Measuring lymphocyte turnover using BrdU: what is the correct model?

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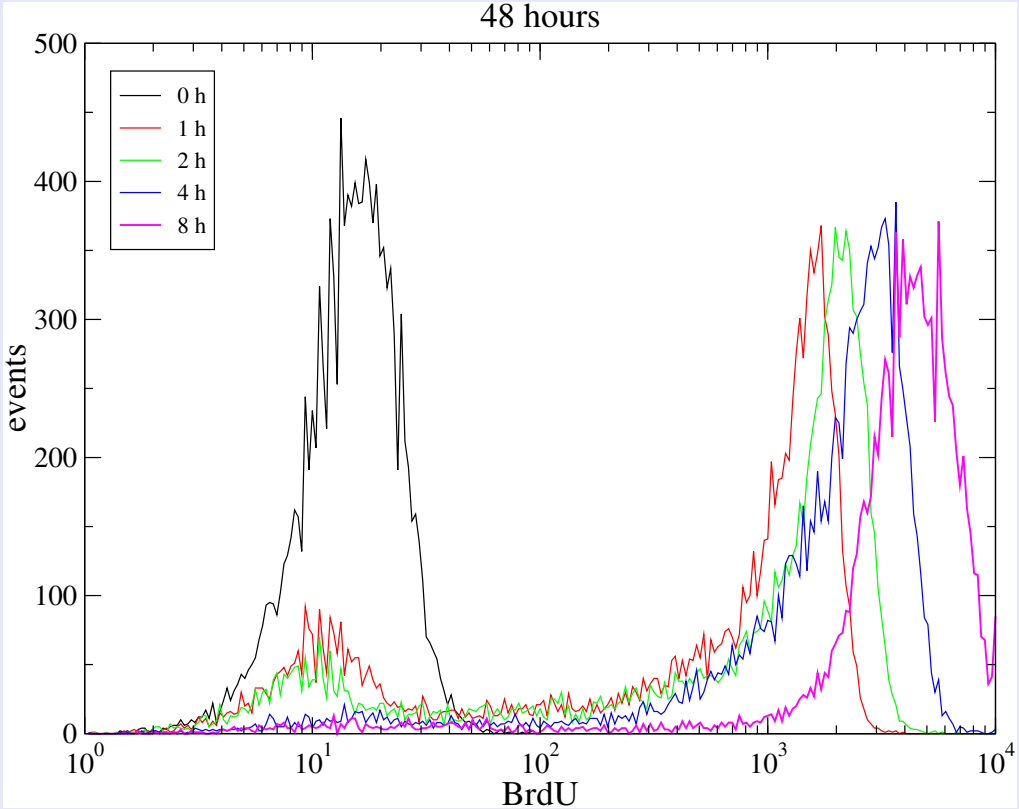
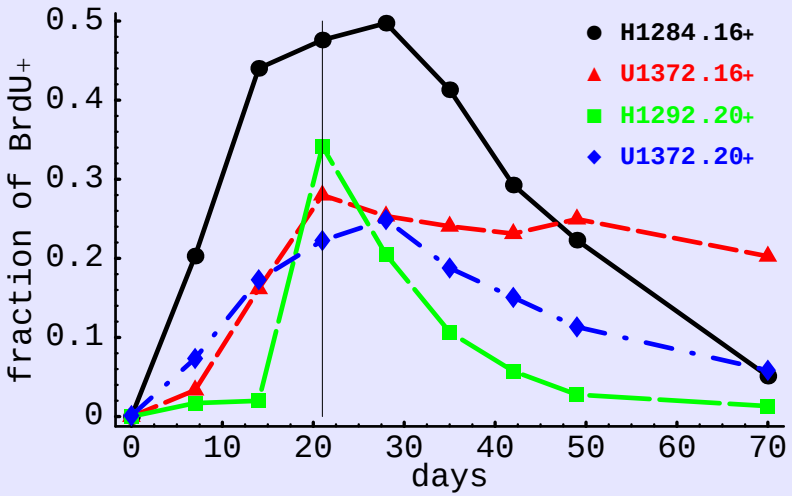
5-bromo-2'-deoxyuridine (BrdU)

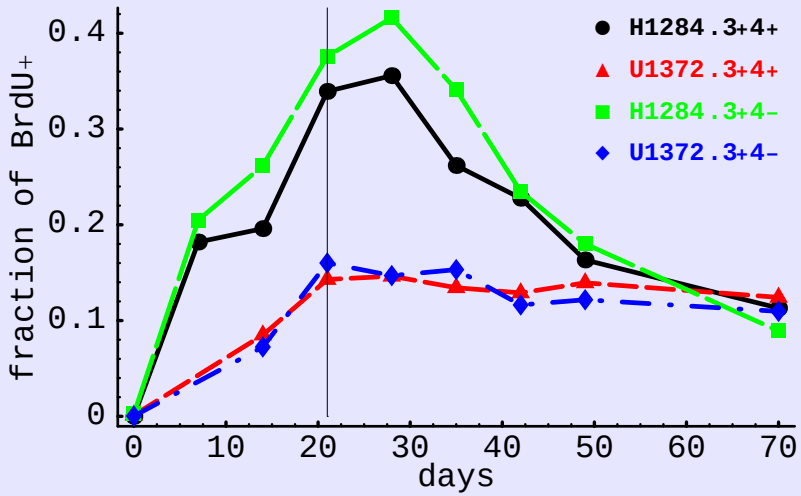
- Thymidine analog.
- Can be detected by specific antibody.
- Level of BrdU incorporated by each cell is measured.



Pulse-chase experiment

- Often measurements of cell turnover are done in two steps:
  - During the **pulse**, BrdU is administered, and the fraction of BrdU+ cells increases over time.
  - During the **chase**, the fraction of BrdU+ cells declines over time.





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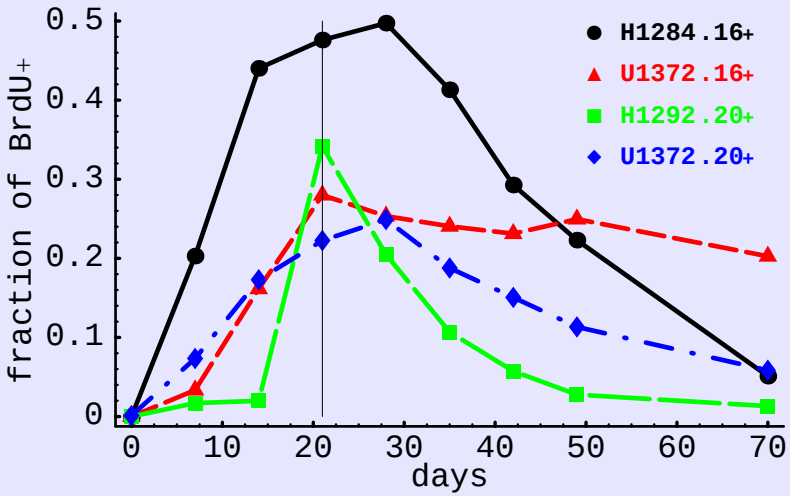
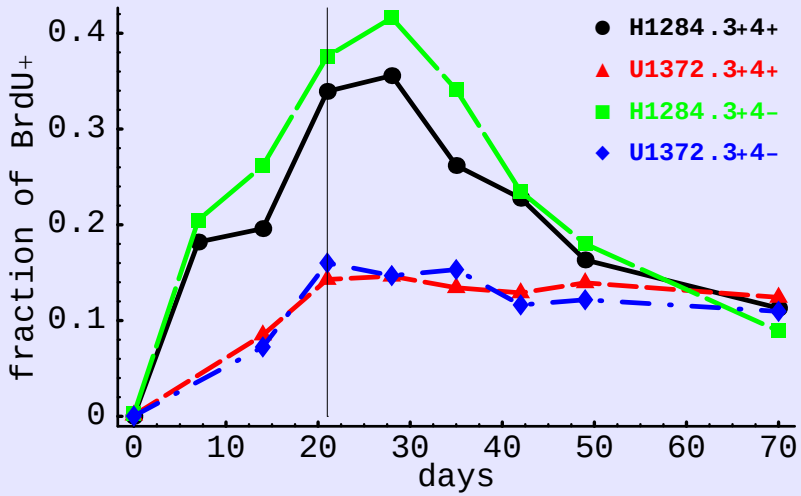
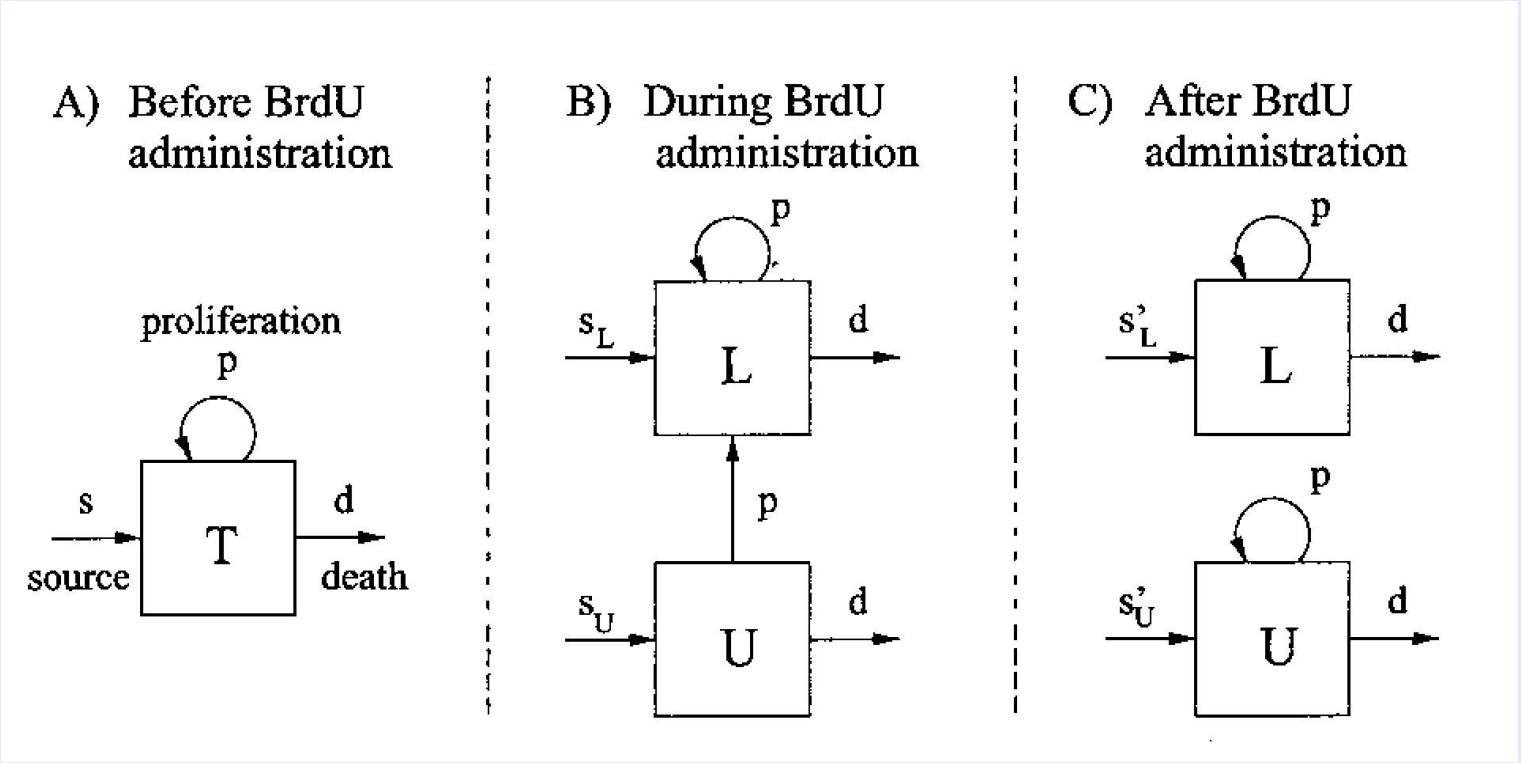




Main models

- Source model
- Kinetic heterogeneity model

The source model



$$L(t) = \begin{cases} A_1(1 - \mathrm{e}^{-(p+d)t}), & \text{if } t < T, \\ A_2 + (L(T) - A_2)\mathrm{e}^{-(d-p)(t-T)}, & \text{otherwise} \end{cases}$$

where $L(t)$ is the fraction of BrdU+ cells in the population.

Mohri et al. 1998

A (simplified) source model

- Assume that only a fraction of cells in the population α is turning over (e.g., activated cells A).
- During pulse: BrdU+ cells can appear by division of unlabeled cells or from a “source”.

$$\frac{\mathrm{d}L(t)}{\mathrm{d}t} = \hat{s} + (p-d)L(t) + 2pU(t)$$

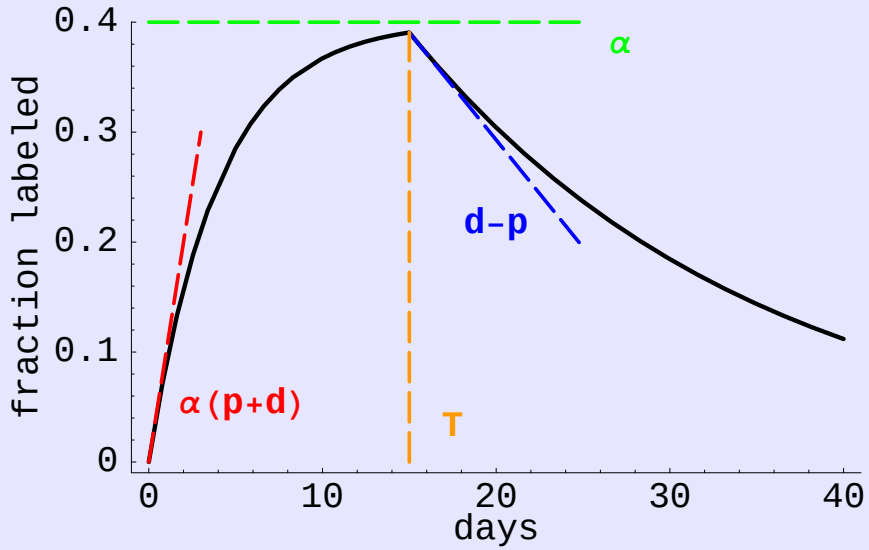
- During chase: BrdU+ cells disappear because new unlabeled cells come from the source.

$$\frac{\mathrm{d}L(t)}{\mathrm{d}t} = (p-d)L(t)$$

De Boer et al. 2003

Solution and properties

$$L(t) = \begin{cases} \alpha(1 - e^{-(p+d)t}), & \text{if } t < T, \\ \alpha(1 - e^{-(p+d)T})e^{-(d-p)(t-T)}, & \text{otherwise,} \end{cases}$$



- Pros: General (3 parameter) model; simple interpretation of the data; can fit various types of data.
- Cons: Source s and proliferation p often cannot be uniquely identified; nature of source is unclear; too large estimate for source for naive T cells.

Is there really a problem with the source model?

- For some cell populations (naive T cells, B cells), there could be clearly a source of new cells.
- In the model, source could also arise because of resting cells becoming activated

$$\begin{aligned} \frac{\mathrm{d}R(t)}{\mathrm{d}t} &= g(R,A) - aR(t), \\ \frac{\mathrm{d}A(t)}{\mathrm{d}t} &= caR(t) + (p-d)A(t) = s + (p-d)A(t). \end{aligned}$$

where $caR(t)$ is the number of activated cells produced by proliferation of resting cells.

Bonhoeffer et al. JI 2000; De Boer et al. PRSL 2003

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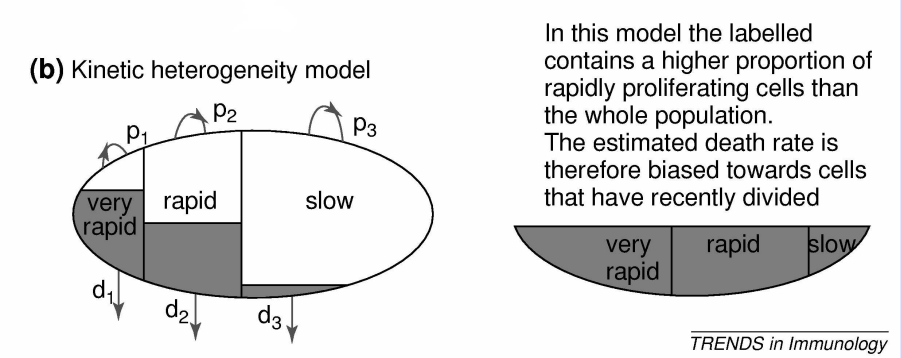
- 3-5% per day production from the source seems too large, unless the expansion factor c is large.

Bonhoeffer et al. JI 2000; De Boer et al. PRSL 2003

Kinetic heterogeneity model



- During pulse: BrdU+ cells appear by division.

$$\frac{\mathrm{d}L(t)}{\mathrm{d}t} = (p-d)L(t) + 2pU(t)$$

- During chase: labeled cells disappear because their death rate d is higher than the average proliferation rate p.

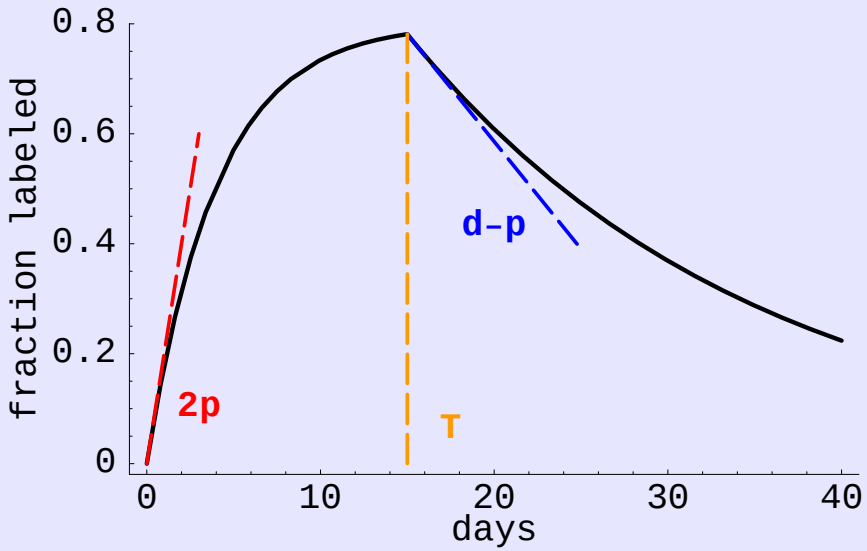
$$\frac{\mathrm{d}L(t)}{\mathrm{d}t} = (p-d)L(t)$$

$L(t)$ — fraction of BrdU+ cells, and $p \leq d$.

Debacq et al. 2002

Solution and properties

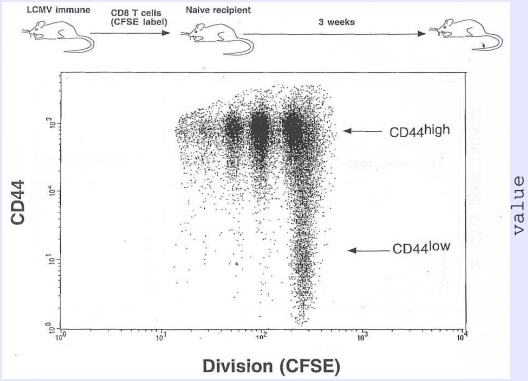
$$L(t) = \begin{cases} \left(\frac{2p}{p+d}\right)(1 - e^{-(p+d)t}), & \text{if } t < T, \\ \left(\frac{2p}{p+d}\right)(1 - e^{-(p+d)T})e^{-(d-p)(t-T)}, & \text{otherwise,} \end{cases}$$

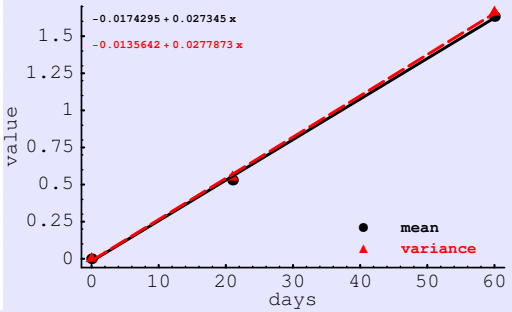


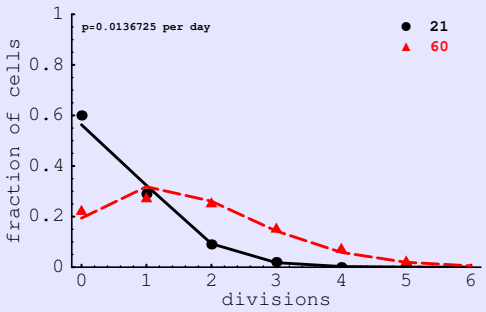
- Pros: Simple, few parameters; somewhat mechanistic.
- Cons: Not all types of heterogeneity will work. No good evidence for a higher death rate of labeled cells.

Problems with kinetic heterogeneity

- Turnover of LCMV-specific and CD44 memory CD8+ T cells in mice is homogeneous







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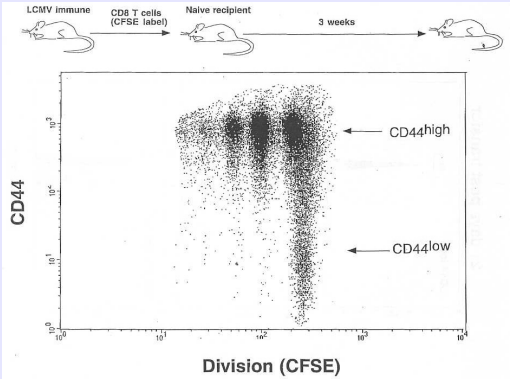
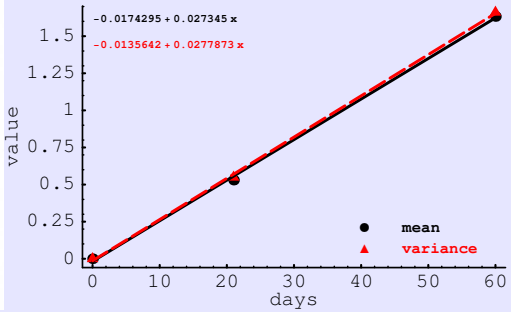
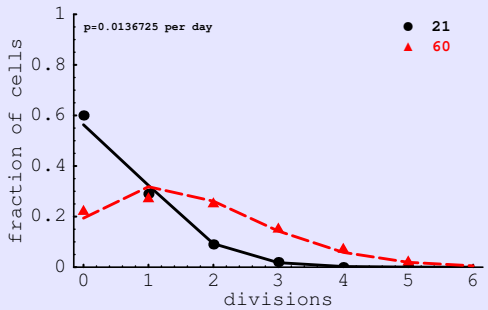
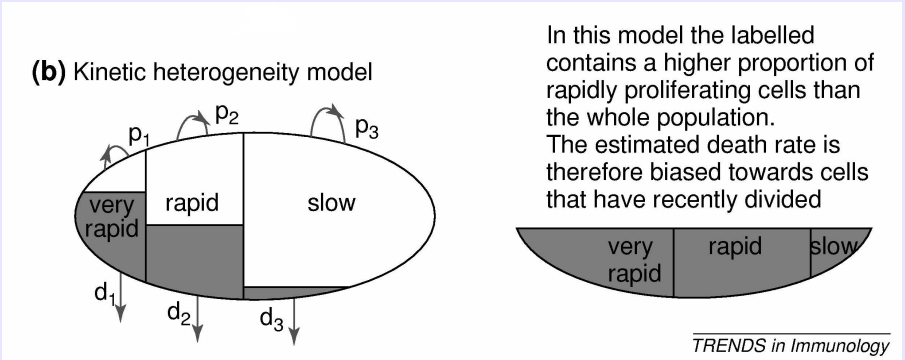






- Simple kinetic heterogeneity model will not allow for the loss of BrdU+ cells during the chase phase



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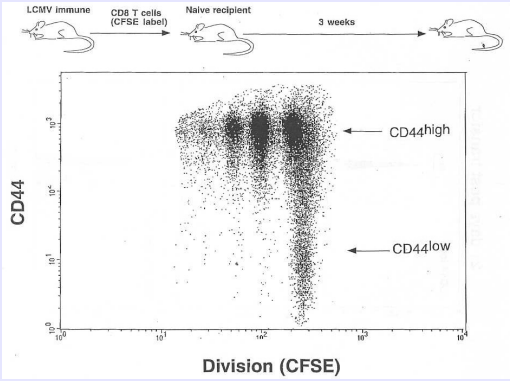

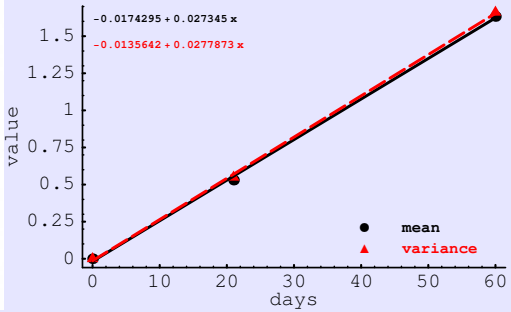

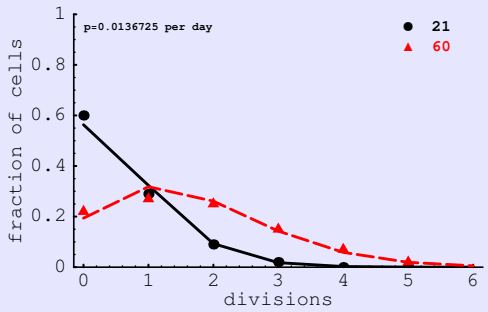

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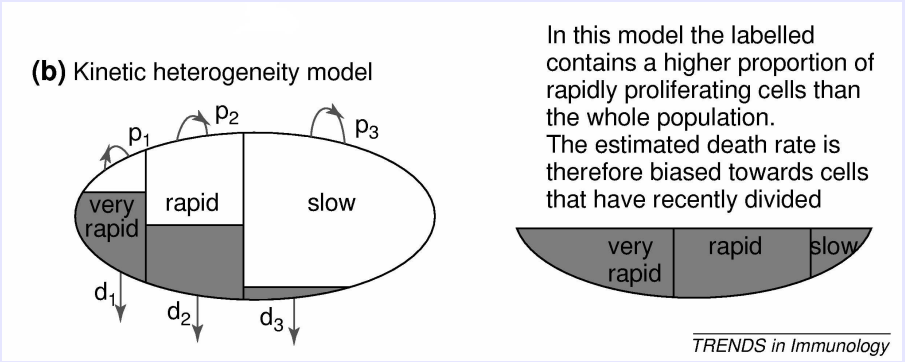
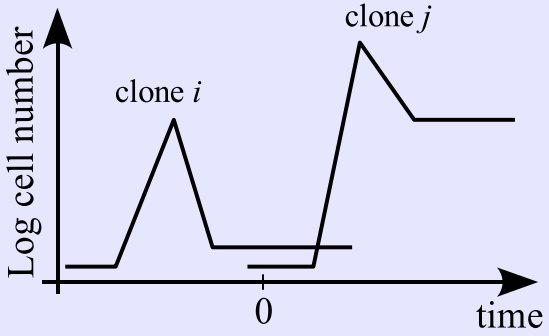
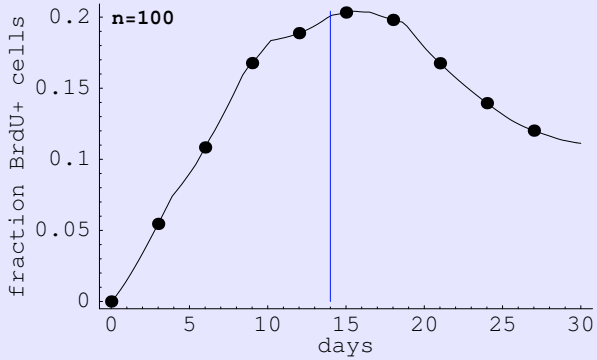


- Temporal heterogeneity may work.

Temporal heterogeneity

- Program-like responses (Ag-specific or non-specific?)





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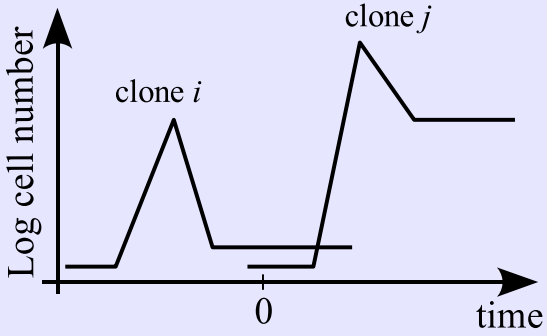

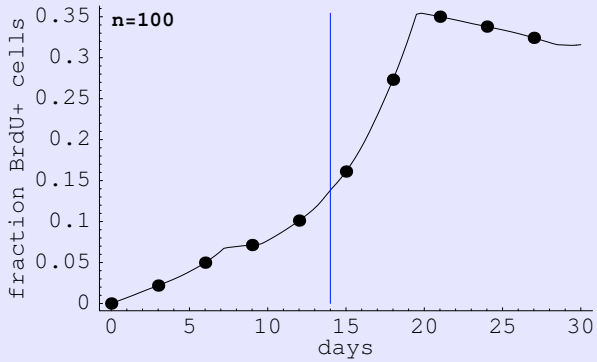

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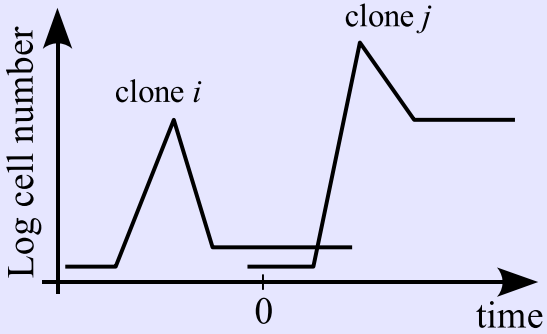

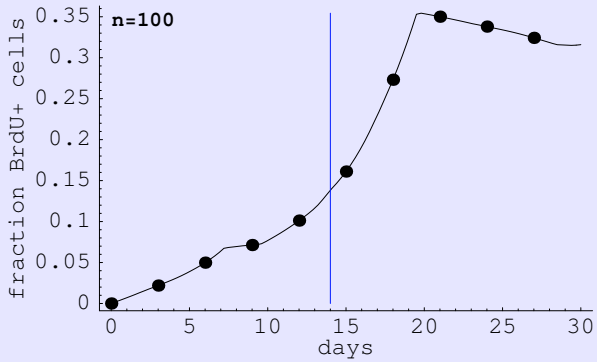

- Activation of resting cells and recruitment of them into the pool of activated/proliferating cells

$$\begin{aligned} \frac{\mathrm{d}R(t)}{\mathrm{d}t} &= g(R,A) - aR(t), \\ \frac{\mathrm{d}A(t)}{\mathrm{d}t} &= aR(t) + (p-d)A(t). \end{aligned}$$

where $aR(t)$ is activation of resting cells (without cell division).

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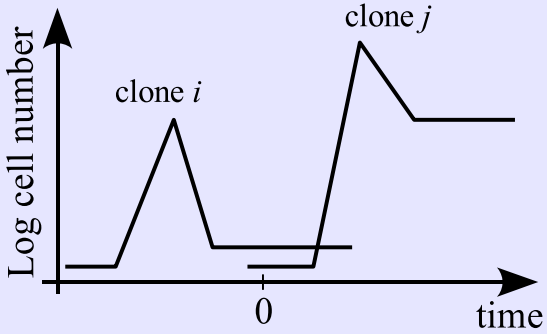

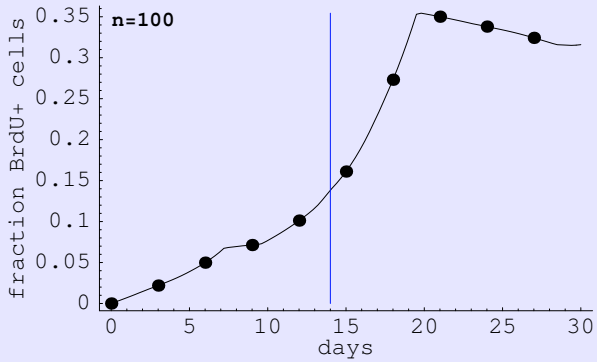

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where $aR(t)$ is activation of resting cells (without cell division).
Also a large "source" term $s = aR^*/X^* \approx d - p$ is estimated.

Similarity between the source and KH models

- source

$$L(t) = \begin{cases} \alpha(1 - e^{-(p+d)t}), & \text{if } t \leq T, \\ L(T)e^{(p-d)(t-T)}, & \text{otherwise,} \end{cases}$$

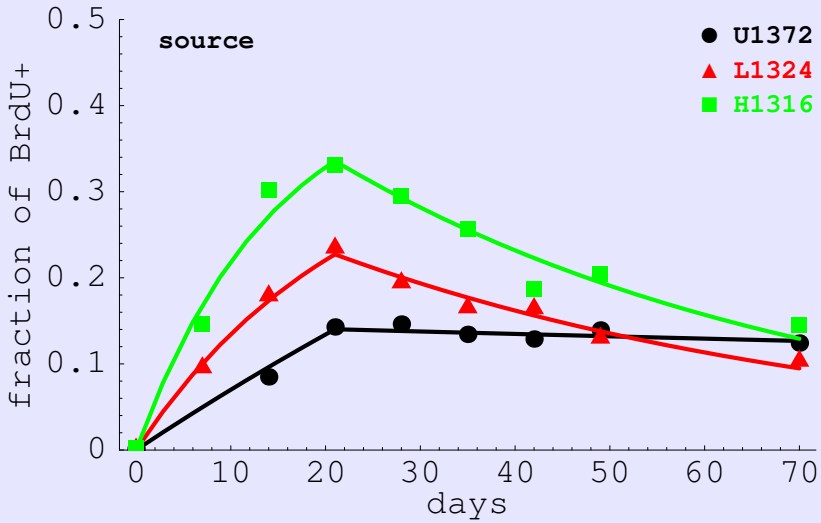
- heterogeneity

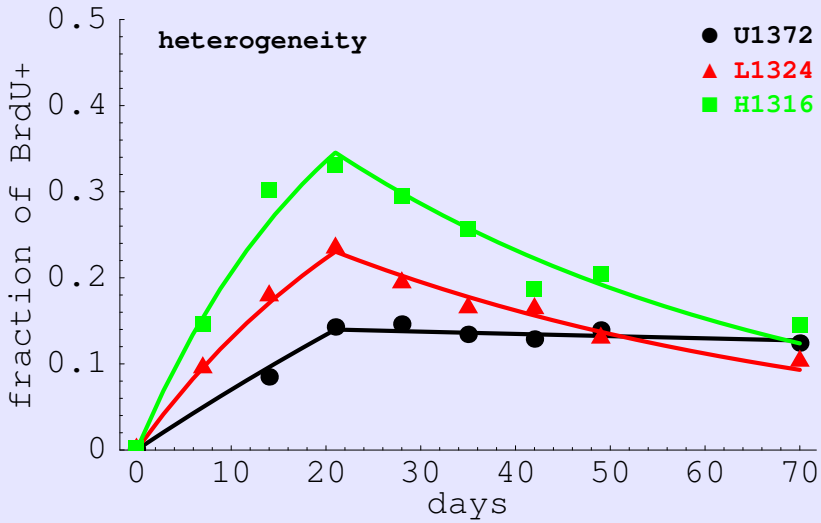
$$L(t) = \begin{cases} \left(\frac{2p}{p+d}\right)(1 - e^{-(p+d)t}), & \text{if } t \leq T, \\ L(T)e^{(p-d)(t-T)}, & \text{otherwise,} \end{cases}$$

Average turnover rate is not the same in two models

$$\alpha \times d \neq p$$

Can both models fit the data?

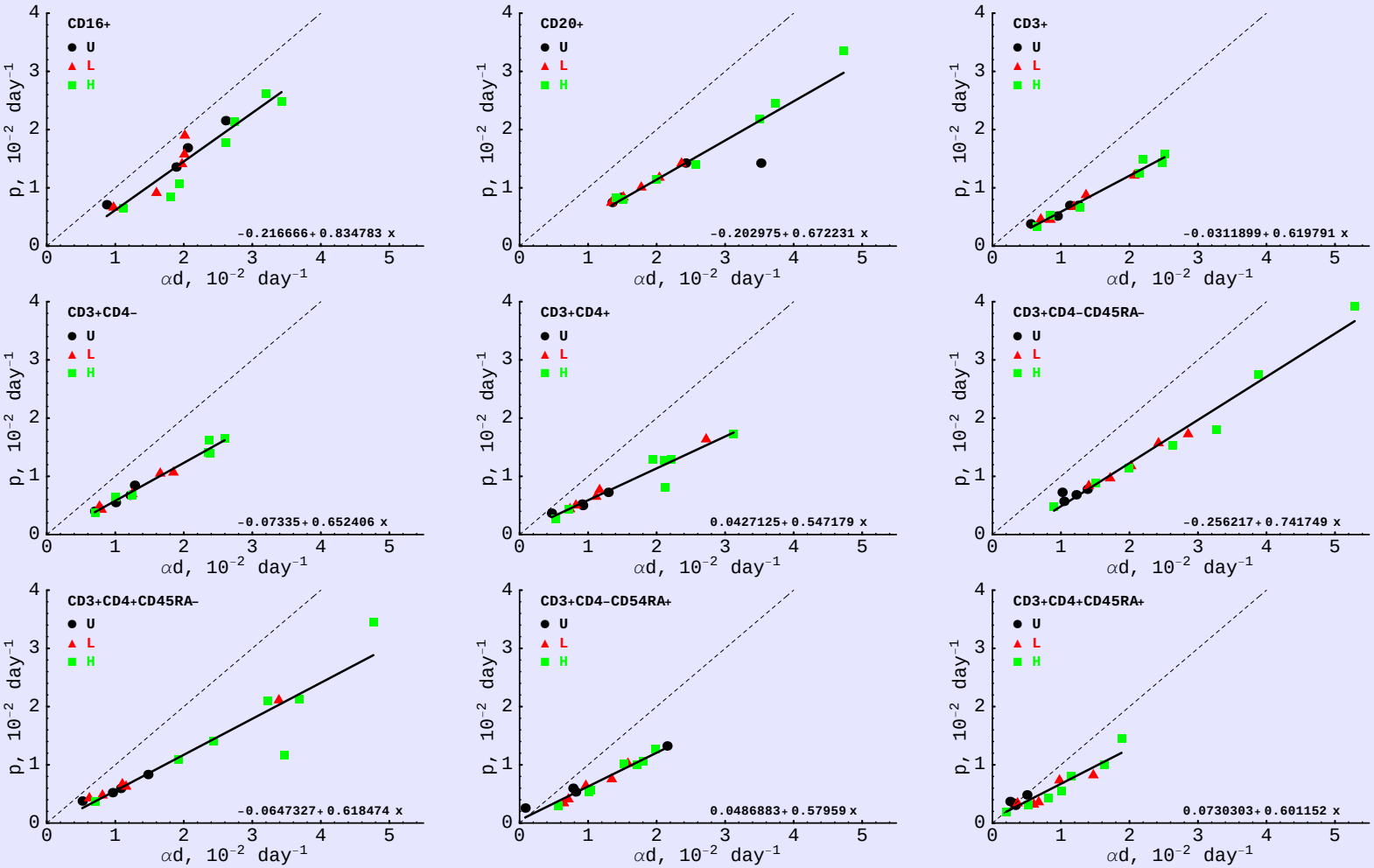




	U1372	L1324	H1316
p	**0.004**	**0.008**	**0.013**
	0.003-0.004	0.007-0.008	0.011-0.015
d	0.006	0.026	0.034
	0.003-0.008	0.023-0.029	0.028-0.040
p	0.003	0.013	0.025
	0.003-0.029	0.002-0.025	0.005-0.051
d	0.005	0.031	0.044
	0.004-0.030	0.021-0.043	0.026-0.068
α	0.837	0.373	0.438
	0.192-0.872	0.291-0.578	0.348-0.735
αd	**0.004**	**0.012**	**0.019**
	0.004-0.006	0.011-0.013	0.017-0.025

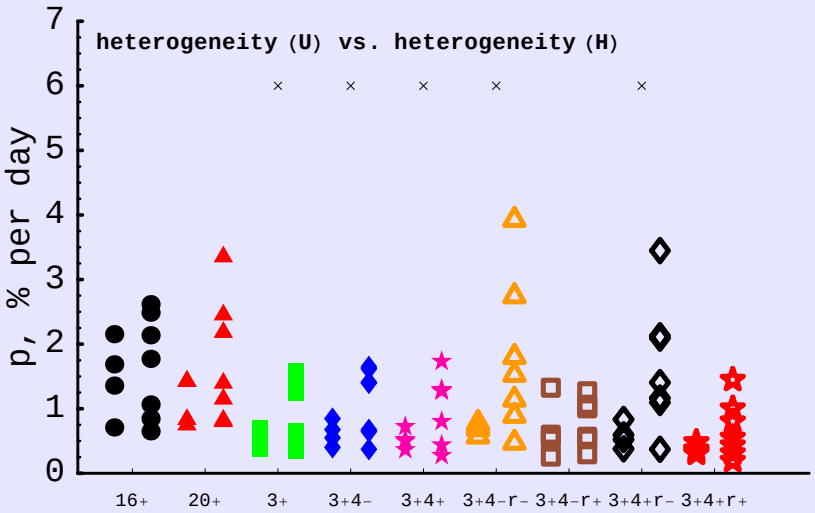
data from Mohri et al. 1998

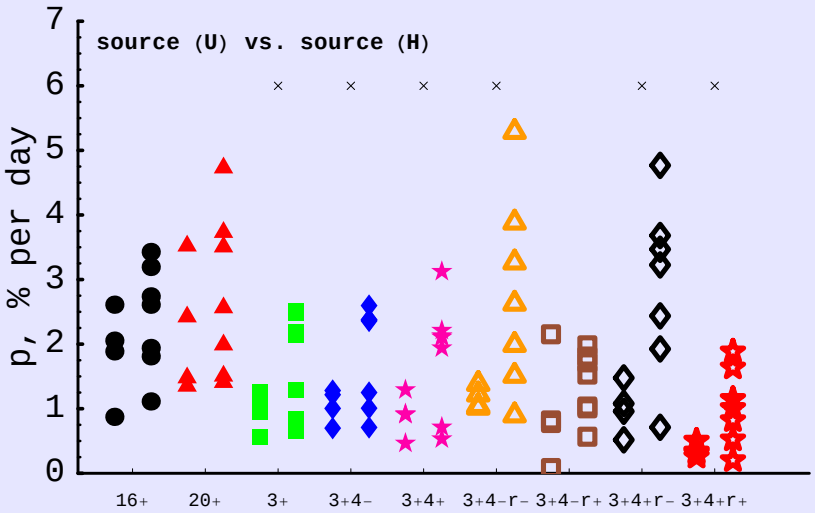
Exploring differences between parameter estimates



data from Mohri et al. 1998

Does SIV infection lead to a higher turnover rate?





$\times$: $p < 0.05$

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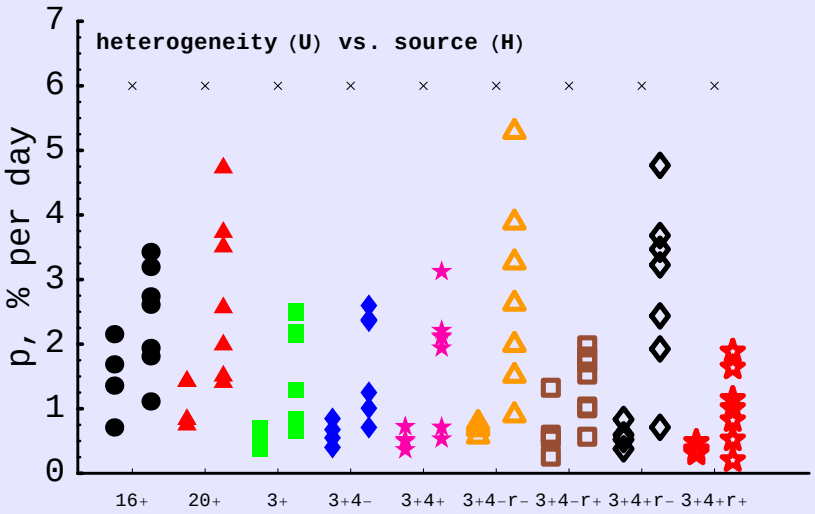

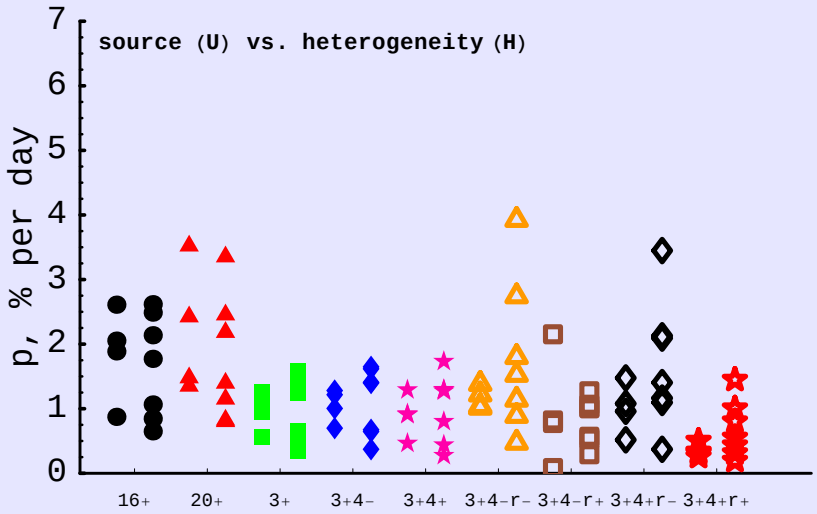

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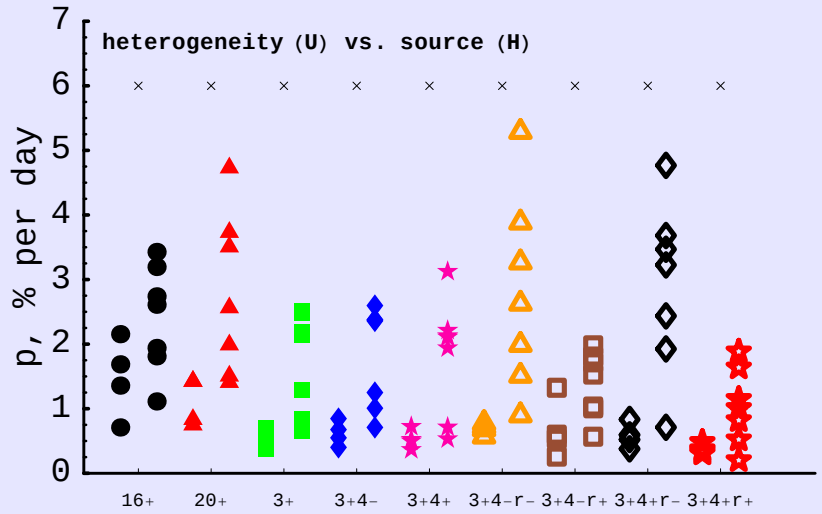
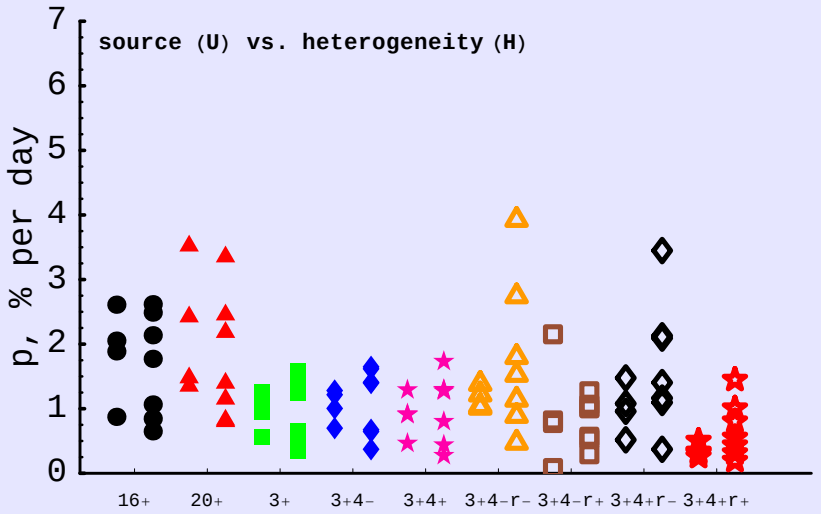


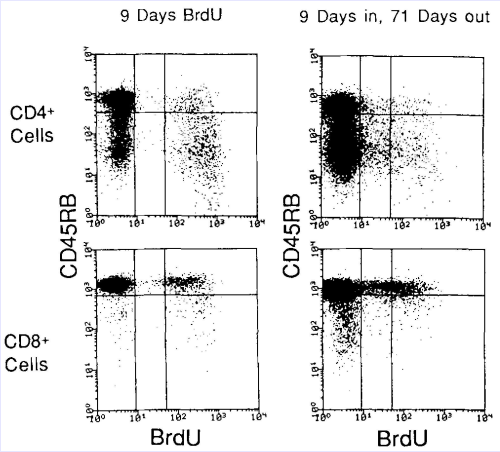


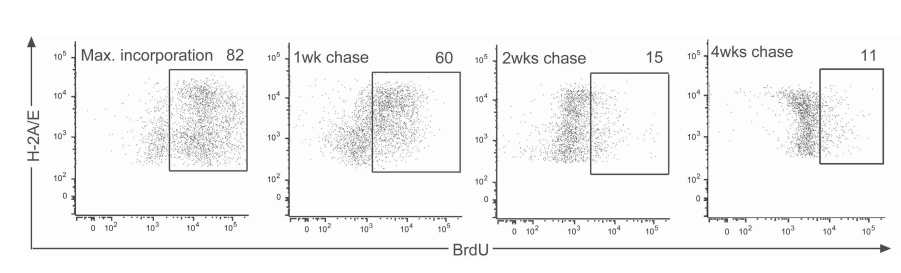
$\times$: $p < 0.05$

Summary:

- more information is needed on the kinetic structure of the population in question!

Reduction of BrdU per cell due to division



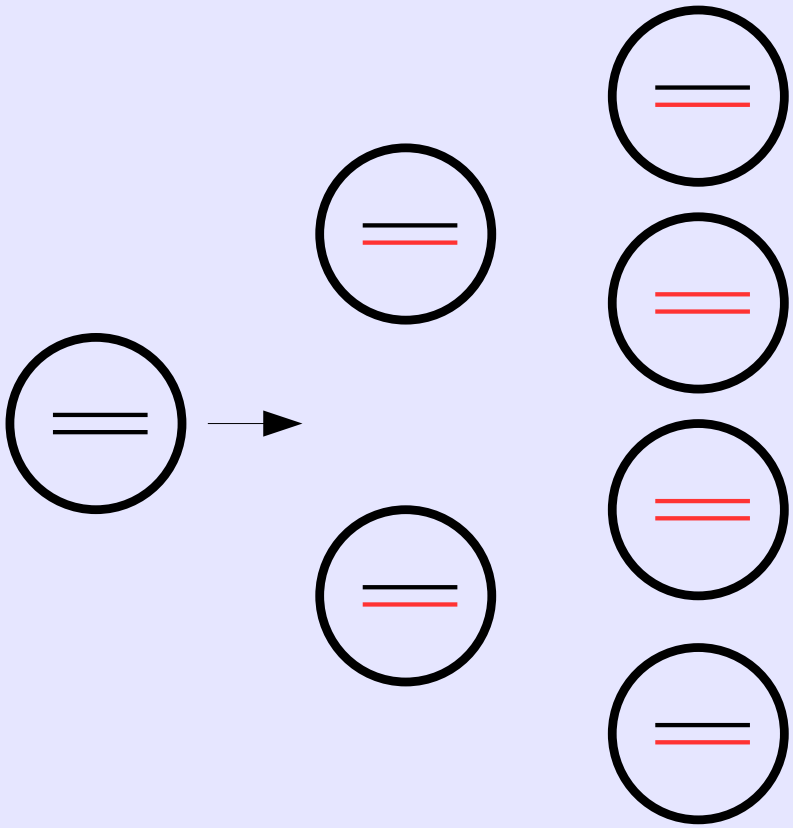


Hints:

- BrdU- cells may arise simply due to dilution of BrdU in dividing BrdU+ cells
- Proliferation of BrdU+ cells should lead to reduction in the BrdU content per cell (MFI)

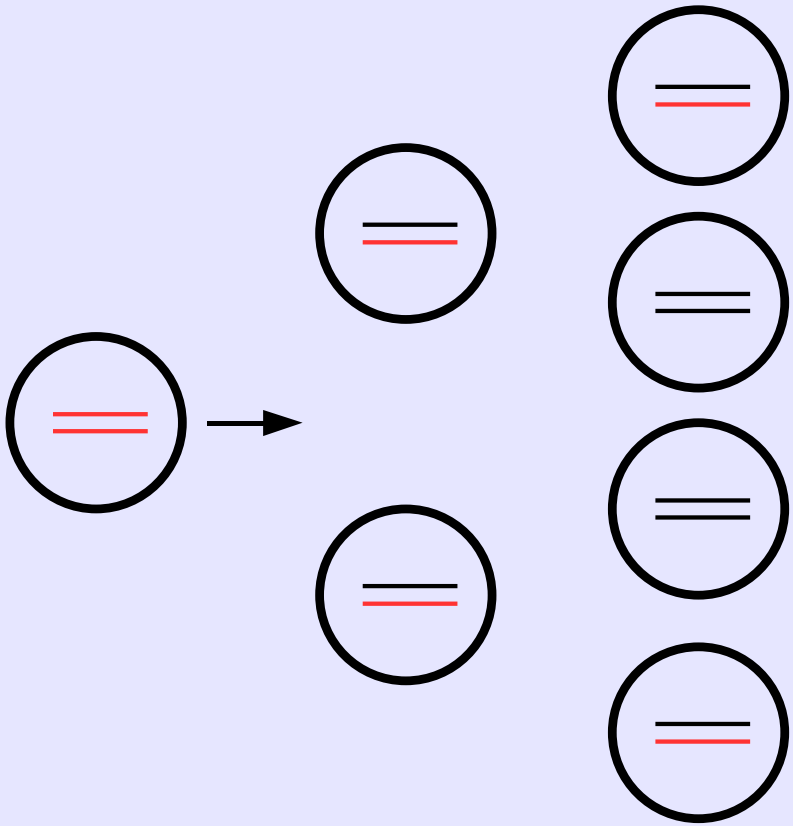
Tough and Sprent JEM 1994; Gray et al JEM 2007

BrdU accumulation (during pulse)



n=0	n=1	n=2	n
$l_0=0$	$l_1=1/2$	$l_2=3/4$	$l_n=1-2^{-n}$

BrdU loss (during chase)



k=0 $l_{\infty 0}$=1

k=1 $l_{\infty 1}$=1/2

k=2 $l_{\infty 2}$=1/4

k $l_{nk}=(1-2^{-n})/2^{k}$

BrdU MFI (ODE model)

Pulse

$$I_M(t) = \sum_{n=1}^{\infty} l_n X_n(t)/X(t) = (1 - \mathrm{e}^{-pt}), \qquad t \leq T$$

Chase

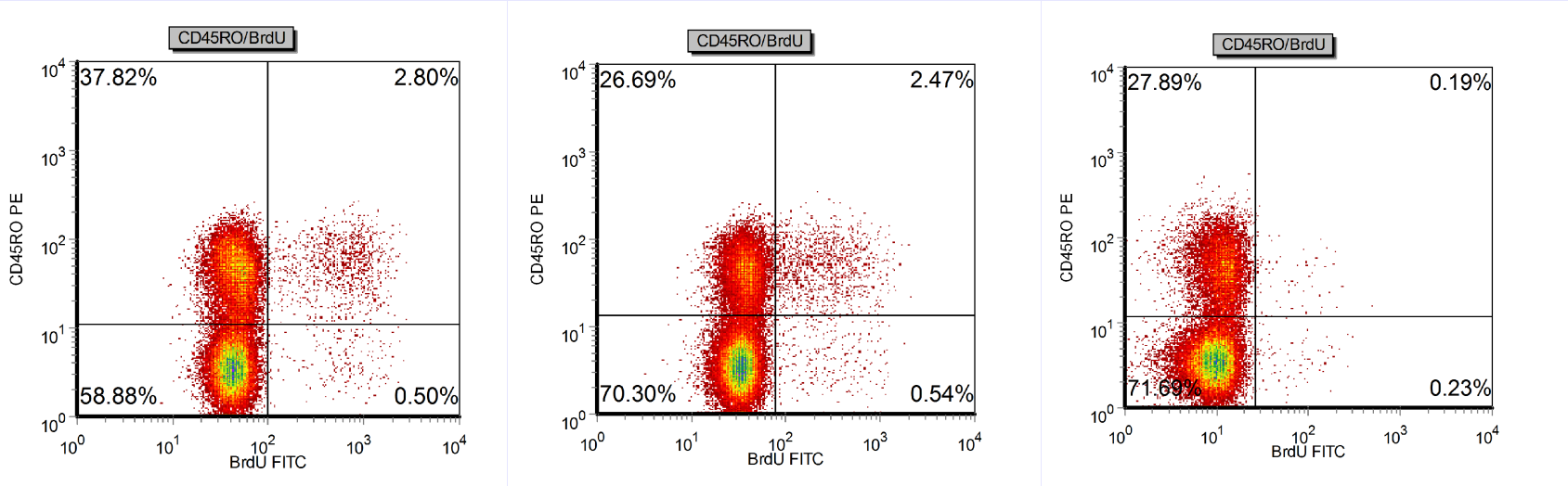
$$I_M(t) = \sum_{n,k} l_{nk} Y_{nk}(t) / \sum_{n,k} Y_{nk}(t) = I_M(T)\mathrm{e}^{-p(t-T)}, \quad t > T$$

Labeling of CD4+ T cells with BrdU in humans

Setup

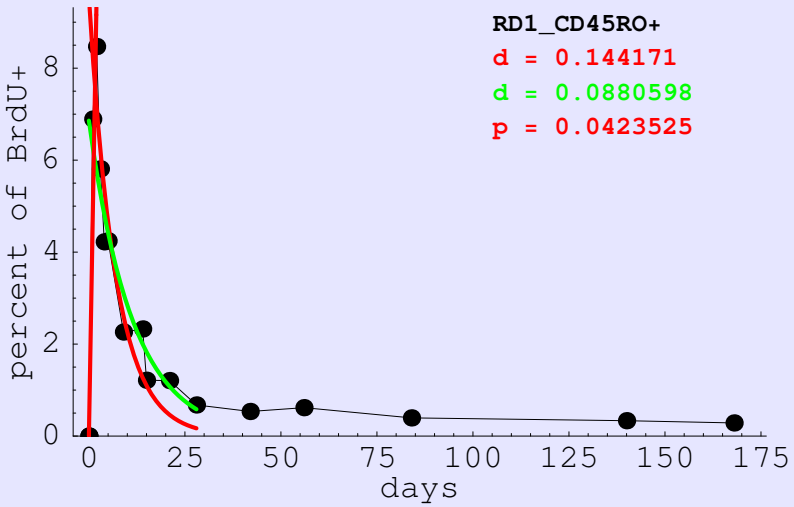
- HIV-infected untreated and HAART-treated patients were given an 30 min infusion of BrdU.
- At different times after the infusion, BrdU incorporation by CD4+CD45RO+ (effector/memory) and CD4+CD45RO- (naive) cells has been measured.

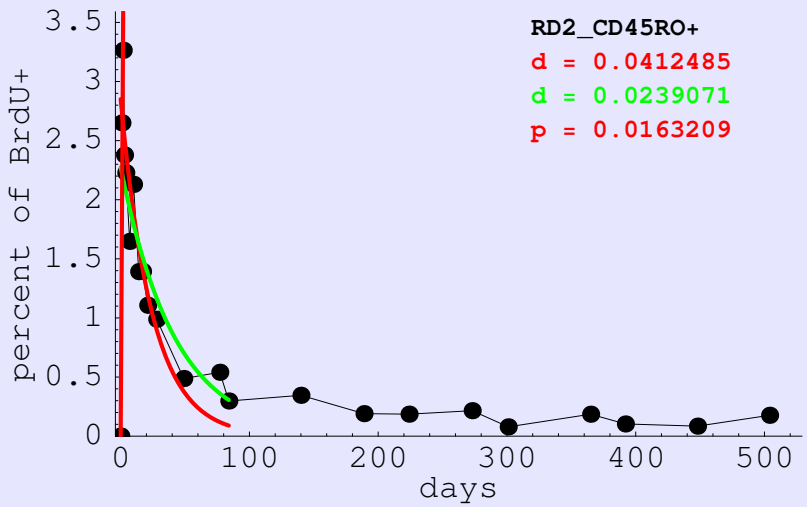
day 1 day 2 day 28



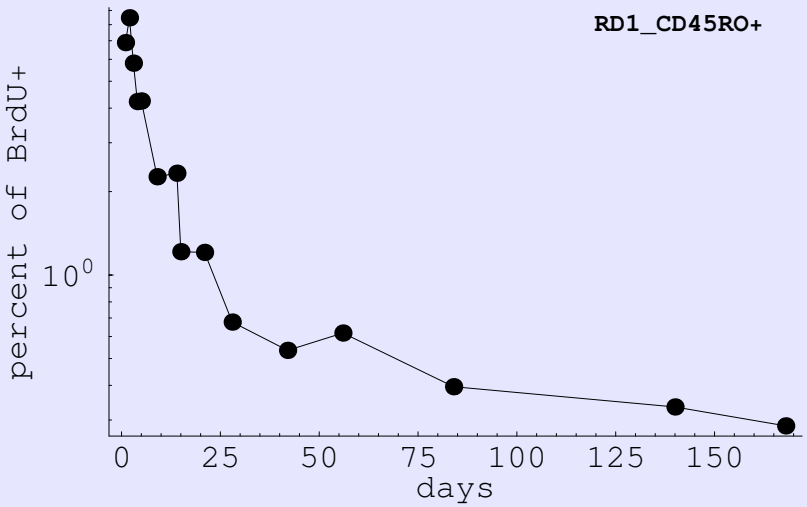
Kovacs et al.; Di Mascio et al. JV 2006

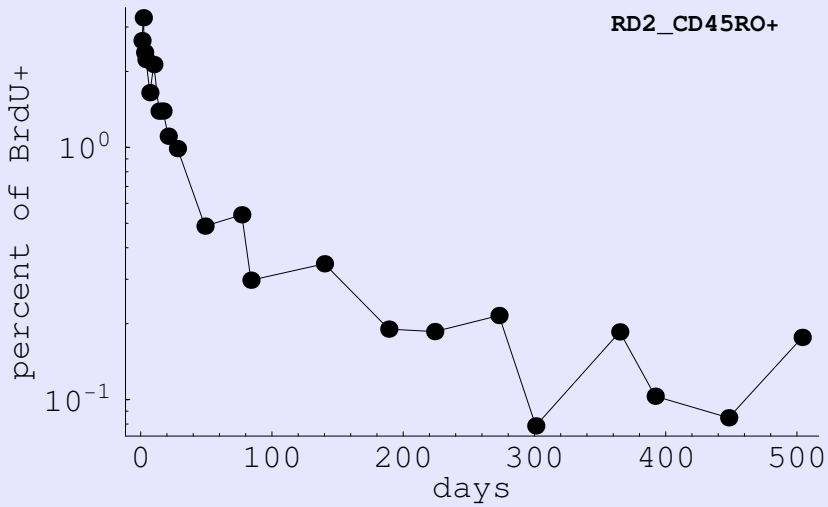
Fraction of BrdU+ cells





Fraction of BrdU+ cells

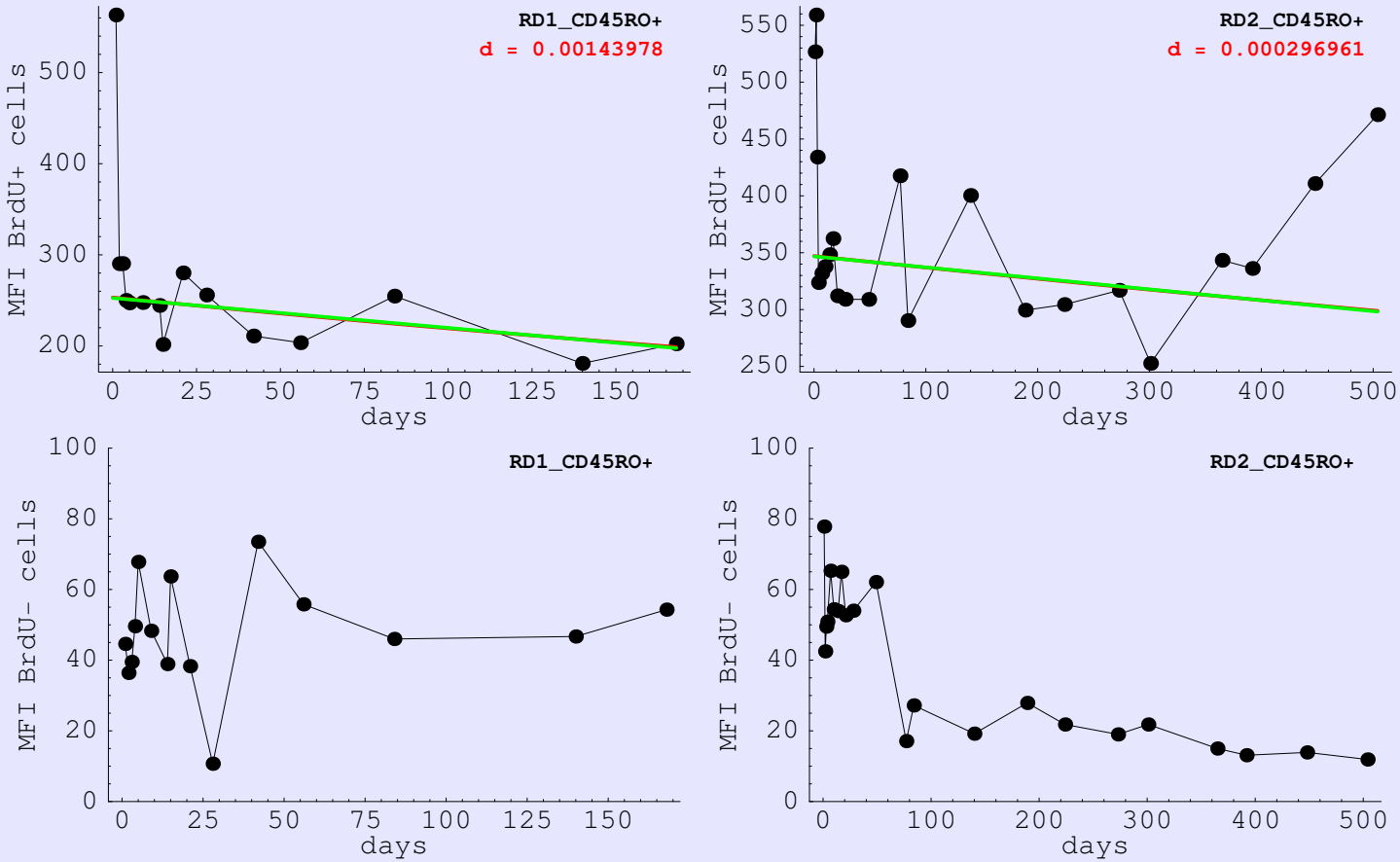




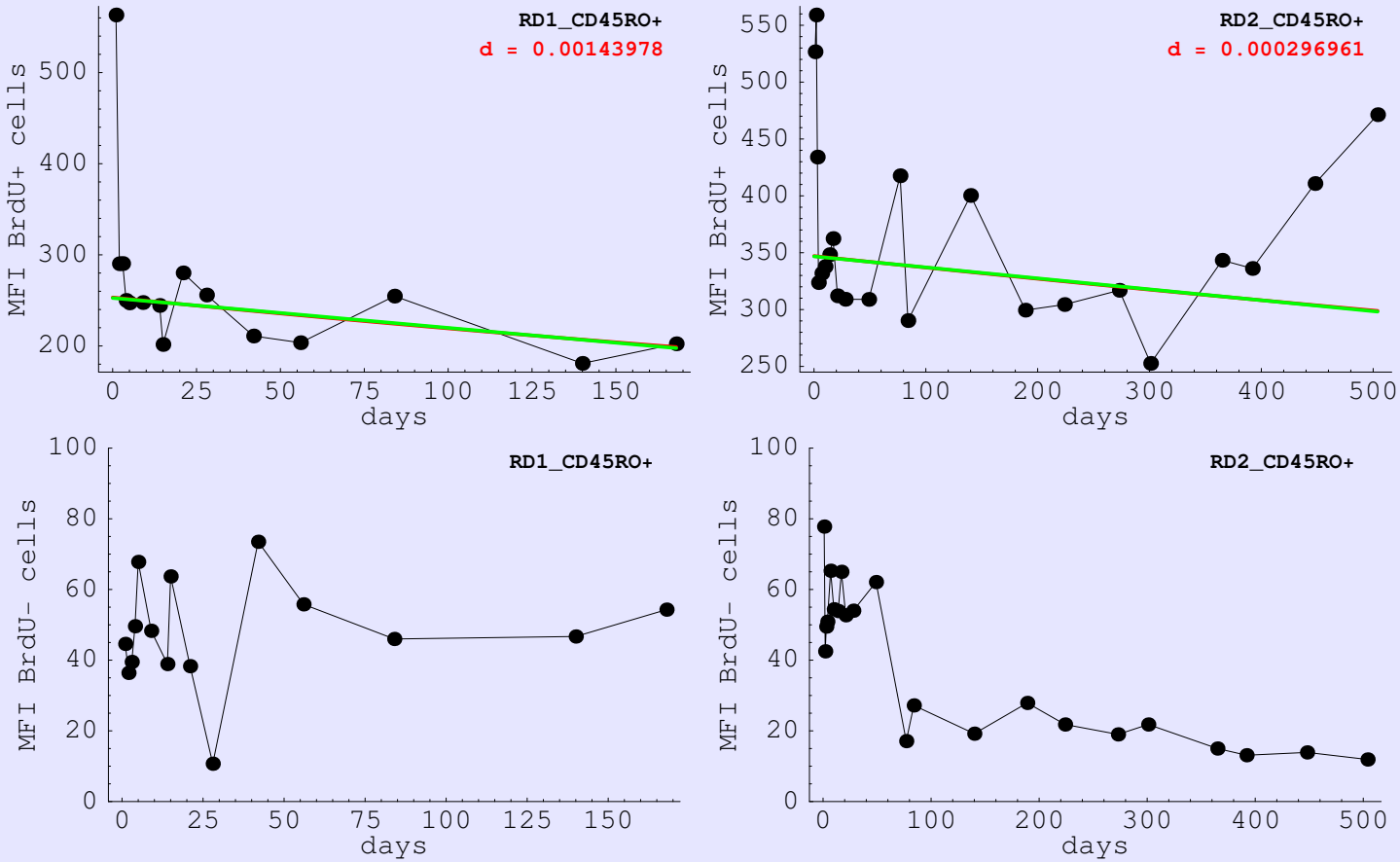
Trend

- Multiphasic decline in the fraction of BrdU+ memory CD4+ T cell is observed

BrdU MFI of BrdU+ cells



BrdU MFI of BrdU+ cells



Trend

- No change in BrdU MFI after the first 2 days

Summary

- Two current models used to describe BrdU data provide different estimates for the average turnover rate.
- Temporal heterogeneity is required to explain the loss of BrdU+ cells in the kinetic heterogeneity model.
- Analysis of the BrdU data suggests that there is little to no proliferation of BrdU+ cells during the chase phase. This feature will need to be explained by (or incorporated in) future models.
- We need better understanding of the kinetic structure of lymphocyte populations and circulation of lymphocytes between different organs (LT, blood, periphery) in healthy and infected individuals.

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