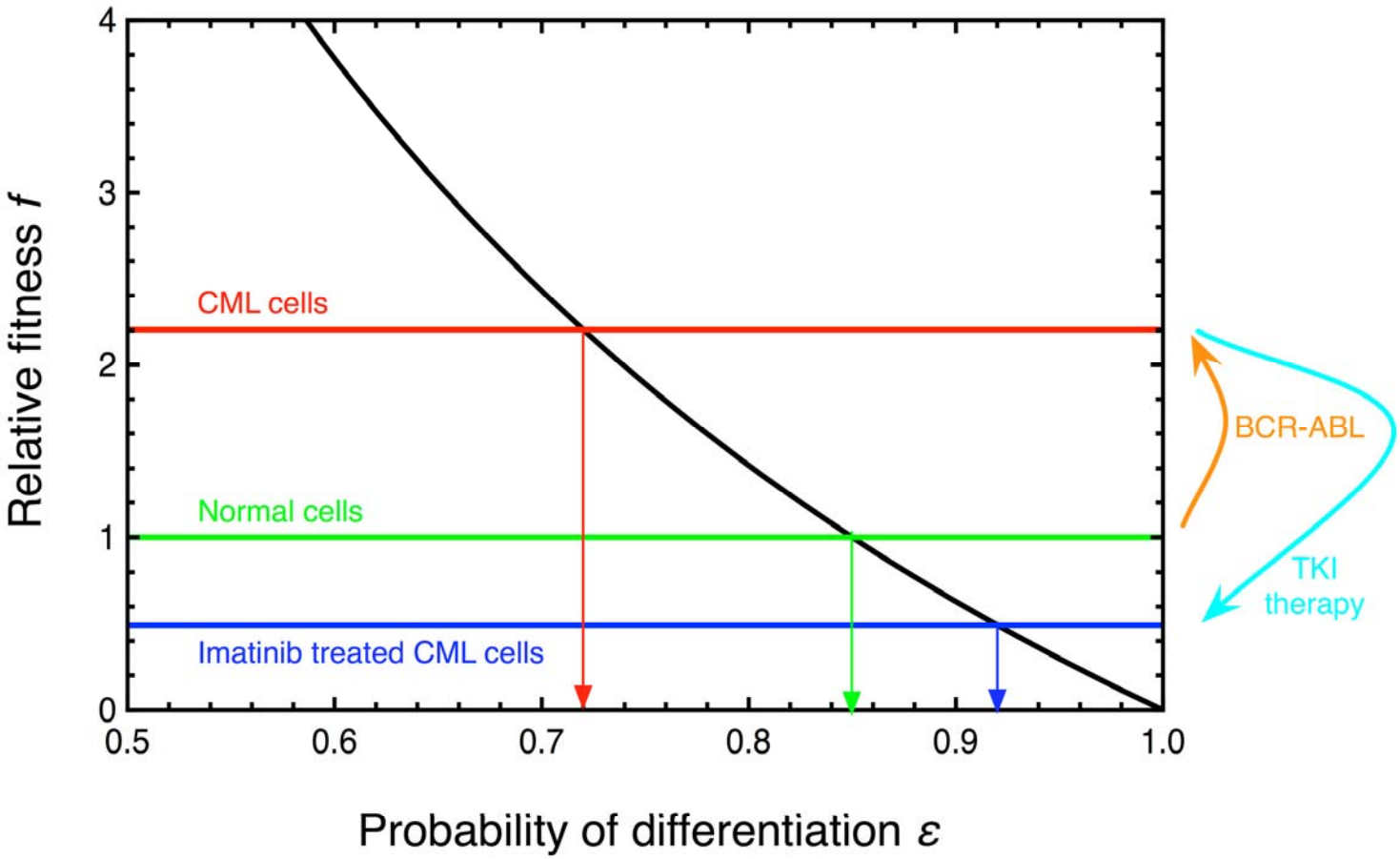
Relative reproductive fitness

$$f_j = \frac{\rho_j}{\rho_0} = \frac{1-\varepsilon_j}{1-\varepsilon_0}\frac{\varepsilon_0}{\varepsilon_j}$$

$$\varepsilon_j < \varepsilon_0 \rightarrow f_j > 1$$

$$\varepsilon_j > \varepsilon_0 \rightarrow f_j < 1$$

Oncogene fitness



Imatinib and Nilotinib

- **Both reversible inhibitors of *Abl***
- **Nilotinib more potent and can inhibit many but not all imatinib resistant mutants**
- **Nilotinib leads to a faster and deeper response**
- **But:**
  - **Neither agent increases apoptosis of $CD34^+$ CML cells**
    - » Jorgensen et al, Blood, 2007
  - **Inhibition of signaling downstream of Bcr-Abl is the same for both drugs**
    - » Konig et al, Leukemia, 2008

Evolutionary dynamics of CML

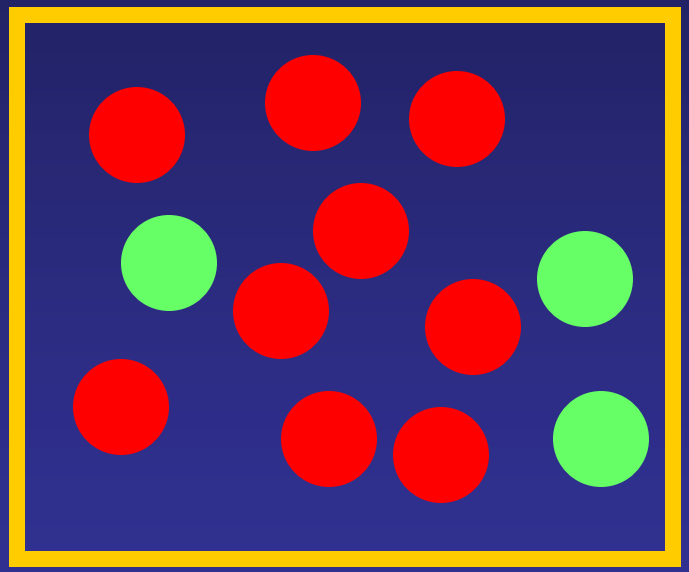
CML progenitor

Normal progenitor

$$T^{+}_{CML}(i) = (1-\varepsilon_{CML})\frac{i}{N_k}$$

$$T^{-}_{CML}(i) = \varepsilon_{CML}\frac{i}{N_k}$$

$$\rho_{CML} = \frac{T^{+}_{CML}(i)}{T^{-}_{CML}(i)} = \frac{1-\varepsilon_{CML}}{\varepsilon_{CML}}$$



$$T^{+}_{0}(i) = (1-\varepsilon_{0})\frac{N_k - i}{N_k}$$

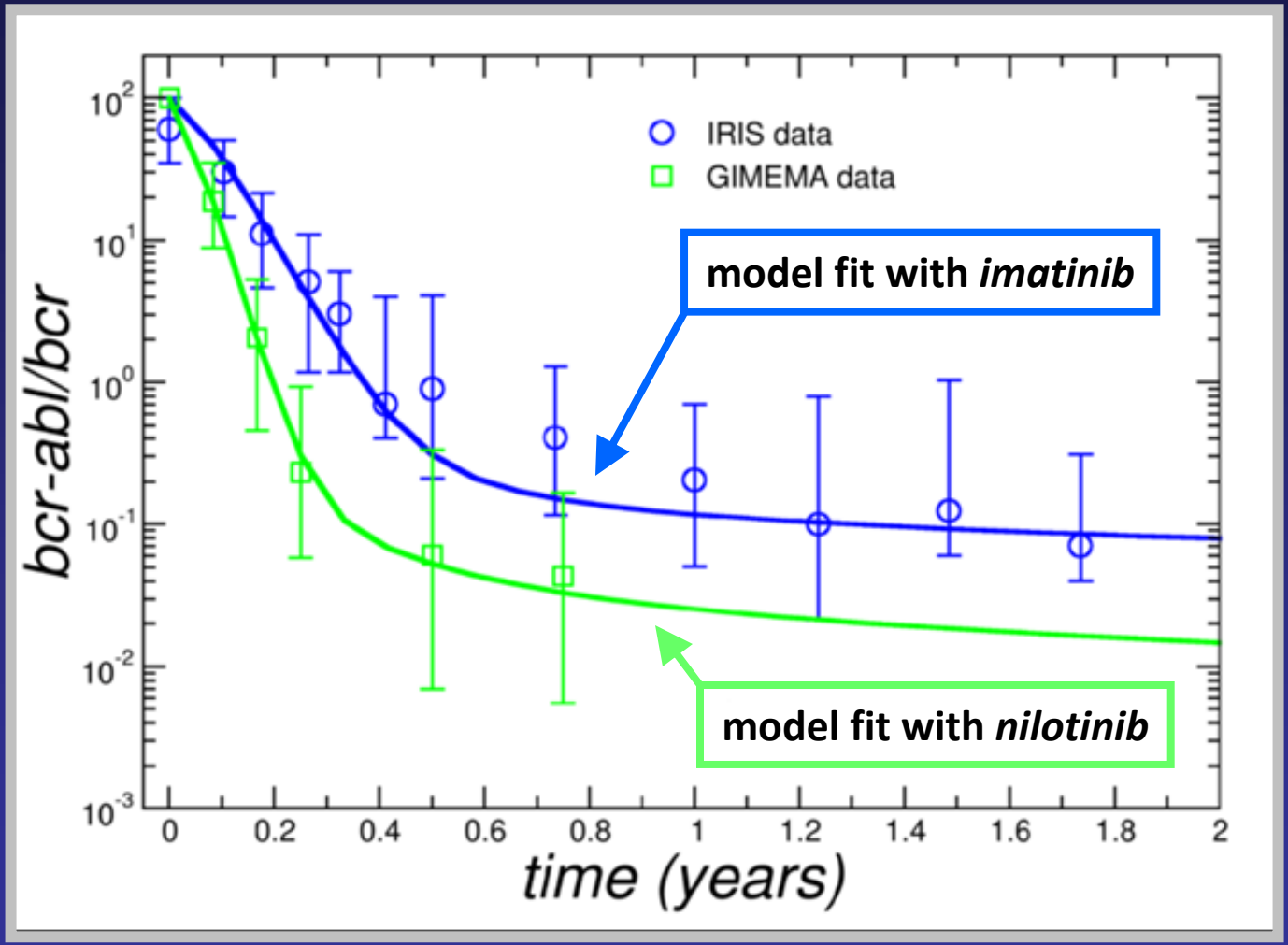
$$T^{-}_{0}(i) = \varepsilon_{0}\frac{N_k - i}{N_k}$$

$$\rho_0 = \frac{1-\varepsilon_0}{\varepsilon_0}$$

$$f_j = \frac{\rho_j}{\rho_0} = \frac{1-\varepsilon_j}{1-\varepsilon_0}\frac{\varepsilon_0}{\varepsilon_j}$$

Traulsen *et al, Cancer Letters*, 2010

Response dynamics



Roeder *et al, Nature Medicine*, 2006

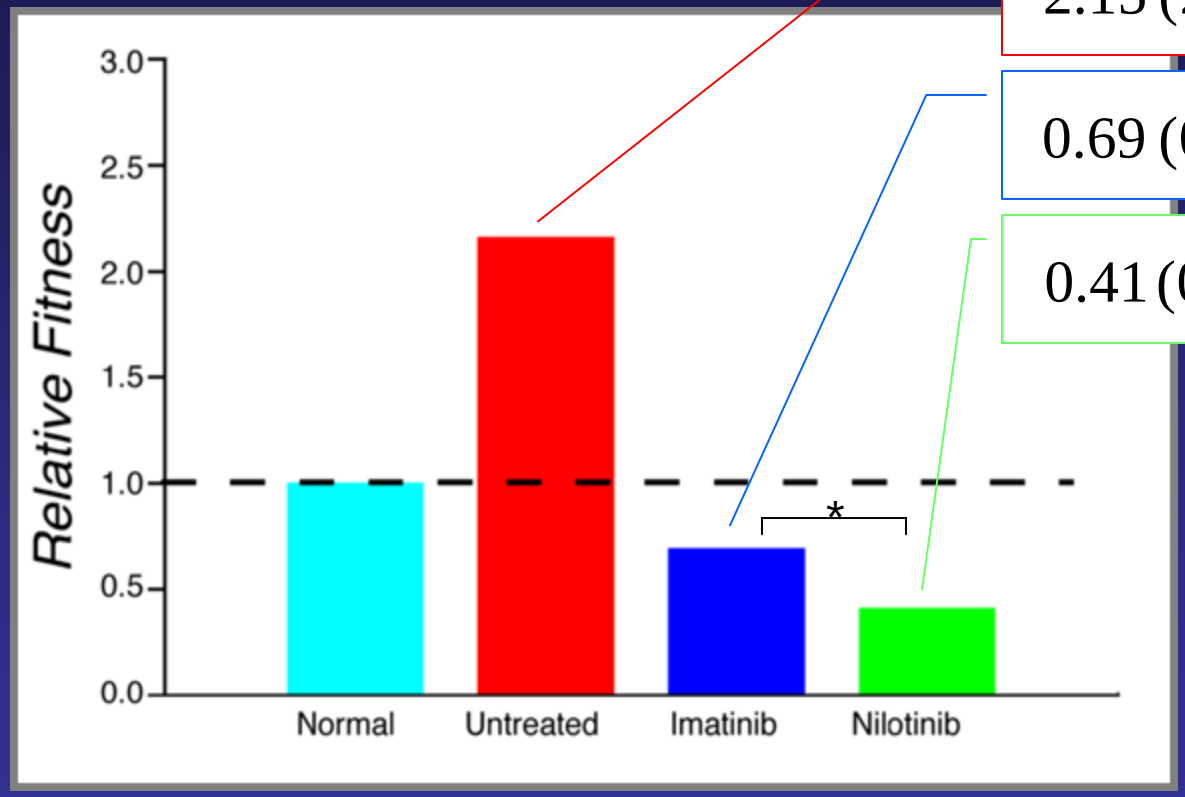
Rosti *et al, Blood*, 2009

Determining model parameters

Data fitting

Parameter	Untreated	*Imatinib*	*Nilotinib*
ε_0	0.85	0.85	0.85
ε_{CML}	0.72	0.72	0.72
	(0.69-0.73)	(0.69-0.73)	(0.69-0.73)
ε_{TKI}	-	0.889	0.932
		(0.881-0.893)	(0.907-0.946)
z_{TKI}	-	0.046	0.083
		(0.046-0.047)	(0.083-0.084)

Relative fitness



2.15 (2.02 – 2.45)

0.69 (0.67 – 0.75)

0.41 (0.32 – 0.57)

CML cells

* p = 0.025

Conclusions

- **The higher affinity of *nilotinib* by itself cannot explain the deeper response observed**
- **The differential impact on self-renewal (1-ε) is small and may be difficult to detect in vitro**
- **This small difference has a major impact on the dynamics**
- **Evolutionary dynamics takes into consideration the environment and competition between populations**
- **These two aspects provide an explanation for the differences in response to the two agents**

- Stem cell dynamics and hematopoiesis
  - Dingli & Pacheco, *PLoS ONE*, 2006
  - Dingli et al, *PLoS Computational Biology*, 2007
  - Dingli et al, *PLoS ONE*, 2007
  - Dingli & Pacheco, *Proceedings of the Royal Society B*, 2007
  - Dingli et al, *Cell Cycle*, 2008
  - Dingli & Pacheco, *Stem Cell Reviews*, 2008
  - Dingli et al, *Proceedings of the National Academy of Sciences*, 2008
  - Traulsen et al, *Stem Cells*, 2008
  - Dingli & Pacheco, *Wiley Interdiscip Rev Syst Biol Med,* 2010
  - Peixoto et al, *Mathematical and Computer Modelling,* 2010
  - Traulsen et al, *BioEssays*, 2010
  - Werner et al, *PLoS Computational Biology*, 2011
  - Traulsen et al, *Journal of the Royal Society Interface*, 2012
- Allometry of hematopoiesis
  - Lopes et al, *Blood*, 2007
  - Dingli et al, *Proceedings of the Royal Society B*, 2008
- Chronic myeloid leukemia
  - Dingli et al, *Clinical Leukemia*, 2008
  - Pacheco et al, *Journal of Theoretical Biology*, 2009
  - Lenaerts et al, *Haematologica*, 2010
  - Traulsen et al, *Cancer Letters*, 2010
  - Dingli et al, *Genes and Cancer*, 2010
  - Lenaerts et al, *Cell Cycle*, 2011

Acknowledgments

- University of Lisbon
  - Jorge M. Pacheco
  - Diogo Peixoto
- Max Planck Institute (Plön)
  - Arne Traulsen
  - Benjamin Werner
- Free University of Brussels
  - Tom Lenaerts
- University of Bologna/GIMEMA
  - Gianantonio Rosti
  - Fausto Castagnetti
- Funding
  - Mayo Clinic
  - MN Partnership
  - NIH
  - FCT Portugal