20th HIV Dynamics & Evolution
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Attempting to quantify T cell turnover

Long-lived memory is maintained by short-lived cells

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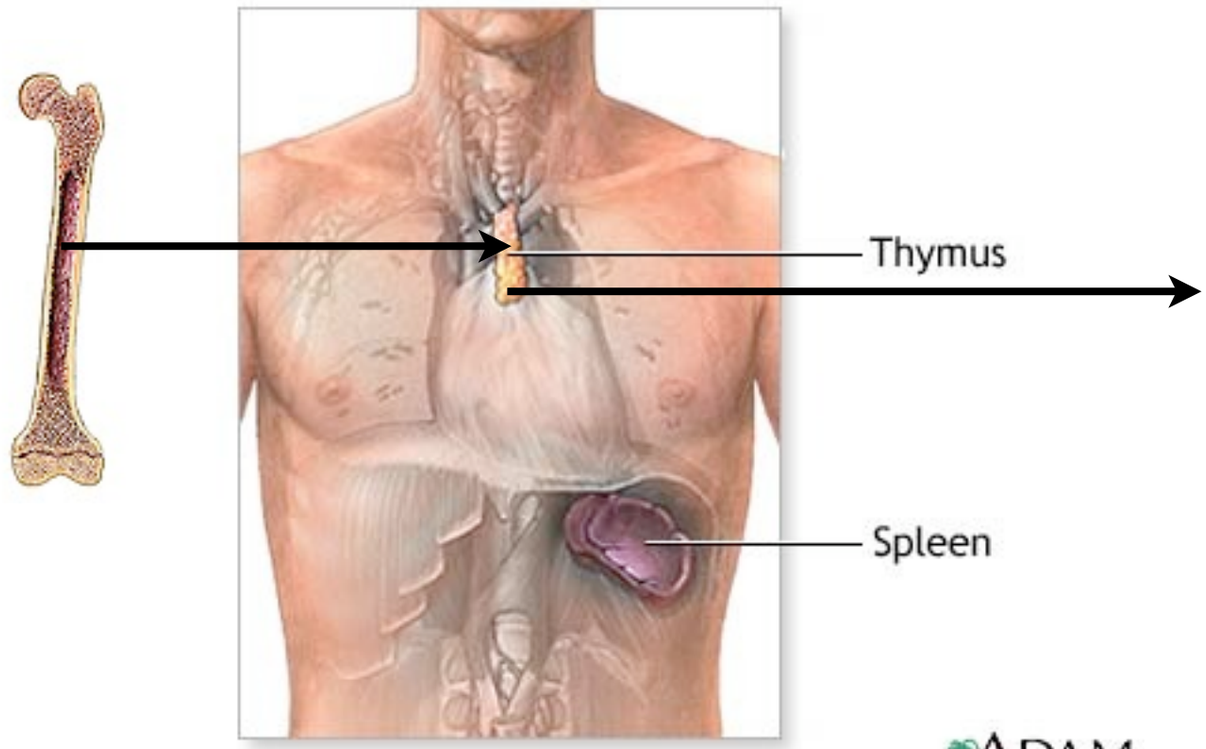


Naive T cells

infection

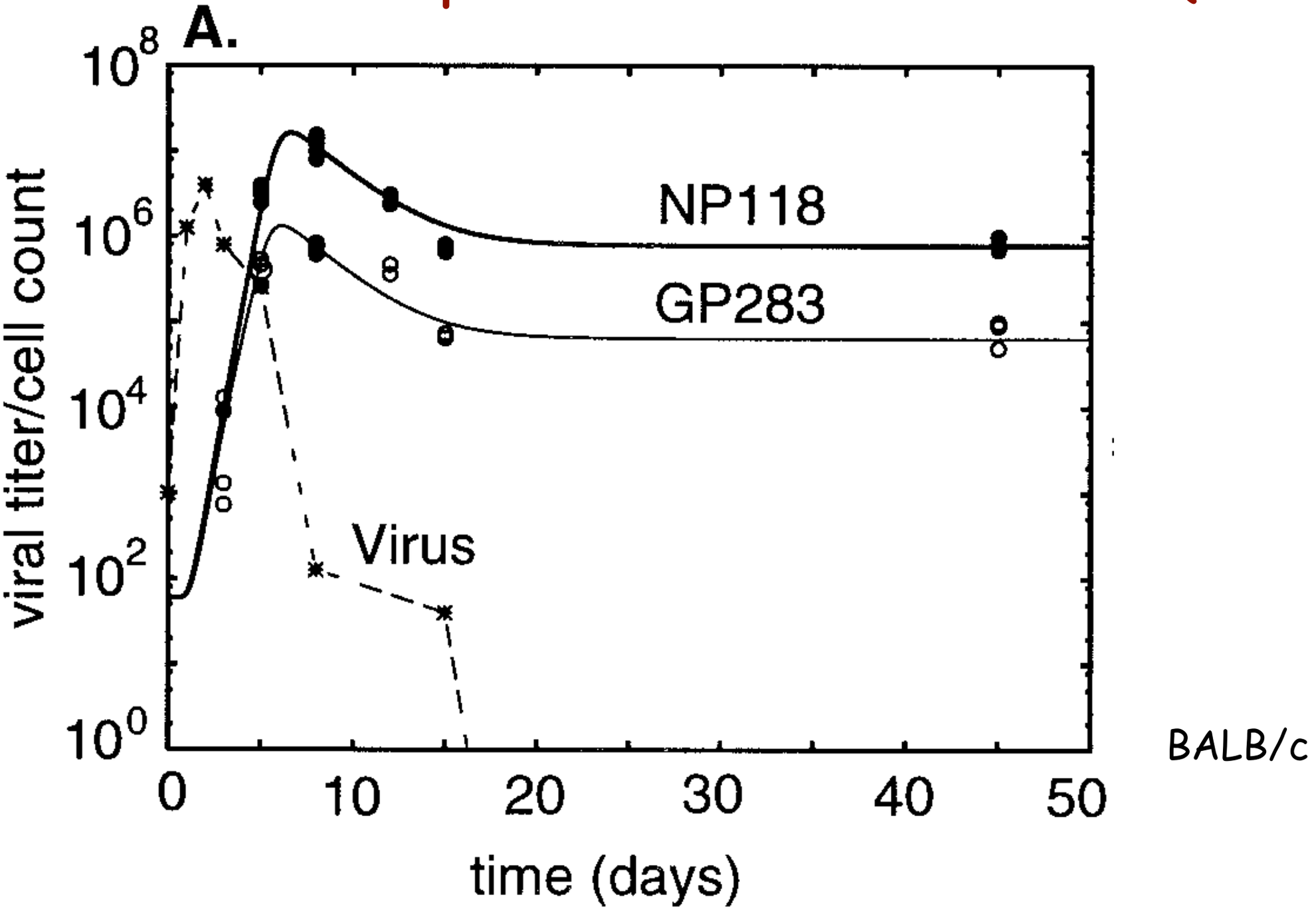
clonal expansion

Activated cells
Effector cells

Memory T cells

$$\frac{\mathrm{d}T}{\mathrm{d}t} = s + (p - d)T$$

A T cell immune response to a mouse virus (LCMV)

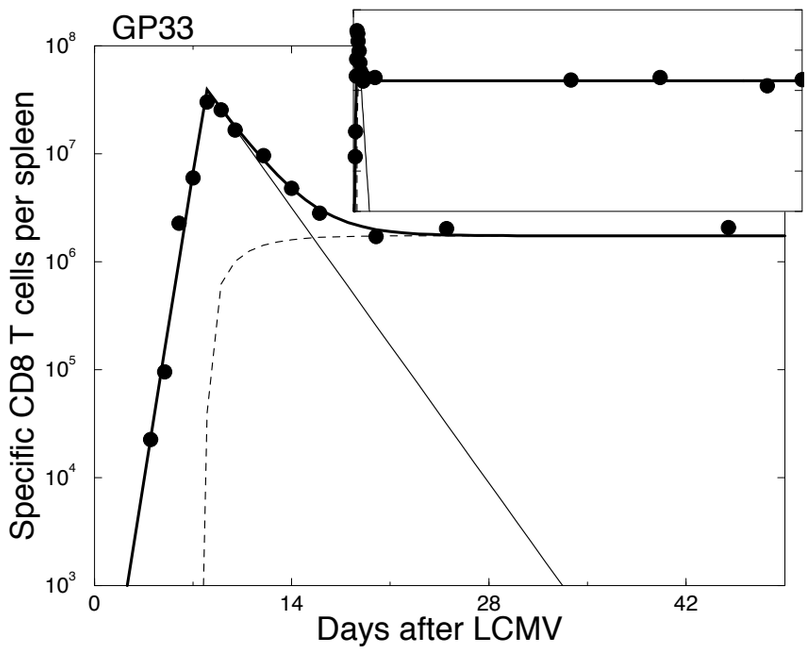


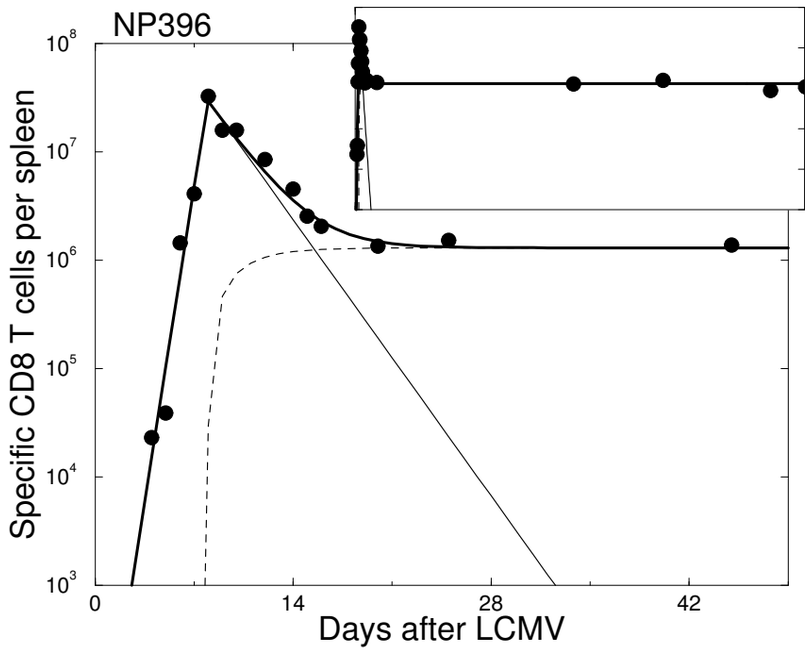
BALB/c

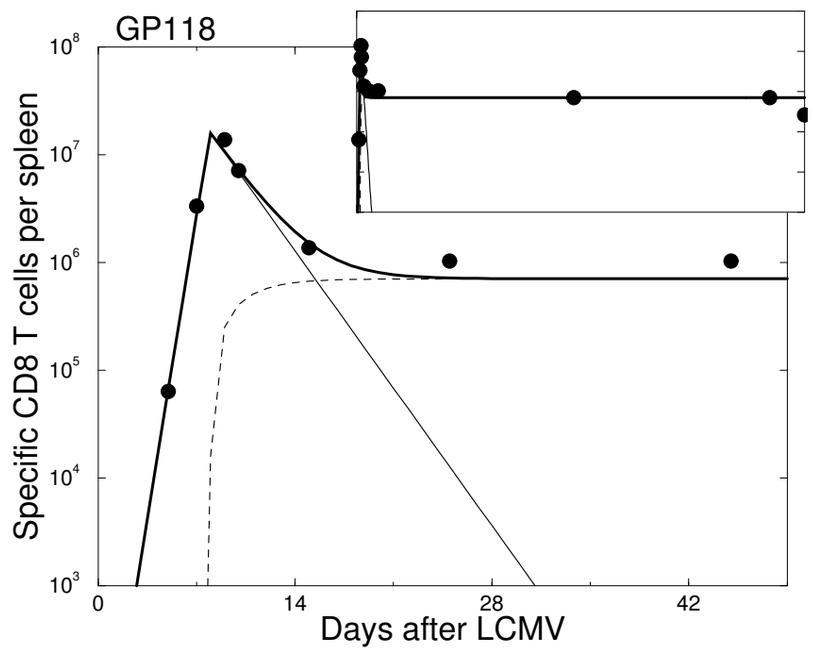
Just a few cells (< 100) start divide around day 3, they peak at day 8, decline, and then persist (memory)

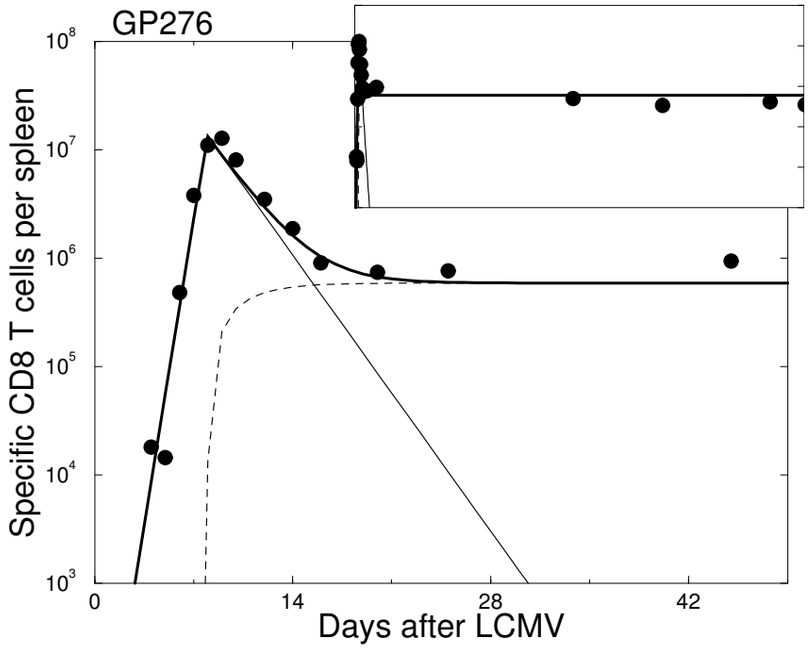
De Boer et al. J. Virol 2001

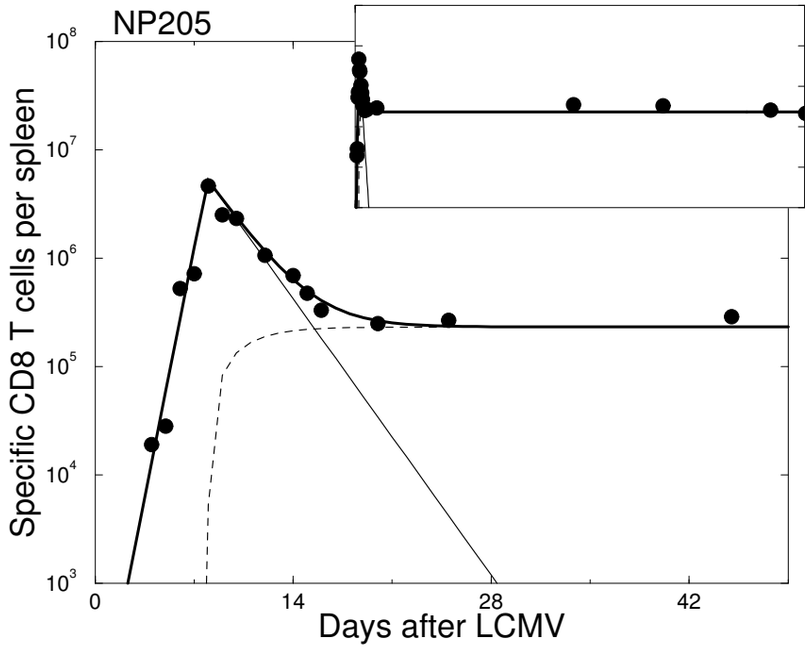
CD8 T cell responses to several "epitopes" of the virus

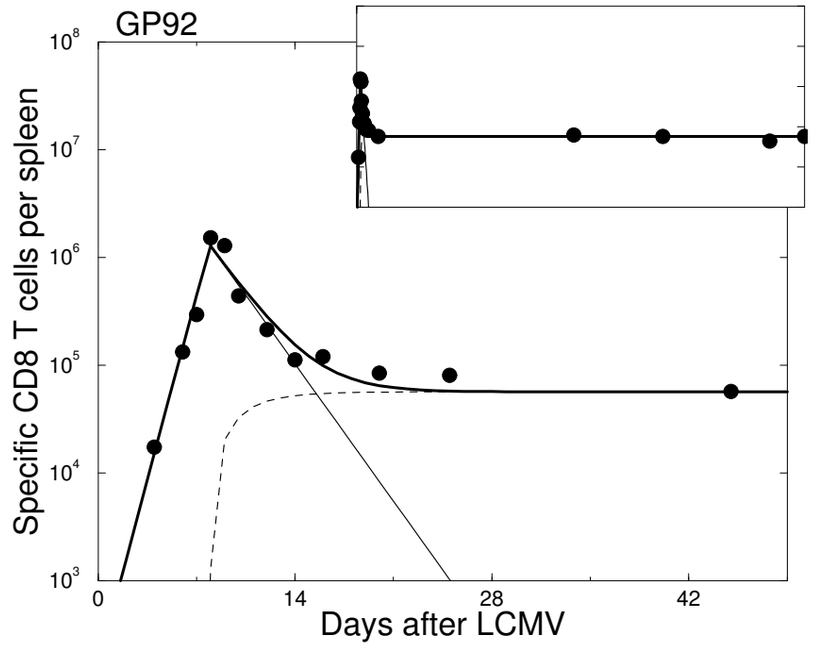












Inset is 912 days: memory lasts "forever"

De Boer et al. J. Imm. 2003

Towards a more quantitative immunology

Systems biology: new type of questions:

What is the expected life span of a normal naive T cell, and how are these cells maintained (thymus/renewal & mouse/man*)?

What is the life span of an effector/memory T cell?

How does a virus like HIV interfere with these normal population dynamics, and how does that cause the depletion of $CD4^+$ T cells?

What fraction of the naive T cell population is composed of short-lived RTE and long-lived truly naive T cells, and what is actually the expected life span within each subset?

Approach:

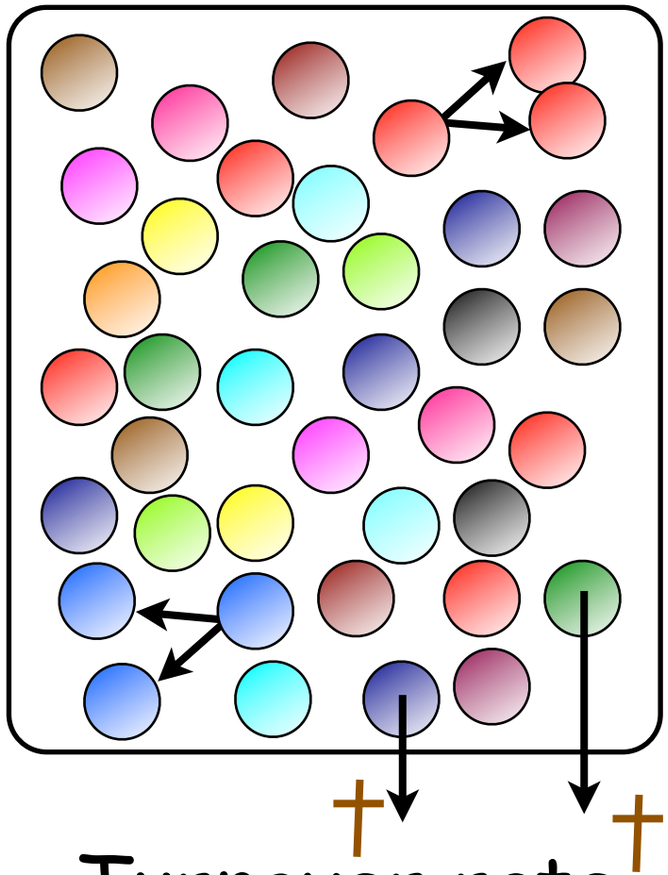
Labeling experiments with deuterium, BrdU and CFSE

Interpret data with appropriate mathematical models

*Den Braber et al. Immunity 2012

Population at steady state maintained by renewal

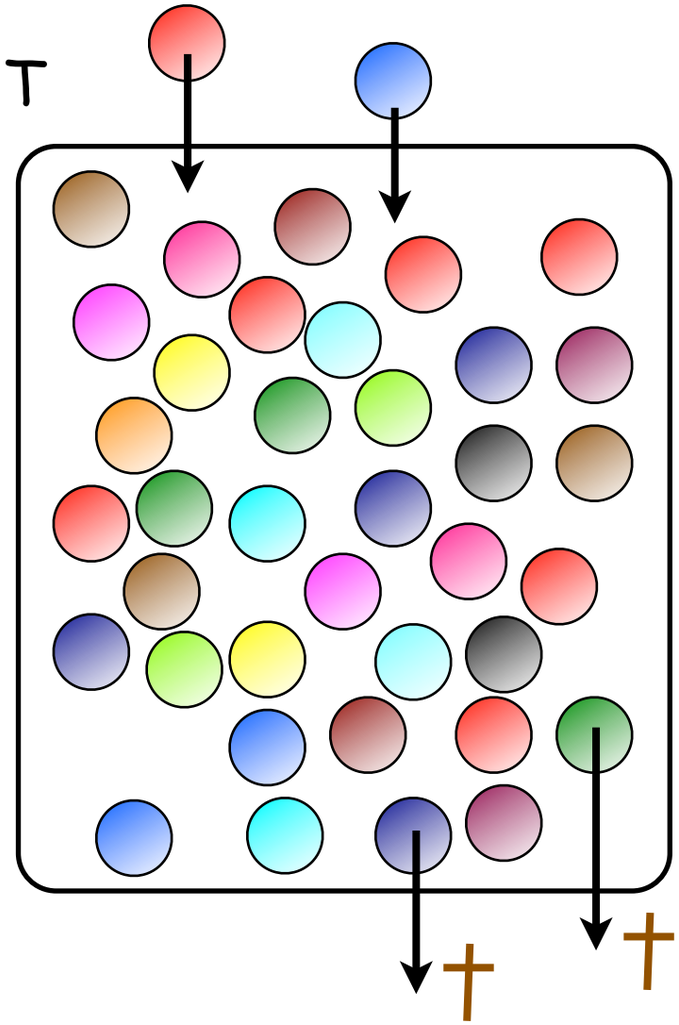
Memory T cells



Turnover rate, average life span, residence time, interdivision time

source

Naive T cells



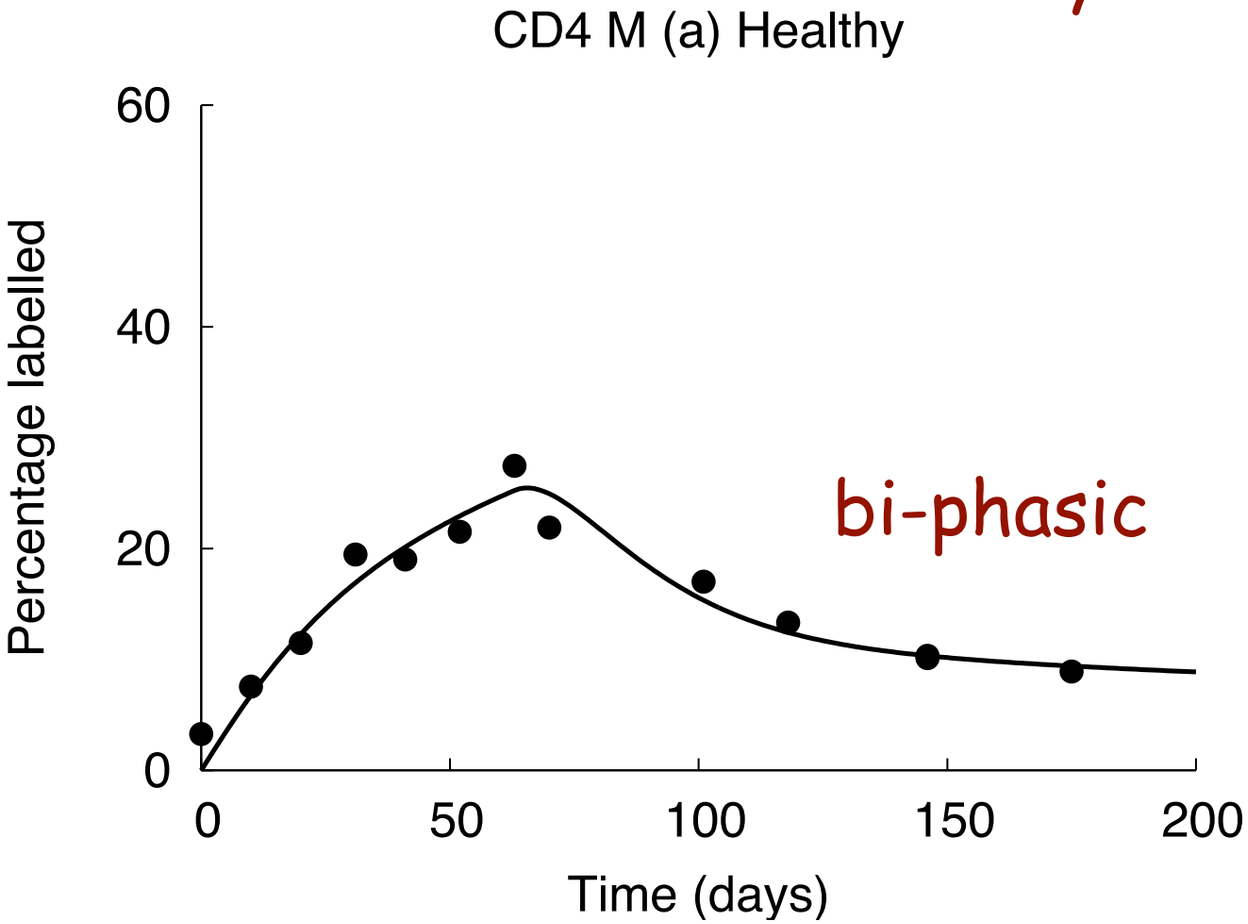
Turnover rate, average life span, residence time

#new cells
=
#cells lost

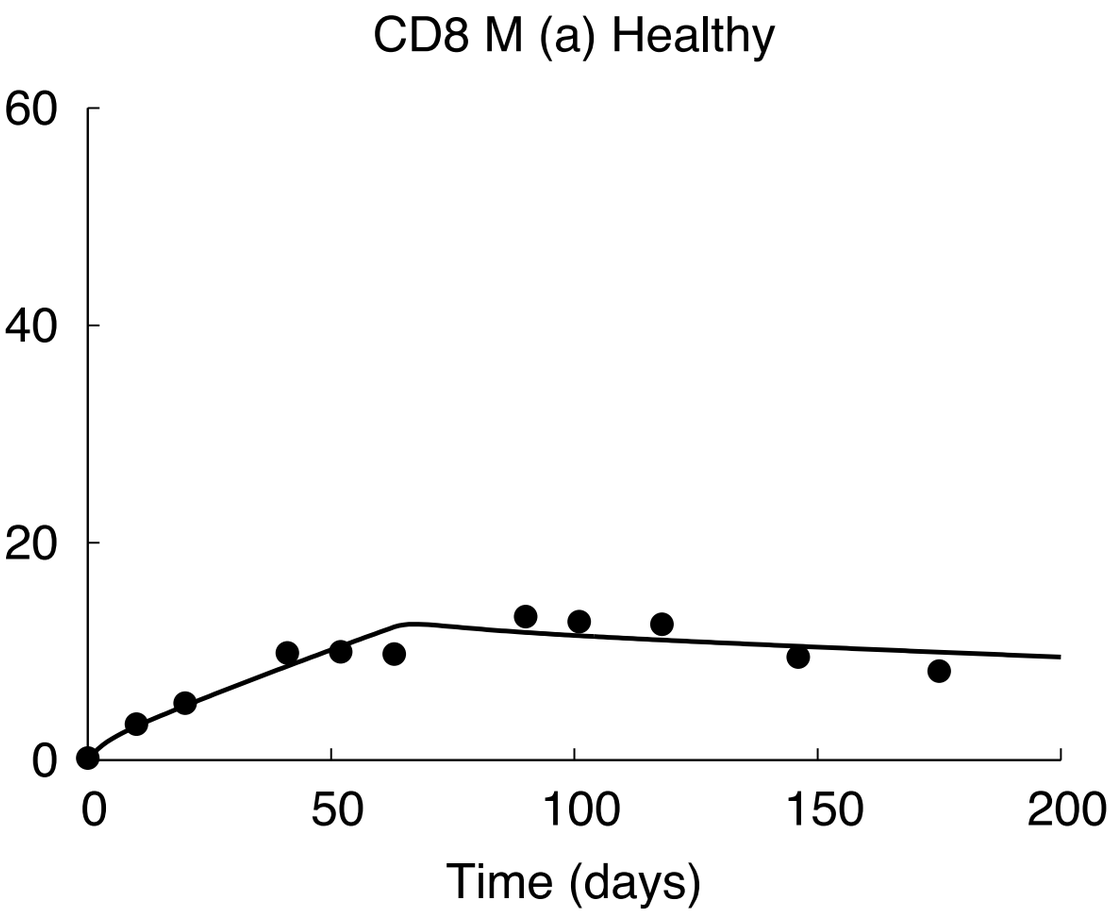
$$\frac{\mathrm{d}T}{\mathrm{d}t} = s + (p - d)T = 0$$

Examples of labeling with deuterium in volunteers

Memory T cells: CD45RO+



bi-phasic

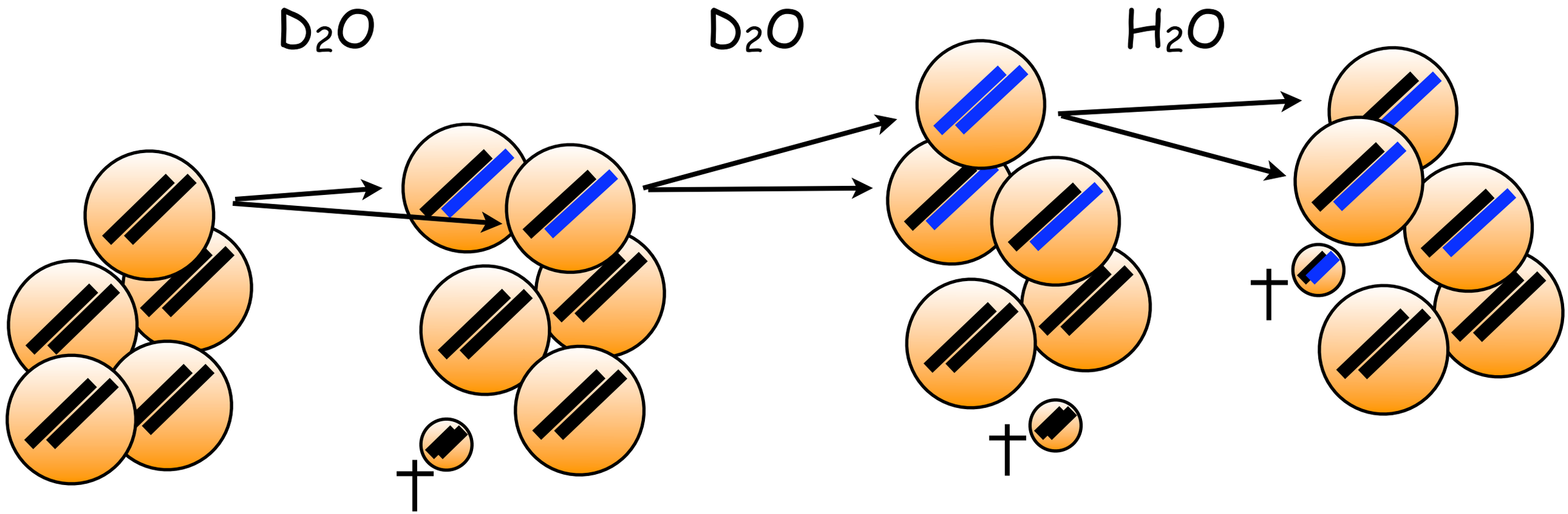


People drink 4% heavy water for T=9 weeks and are followed for another 16 weeks. Naive and memory T cells are sorted from the blood and the deuterium enrichment in their DNA is measured my mass-spec

Deuterium labeling much easier to interpret than BrdU labeling

Vrisekoop et al., PNAS 2008

Modeling seems relatively easy



L:U 0:10 2:10 4:10 3:10

DNA strands largely disappear by cell death.

Model loss of unlabeled strands during up-labeling:

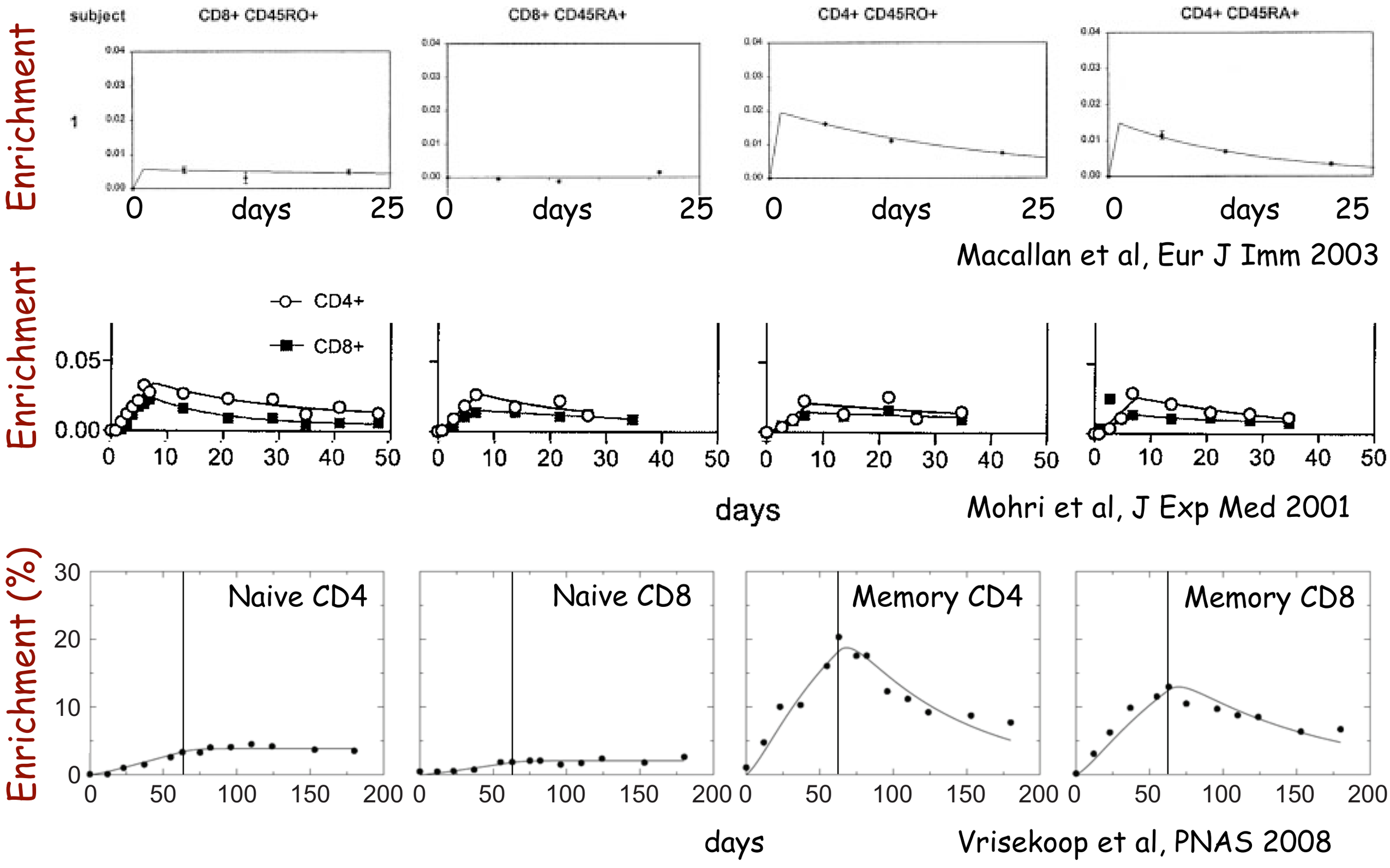
$dU/dt = -dU$ or $L(t) = 1-e^{-dt}$

and the loss of labeled strands during down-labeling:

$dL/dt = -dL$ or $L(t) = L(T)e^{-d(t-T)}$

T: labeling period

Three examples of quite different deuterium studies



Macallan et al, Eur J Imm 2003

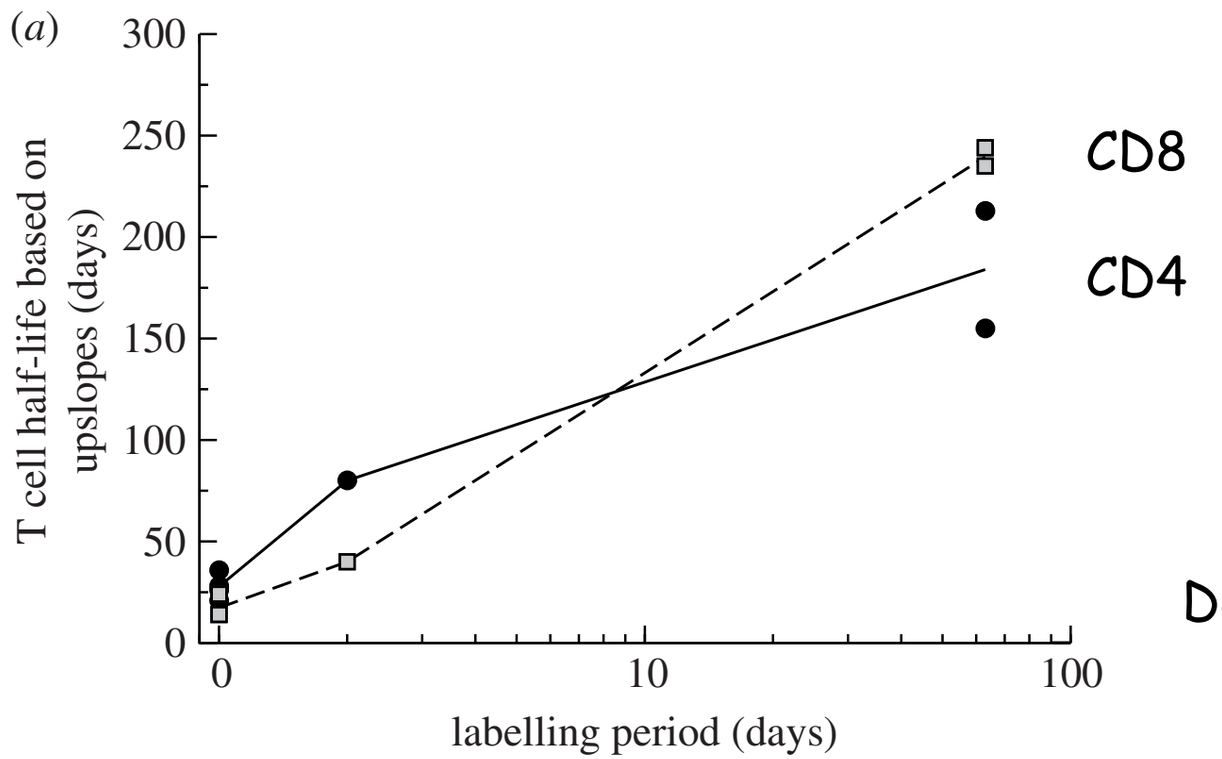
Mohri et al, J Exp Med 2001

Vrisekoop et al, PNAS 2008

Short studies D-glucose and long study D-water

Length of labeling period increases estimated life spans

Memory T cells



De Boer et al, J Roy Soc Int 2012

Table 1. **Average half-lives (in days) of different T-cell populations in healthy individuals estimated by stable isotope labeling**

Reference	(2)	(53)	(54)	(7)	(8)	(55)	(56)	(57)		(Vrisekoop *et al.*)
Method	$^2H_2$-glucose	$^2H_2$-glucose	^2H_2O	$^2H_2$-glucose	$^2H_2$-glucose	$^2H_2$-glucose	$^2H_2$-glucose	$^2H_2$-glucose		^2H_2O
Label period	2 days	2 days	9 weeks	1 week	1 week	1 day	1 day	1 day		9 weeks
Model	pp	pp	pp	1comp	2comp	Asq	Asq	Asq		Asq
CD4	87	82	385	173	154					
CD8	77	139	420	231	257					
Naive CD4		187				118	361	119	184	1517
Naive CD8		204				154		131	112	2398
Memory CD4		80	213			26	21	28	36	155
Memory CD8		40	235			14		18	24	244

Borghans & De Boer, Imm Rev 2007; De Boer & Perelson, JTB, submitted.

Data have mostly been interpreted with models having one exponential

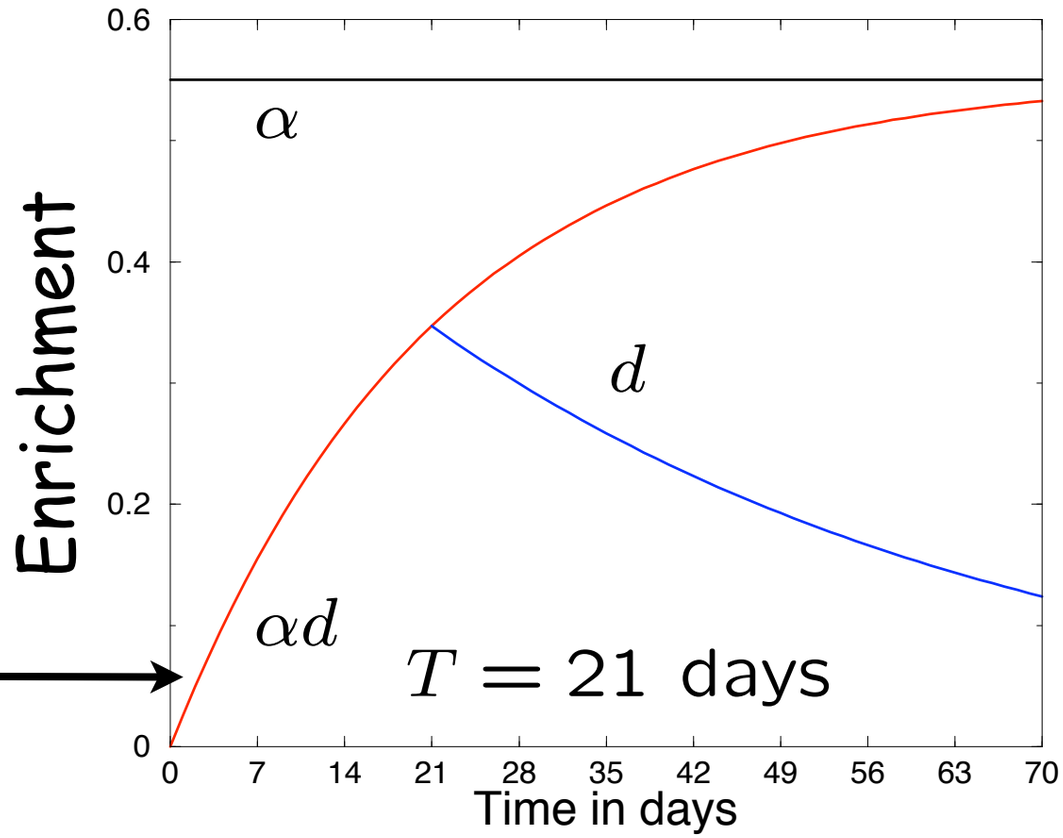
$$L(t) = \begin{cases} \alpha(1 - e^{-dt}) \ , & \text{if } t \leq T_{\text{end}} \ , \\ L(T_{\text{end}})e^{-d(t-T_{\text{end}})}, & \text{otherwise} \ , \end{cases}$$

with asymptote α, turnover rate d & average turnover rate αd.

(models used by Asquith (p= αd) and Mohri are mathematically equivalent)

So why then are the estimates of αd so different?

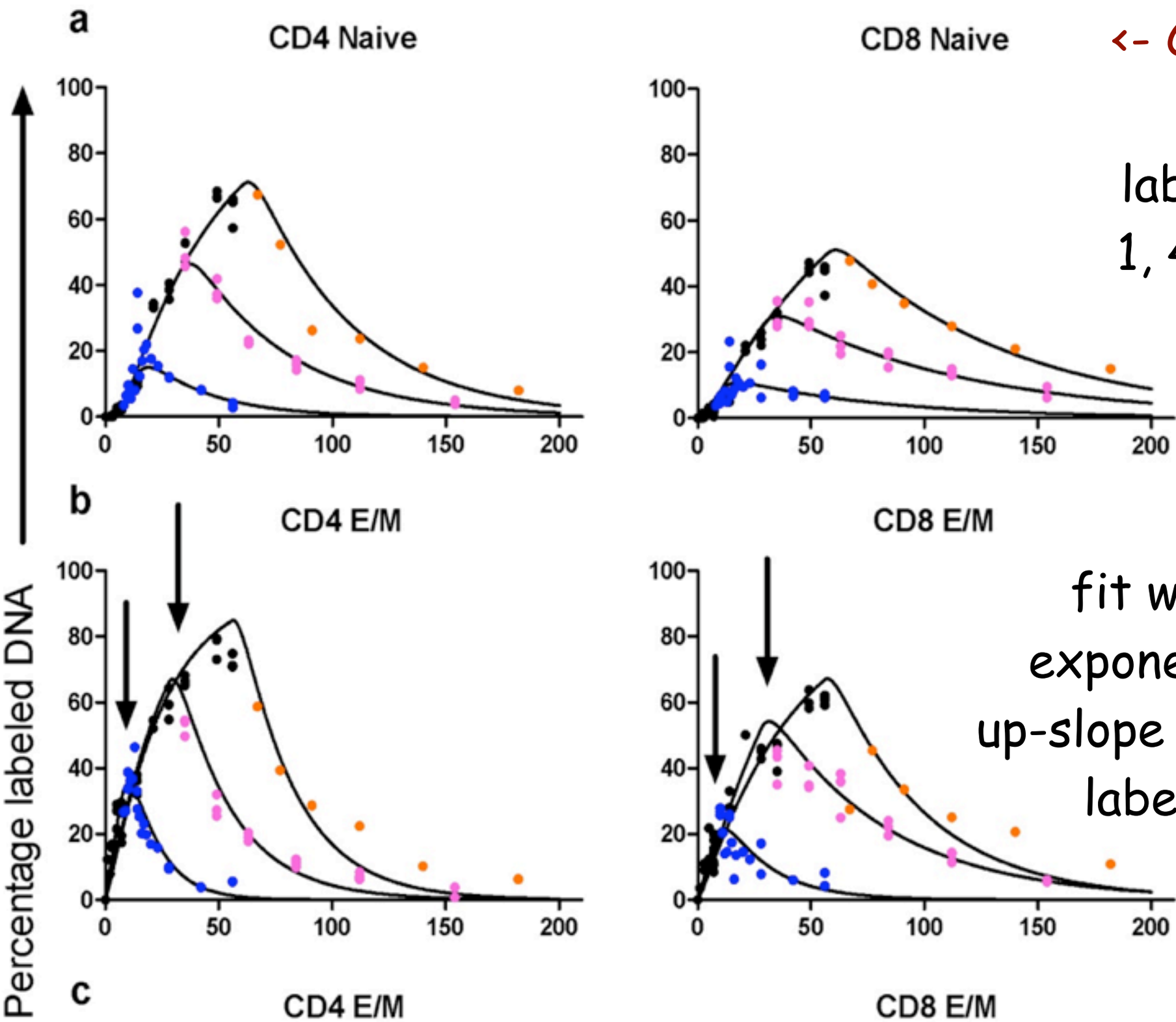
Initial slope: αd



Estimates of d are expected to be different [Asquith TI02]

Ganusov et al, Plos Comp Biol 2010

Repeat the effect of labeling period in one experiment



<- $CD44^{-}CD62L^{+}$

label mice for 1, 4 & 8 weeks and

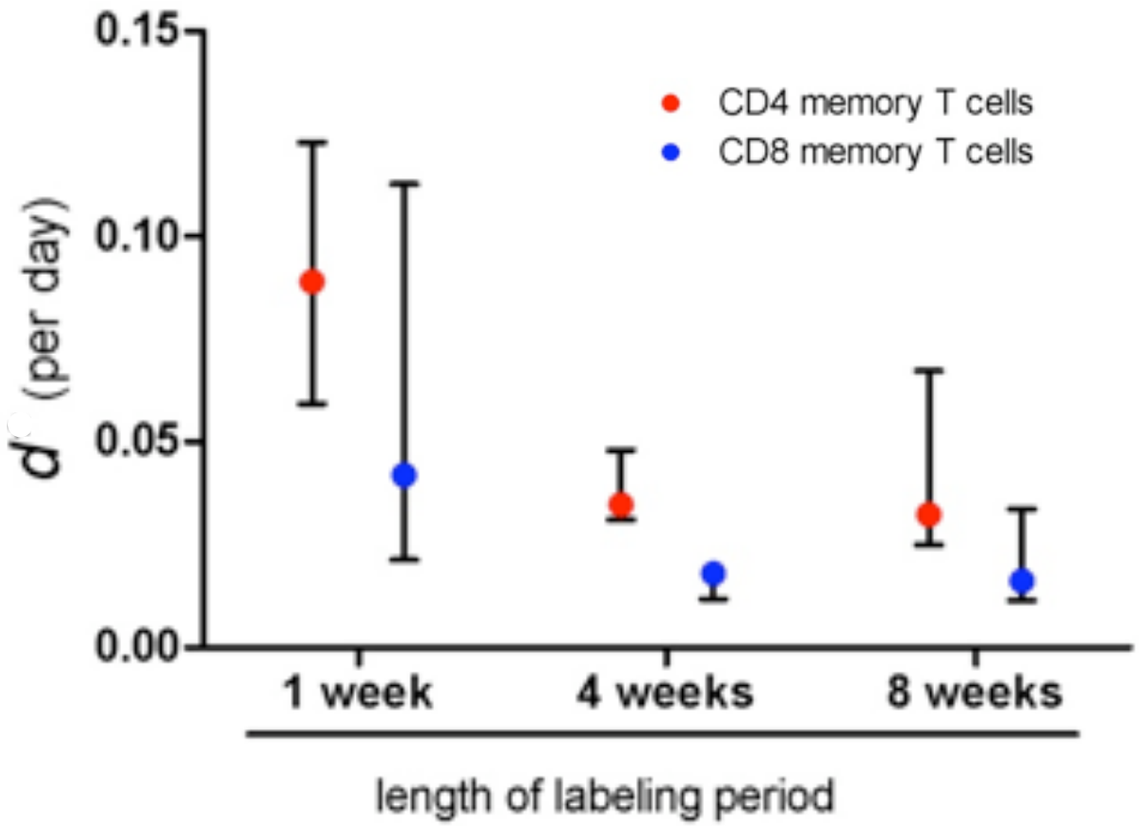
<- $CD44^{+}$

fit with "single-exponential" model: up-slope decreases with labeling period

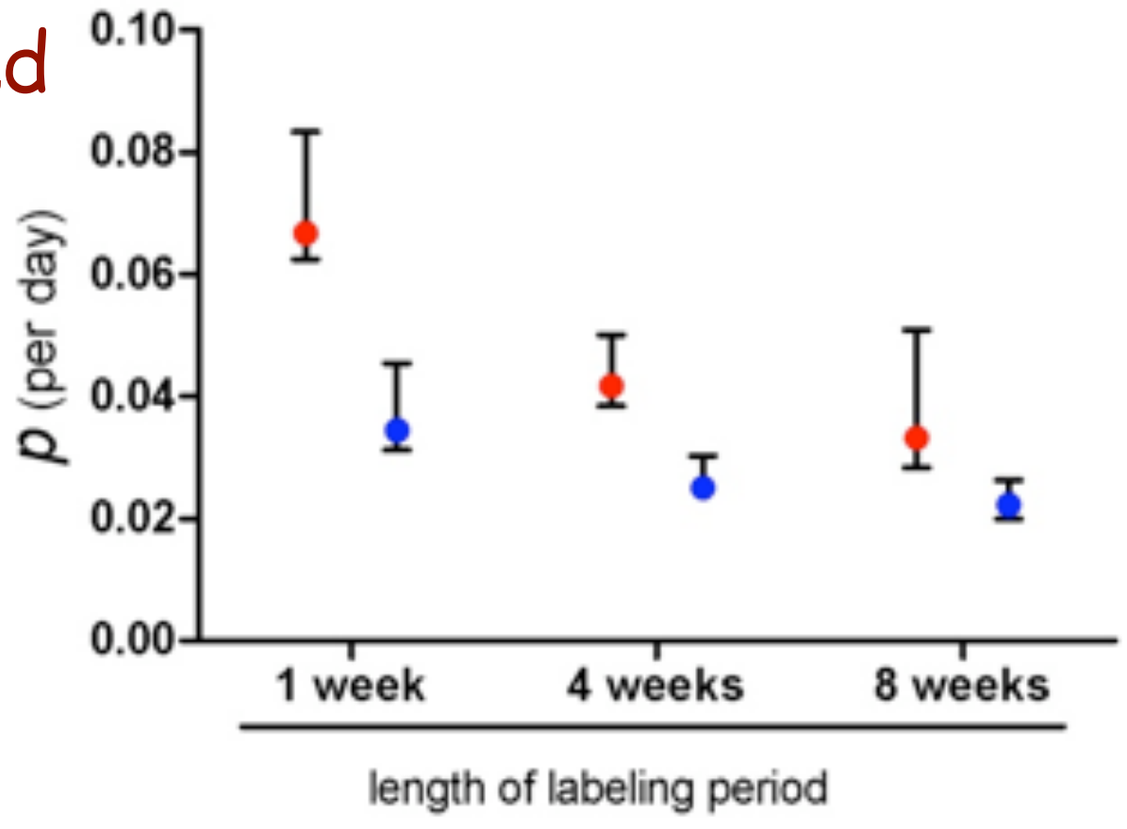
Westera et al. Submitted

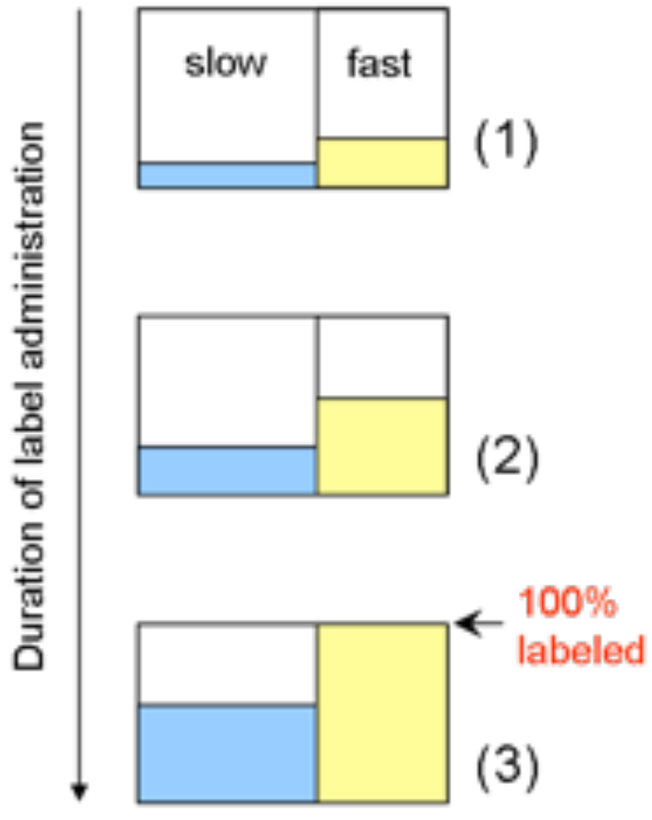
While there can only be one true up-labeling curve

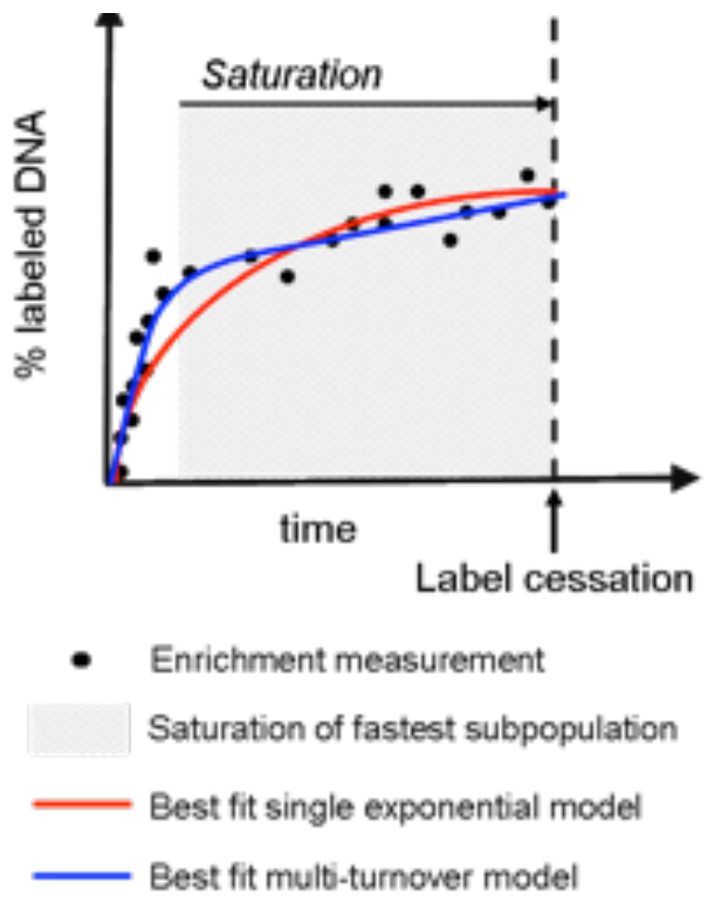
Memory T cells form a kinetically heterogeneous population



αd







Heterogeneity cannot be captured by single exponential models: compromise at long labeling periods

Westera et al. Submitted
Asquith Trends Imm 2002

Generalize into explicit kinetic heterogeneity model

From

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

to

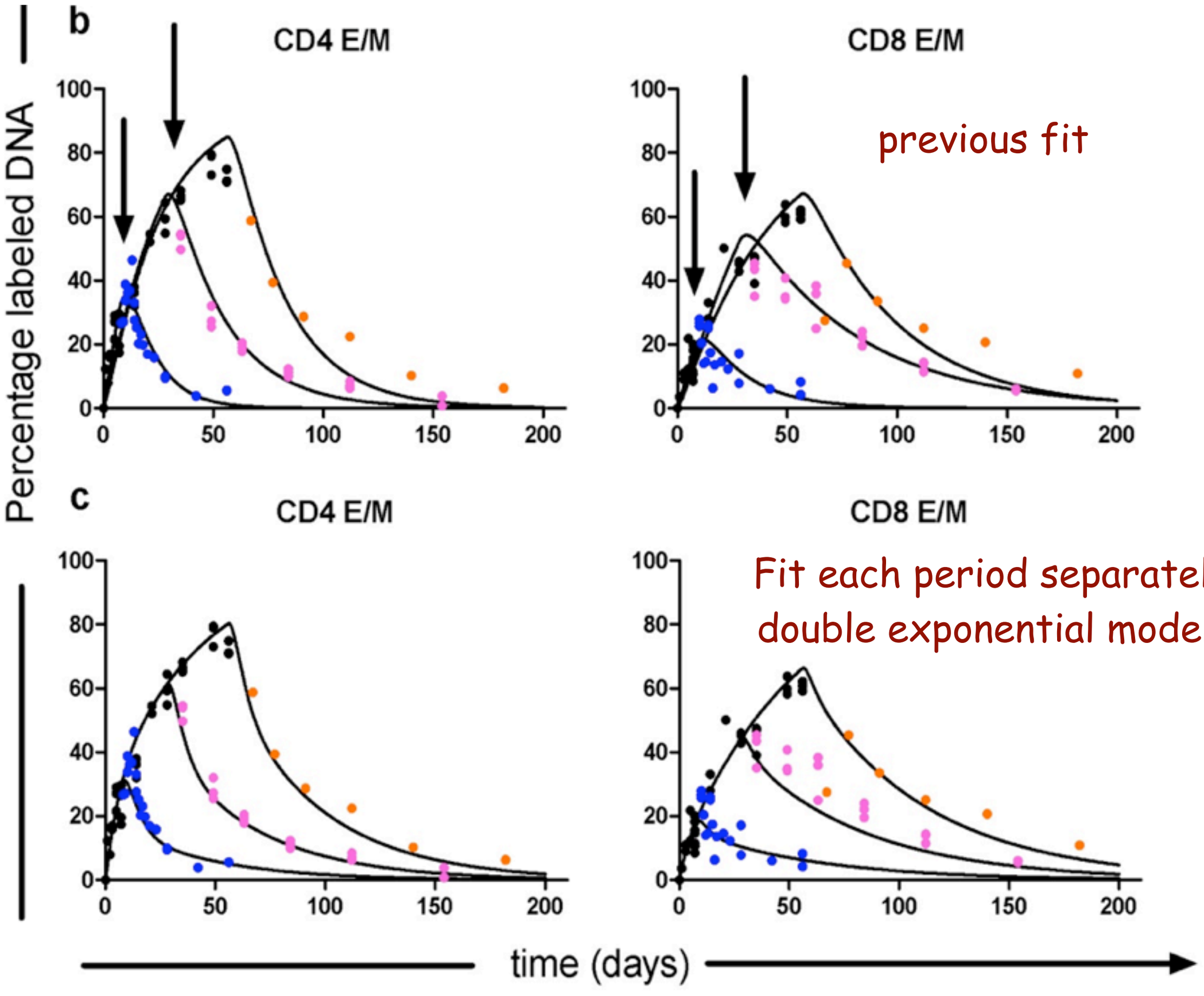
$$L(t) = \begin{cases} \sum \alpha_i(1 - \mathrm{e}^{-d_i t}) \ , & \text{if } t \leq T_{\text{end}} \ , \\ \sum \alpha_i(1 - \mathrm{e}^{-d_i T_{\text{end}}})\mathrm{e}^{-d_i(t-T_{\text{end}})}, & \text{otherwise} \ , \end{cases}$$

where α_i is the fraction of cells with turnover rate d_i

Fit model for n = 1, 2, .. compartments until increasing the number of compartments no longer changes the estimated average turnover rate $d = \Sigma\ \alpha_i d_i$

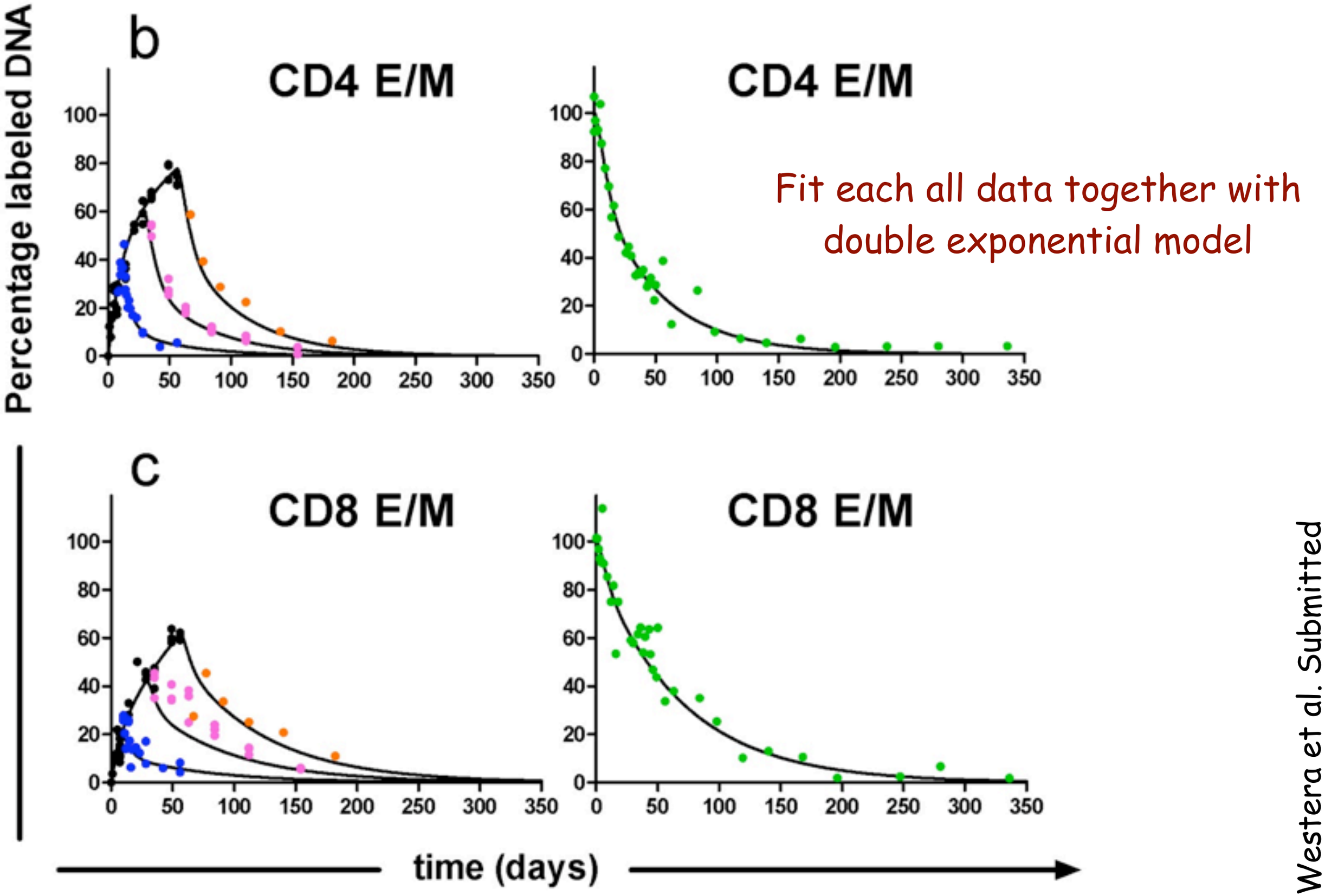
Ganusov, Borghans & De Boer, Plos Comp Biol 2010

Bi-phasic labeling curves call for more exponentials



Westera et al. Submitted

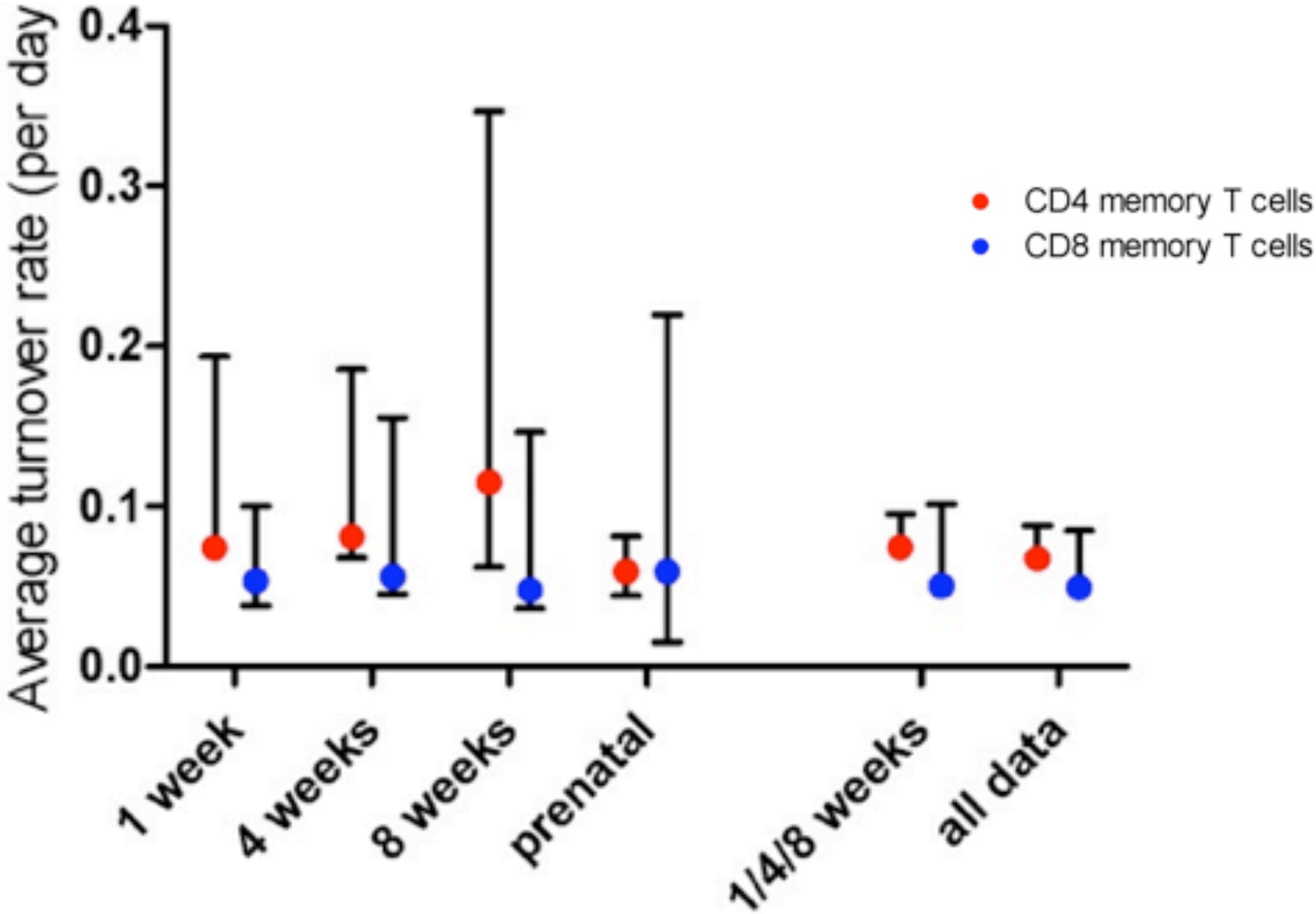
Bi-exponential model also describes prenatal labeling



Fit each all data together with double exponential model

Westera et al. Submitted

Fit all mouse data together with double exponential model



CD4 E/M T cells: 15 days (11.4-15.4)

CD8 E/M T cells: 20 days (11.7-22.4)

CD4 naive T cells: 50 days (48-53); CD8 naive T cells: 102 days (92-110)

Westera et al. Submitted

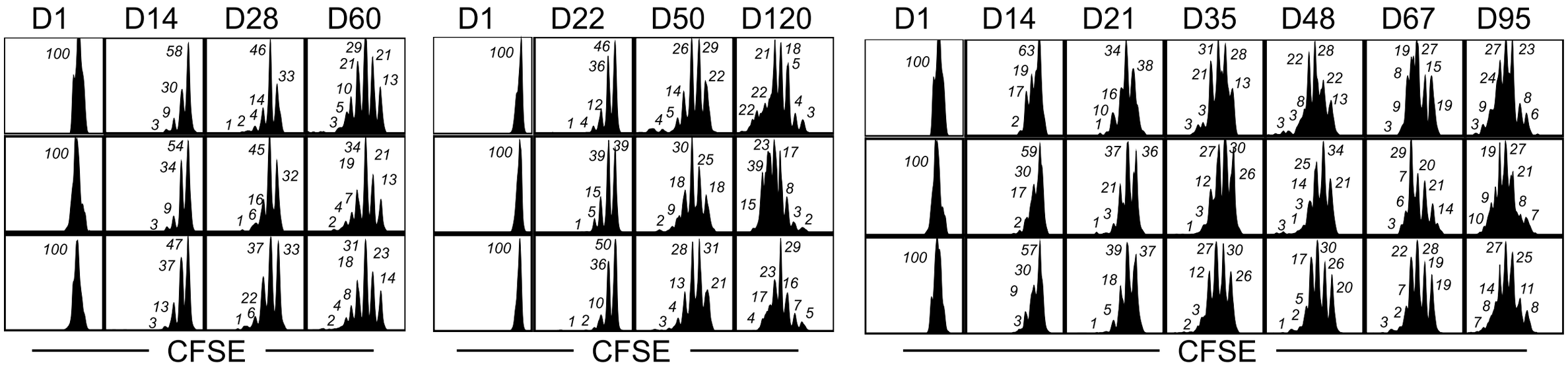
But "true" memory cells live 50 days?

Homeostatic Turnover of Virus-Specific Memory CD8 T Cells Occurs Stochastically and Is Independent of CD4 T Cell Help

The Journal of Immunology 2010

Daniel K. Choo,* Kaja Murali-Krishna,† Rustom Anita,‡ and Rafi Ahmed*

B



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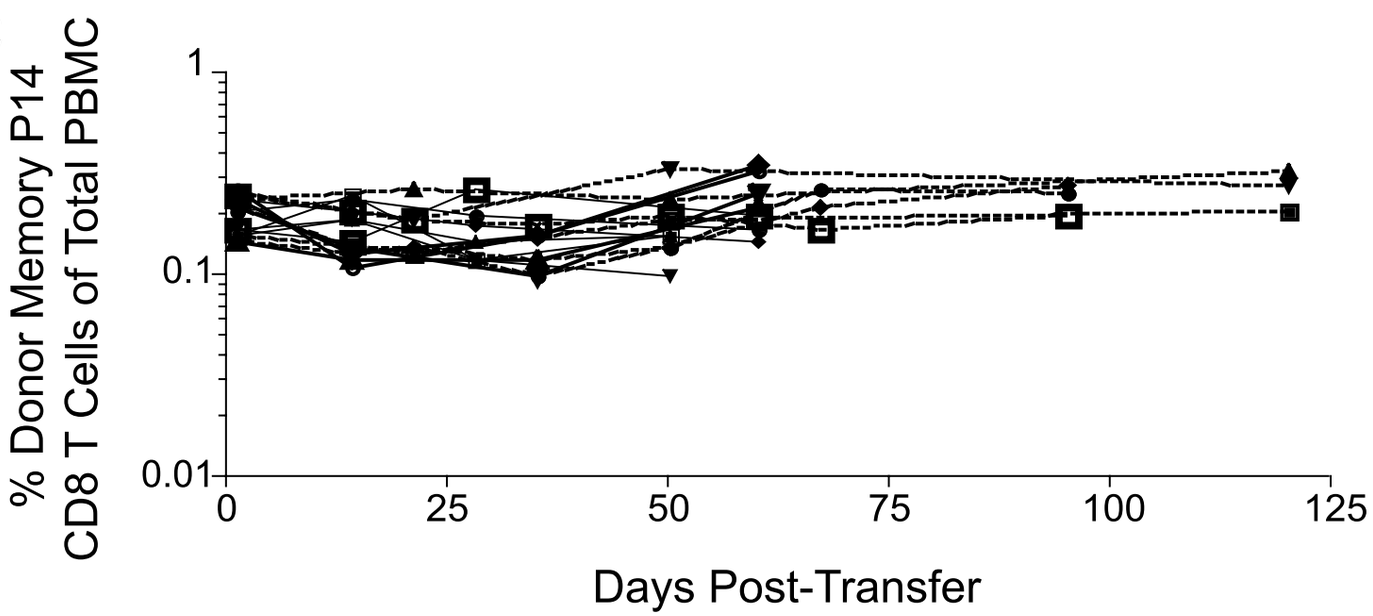


$$x_n = f_n e^{(\lambda - d)t}$$

$$f_n = \frac{(2\lambda t)^n e^{-(2\lambda t)}}{n!}$$

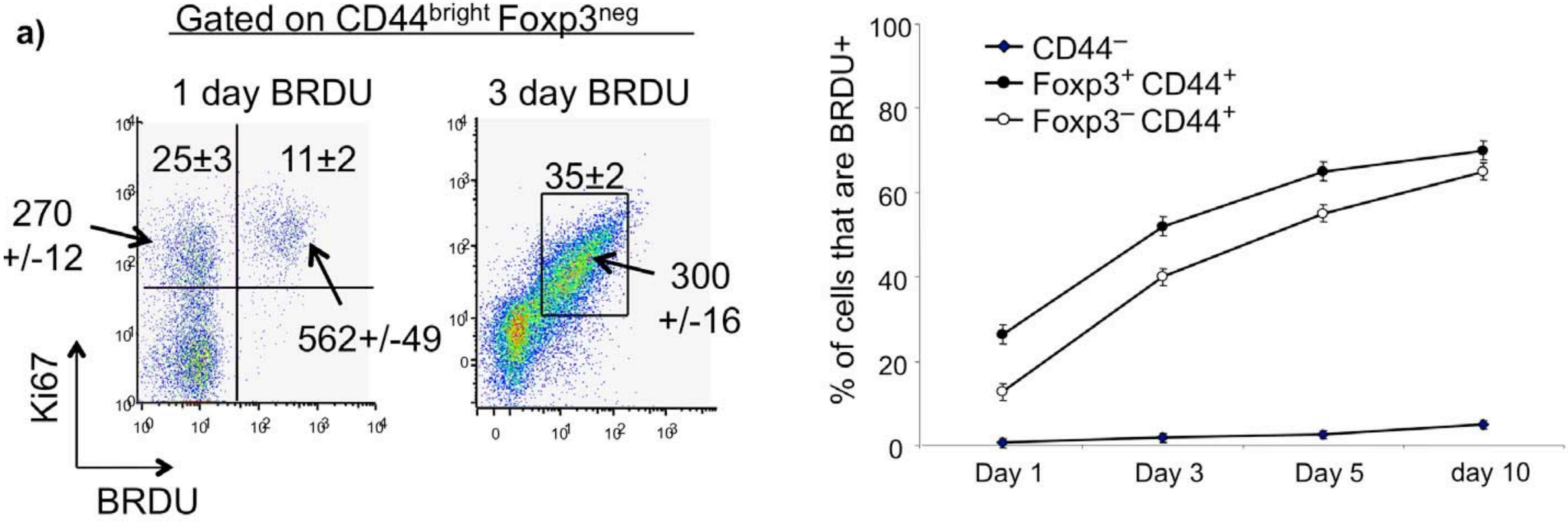
LCMV specific memory CD8+ T cells divide once every 50 days

OPEN ACCESS Freely available online

PLoS BIOLOGY 2011

Memory Phenotype CD4 T Cells Undergoing Rapid, Nonburst-Like, Cytokine-Driven Proliferation Can Be Distinguished from Antigen-Experienced Memory Cells

Souheil-Antoine Younes, George Punkosdy, Stephane Caucheteux, Tao Chen, Zvi Grossman, William E. Paul*



Combining BrdU and Ki67:
LCMV specific CD4+ T cells divide once every 50 days
while other CD44+ memory cells divide every 2-3 weeks

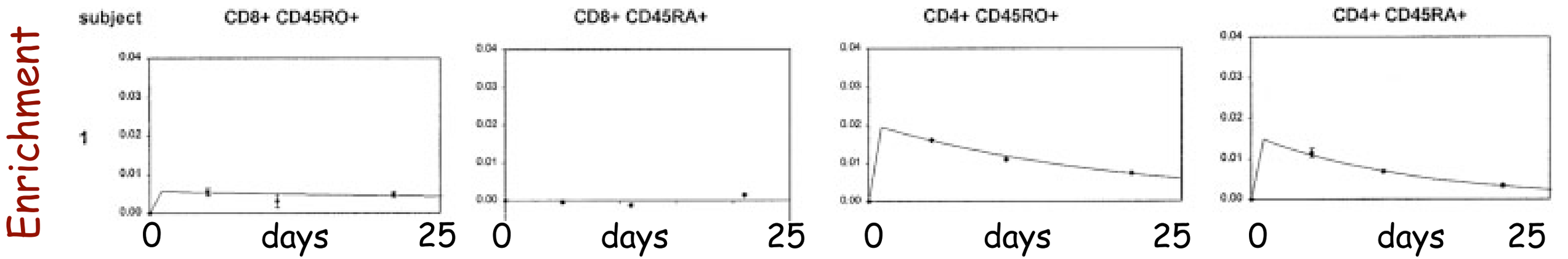
Conclusions on T cell kinetics in mice

Life span	Range		T cell type	Method	Model	Ref.	Remarks
mouse							
68 d	65–71 d	CI	naive $CD8^+$	BrdU	Eq. (13)	Parretta *et al.* [172]	thymectomized mice
47 d	41–54 d	CI	naive $CD4^+$	^2H_2O	Eq. (23)	Den Braber *et al.* [56]	young adult mice
80 d	67–92 d	CI	naive $CD8^+$	^2H_2O	Eq. (23)	Den Braber *et al.* [56]	young adult mice
41 d	36–47 d	CI	naive $CD4^+$	^2H_2O	Eq. (23)	Den Braber *et al.* [56]	old mice
116 d	94–139 d	CI	naive $CD8^+$	^2H_2O	Eq. (23)	Den Braber *et al.* [56]	old mice
90 d	64-133 d	CI	memory $CD8^+$	BrdU	Eq. (18)	Parretta *et al.* [172]	no source: $\sigma = 0$, no de-labeling
50 d	—		memory $CD8^+$	CFSE	Eq. (15)	Choo *et al.* [35]	LCMV specific memory cells
14–22 d	—		memory $CD4^+$	BrdU	*	Younes *et al.* [238]	memory phenotype cells
50 d	—		memory $CD4^+$	Ki67	—	Younes *et al.* [238]	LCMV specific memory cells
15 d	11–15 d	CI	memory $CD4^+$	^2H_2O	Eq. (26)	Westera *et al.* [226]	3 different labeling periods
20 d	12–22 d	CI	memory $CD8^+$	^2H_2O	Eq. (26)	Westera *et al.* [226]	3 different labeling periods

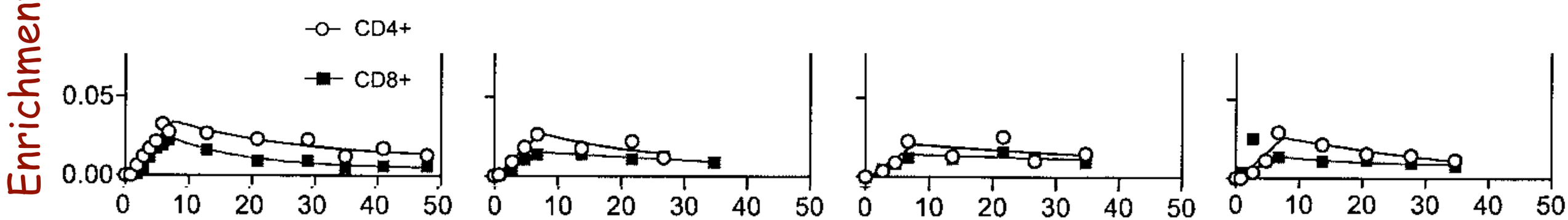
Memory phenotype cells turn over faster
than "true" memory T cells?
Naive T cells live longer than memory T cells.
$CD8^+$ naive T cells live longer than $CD4^+$ naive T cells.

Review: De Boer & Perelson (JTB, submitted)

Back to the kinetics of human T cells

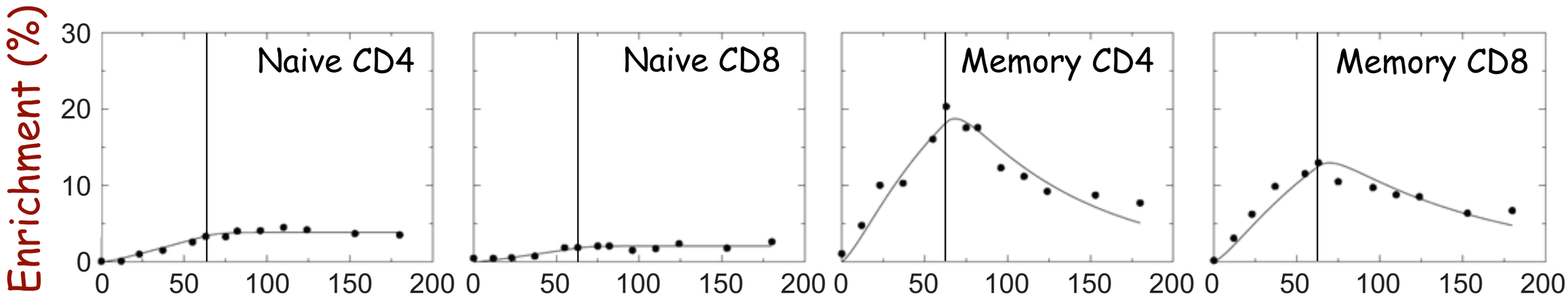


Macallan et al Eur J Imm 2003



days

Mohri et al J Exp Med 2001

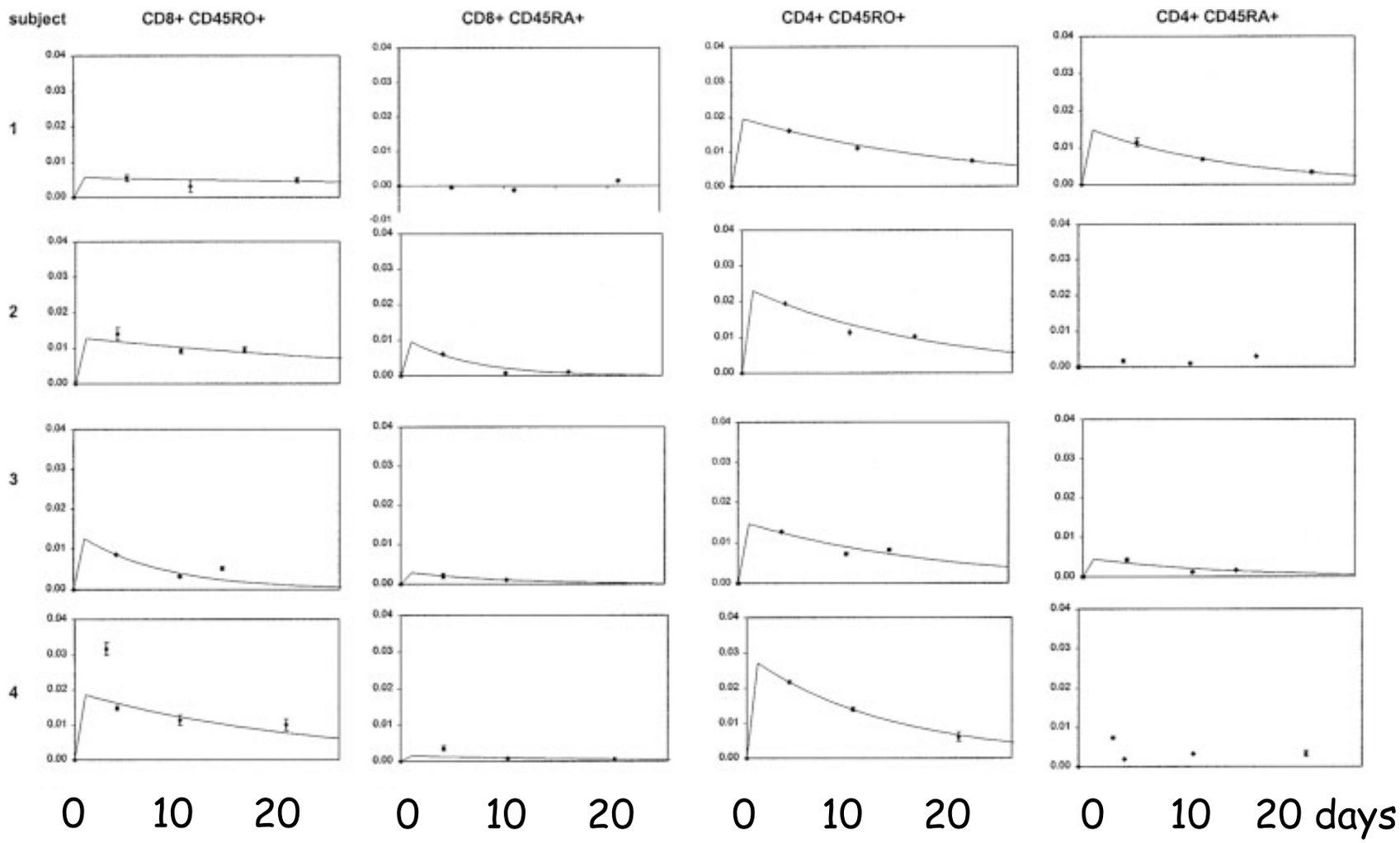


days

Vrisekoop et al, PNAS 200

Short studies D-glucose and long study D-water

Very short term labeling with glucose also problematic



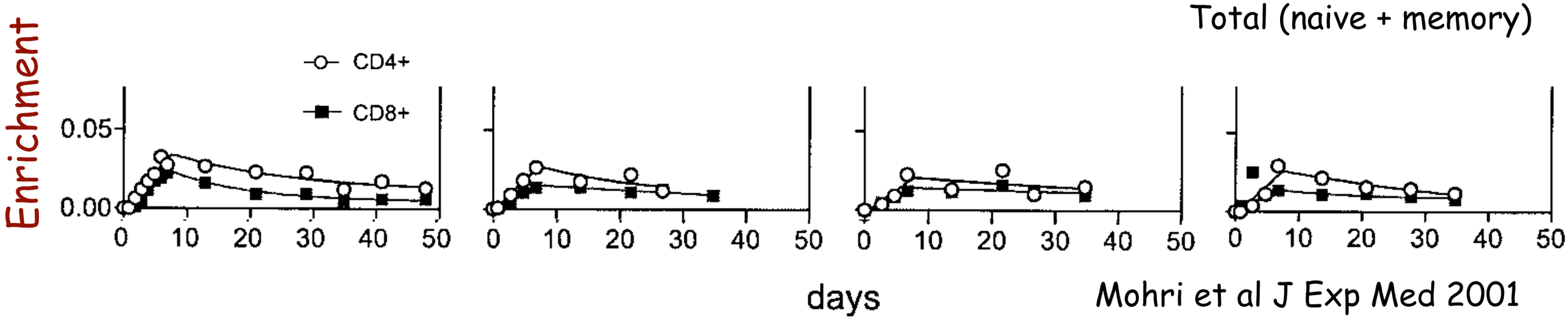
One day of labeling: but "peak" observed only at day three.

Assume true peak at day one and extrapolate down-slope backwards to back-calculate true peak at day 1.

Short term labeling suffers from unknown exit rates from lymphoid tissue to blood and poorly estimates initial up-slope.

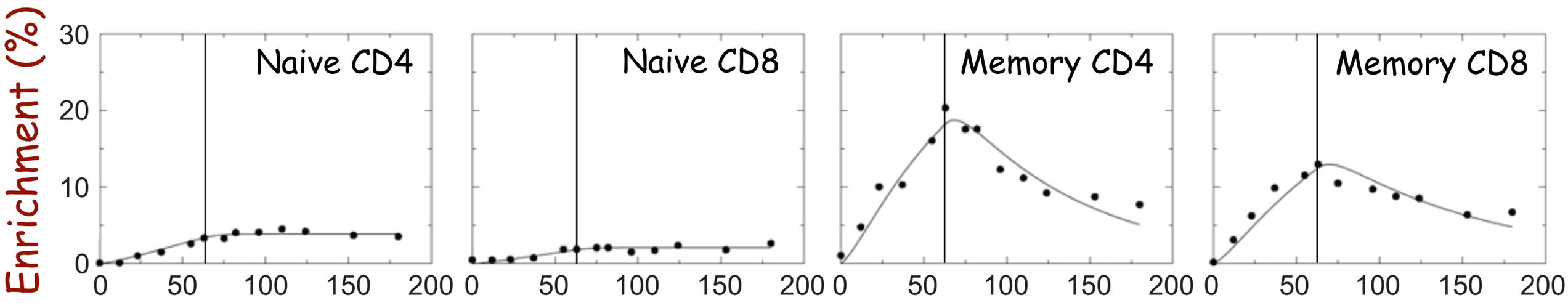
Life spans remain different between two long-term studies

7 days D-glucose



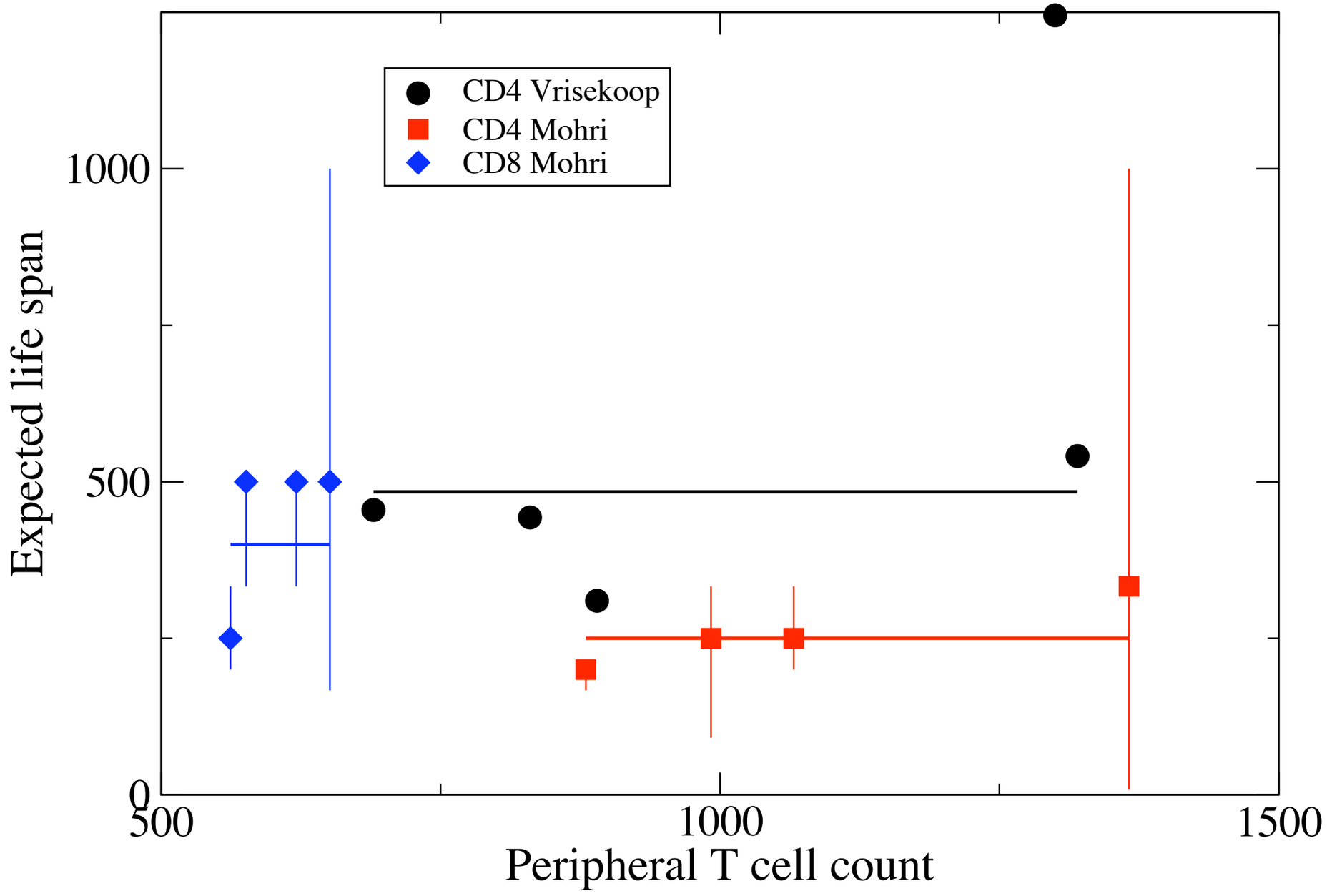
Mohri et al J Exp Med 2001

9 weeks D-water



Vrisekoop et al, PNAS 2008

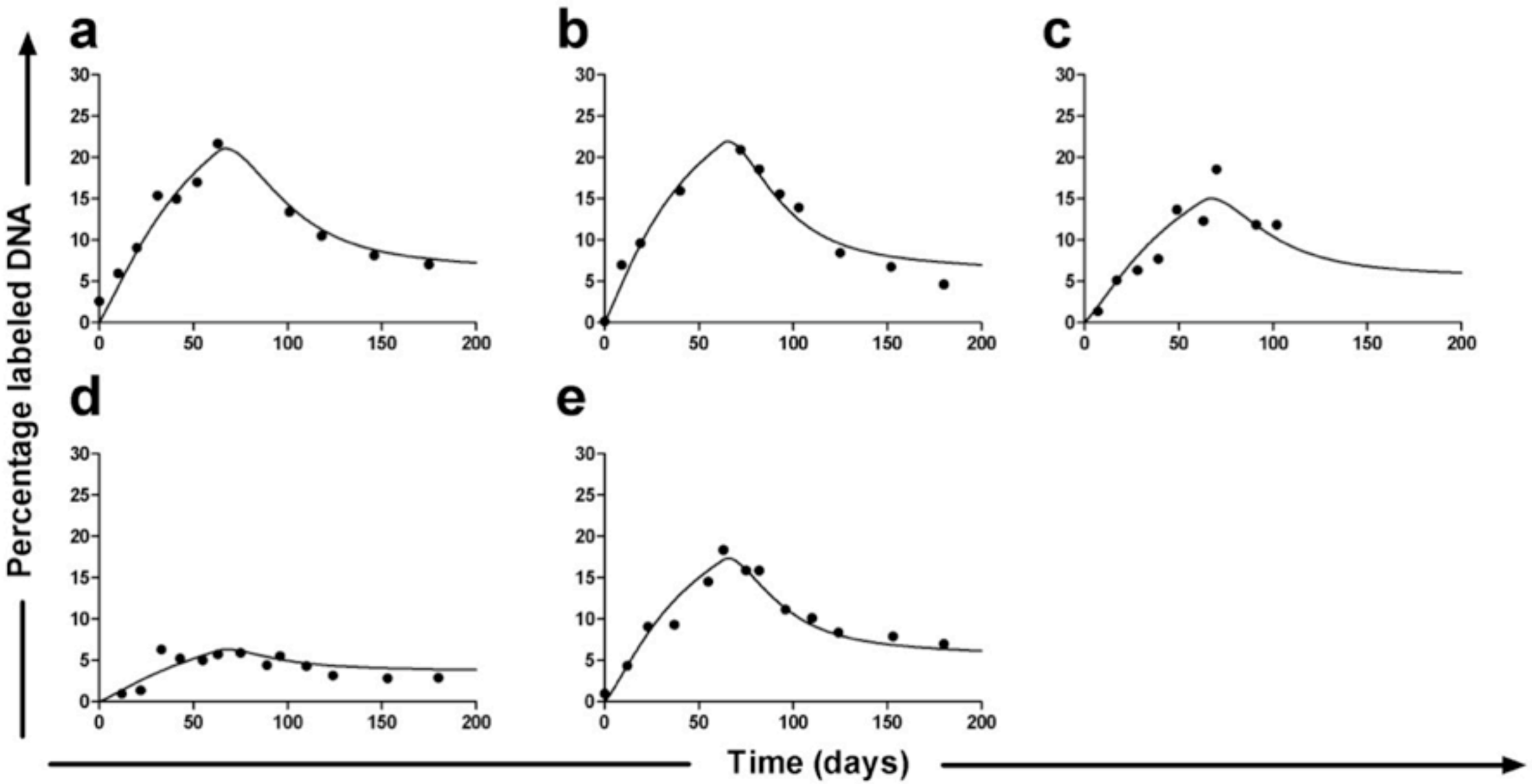
Published expected life spans of human CD4⁺ T cells



Vrisekoop naive & memory data were recalculated into total CD4

Mohri (JEM 2001): 1 week D-glucose & Vrisekoop (PNAS 2008): 9 weeks D-water

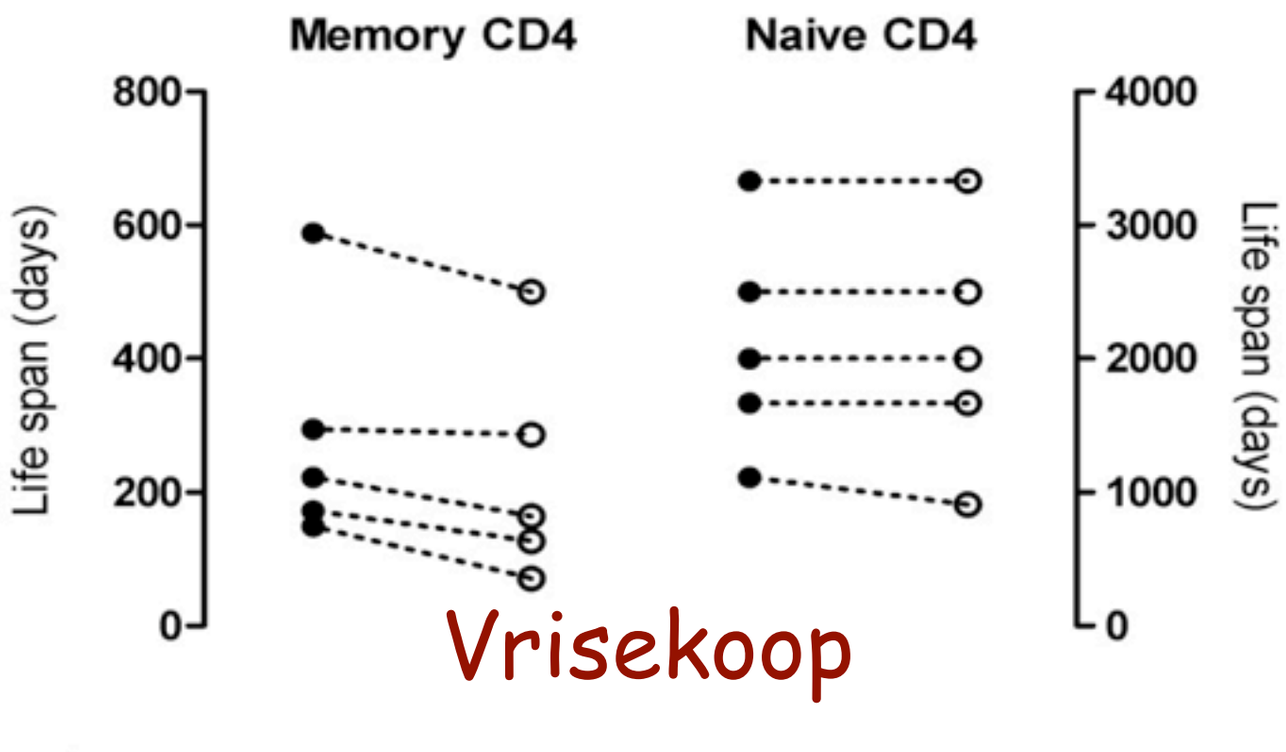
Refit both data sets using double exponent models



Memory CD4$^+$ T cells from Vrisekoop (PNAS 2008): fits significantly better than original model

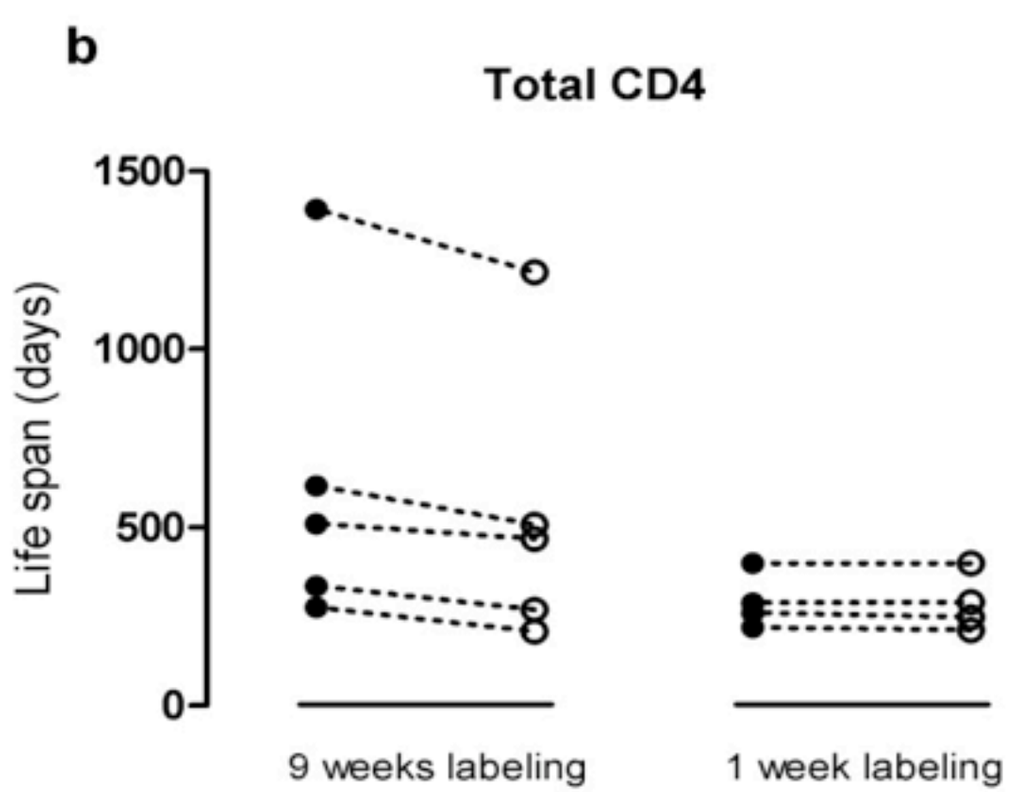
Westera et al. Submitted

Shorter life spans using double exponent models



Vrisekoop

- ● single exponential
- ○ double exponential



Vrisekoop Mohri

Difference between Mohri and Vrisekoop becomes smaller

Westera et al. Submitted

Conclusions from 5 human volunteers labeled with D-water

Expected life spans (medians)

Naive $CD4^+$ T cells: 2000 days (5.5 years)

Naive $CD8^+$ T cells: 3300 days (9.1 years)

Effector/memory $CD4^+$ T cells: 160 days (0.45 years)

Effector/memory $CD8^+$ T cells: 160 days (0.45 years)

Compartments:

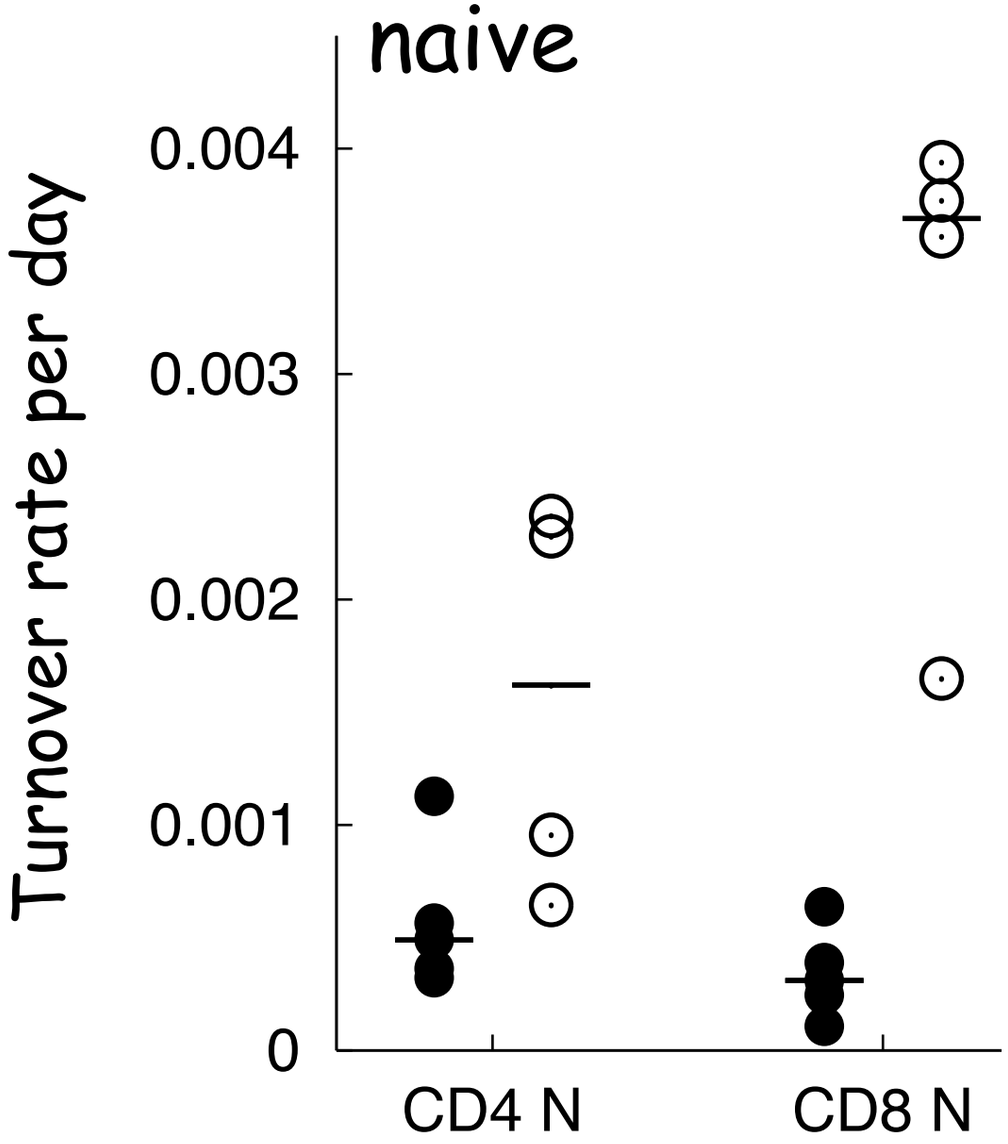
Naive T cell data typically requires only one exponent

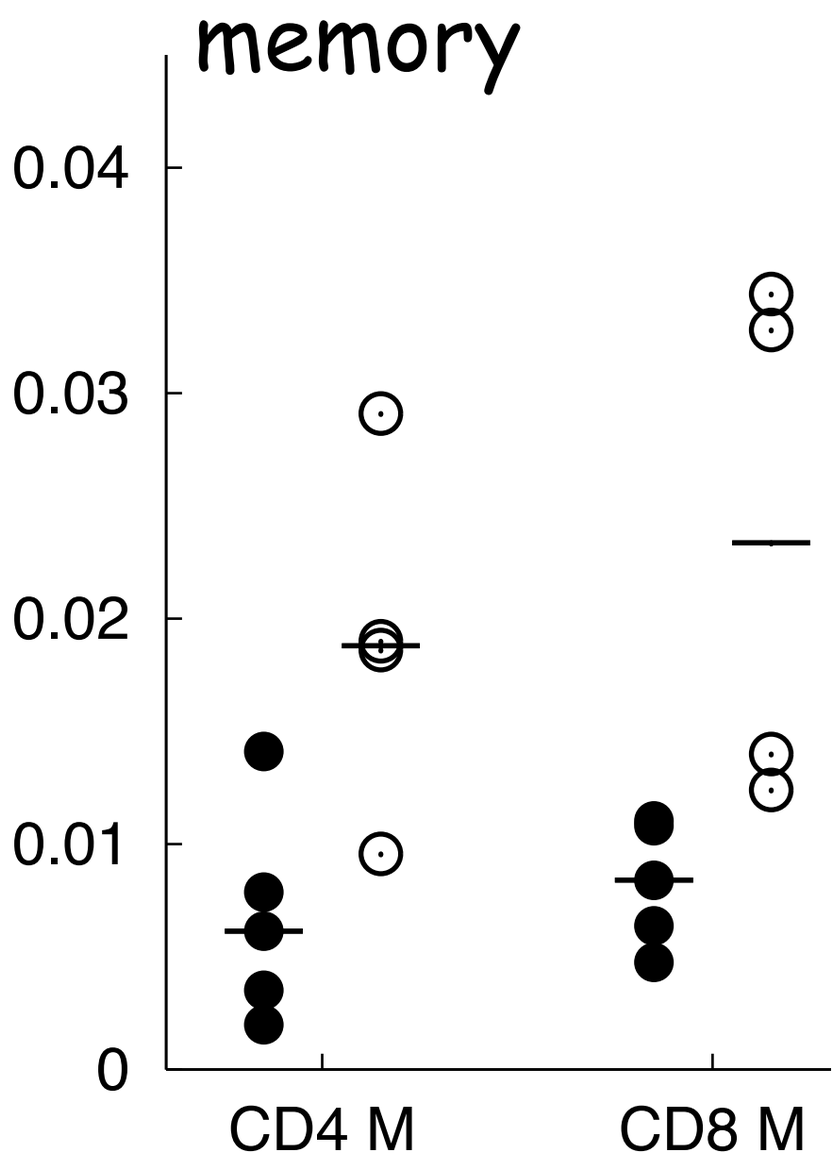
Memory data do require 2 compartments: heterogeneity

Immunological memory is maintained by short-lived cells

Vrisekoop et al PNAS 2008
Westera et al Submitted

5 human volunteers (●) and 4 HIV-1⁺ Patients (⊙)





Life span: 6.2y 9.1y 5.5m 4.0m

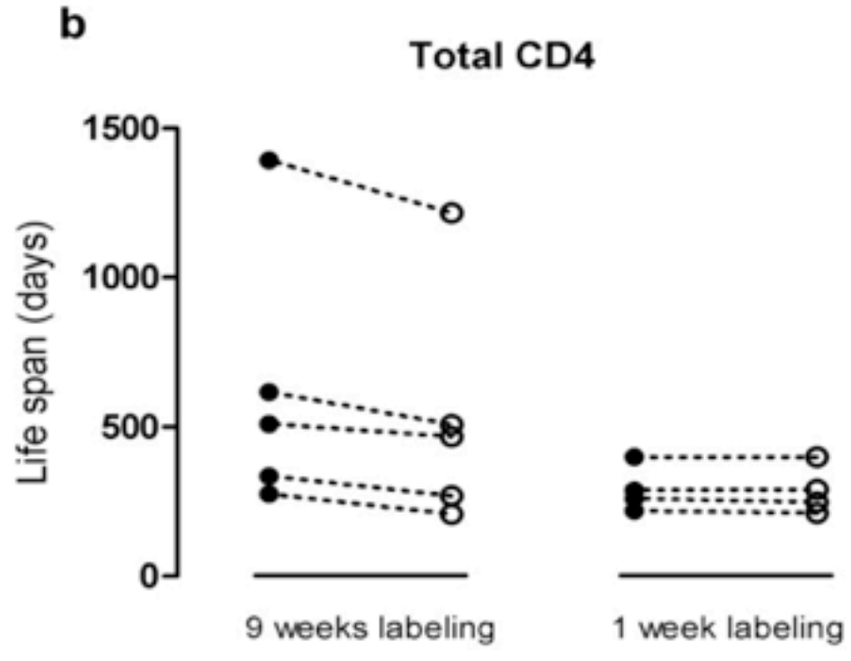
HIV-1⁺: 1.7y 0.7y 1.8m 1.4m

HIV-1 infection increases cellular turnover rates (production)

Fitted with a single exponential model

some problems remain …

Is the difference between
D-glucose and D-water
really solved?



Also differences between the 2 day and the 1 week glucose data
(to be worked on here with Becca Asquith)

Biology of the n-compartment model should not be
taken too seriously

Kinetic vs Temporal heterogeneity

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Modelling deuterium labelling of lymphocytes with temporal and/or kinetic heterogeneity

Rob J. De Boer[1,3,*], Alan S. Perelson[2,3] and Ruy M. Ribeiro[2]

Model with resting and recently divided cells:

$$\left.\begin{aligned}\frac{\mathrm{d}R}{\mathrm{d}t} &= rA - (a + d_{\mathrm{R}})R \\ \frac{\mathrm{d}A}{\mathrm{d}t} &= caR - (r + d_{\mathrm{A}})A.\end{aligned}\right\}$$

c=2 models the
Choo et al LCMV CFSE data.
Typically $d_A > d_R$

One can find a solution:

$$\left.\begin{aligned}\frac{\mathrm{d}U_\mathrm{R}}{\mathrm{d}t} &= rU_\mathrm{A} - (a + d_\mathrm{R})\,U_\mathrm{R}\\ \frac{\mathrm{d}U_\mathrm{A}}{\mathrm{d}t} &= aU_\mathrm{R} - (r + d_\mathrm{A})\,U_\mathrm{A}\end{aligned}\right\},$$

labeling phase

$$L(t) = 1 - \alpha \mathrm{e}^{-e_1 t} - (1-\alpha)\mathrm{e}^{-e_2 t},$$

de-labeling phase:

$$L(t) \simeq \alpha(1 - \mathrm{e}^{-e_1 t_\mathrm{end}})\mathrm{e}^{-e_1 (t - t_\mathrm{end})} + (1-\alpha)(1 - \mathrm{e}^{-e_2 t_\mathrm{end}})\mathrm{e}^{-e_2 (t - t_\mathrm{end})},$$

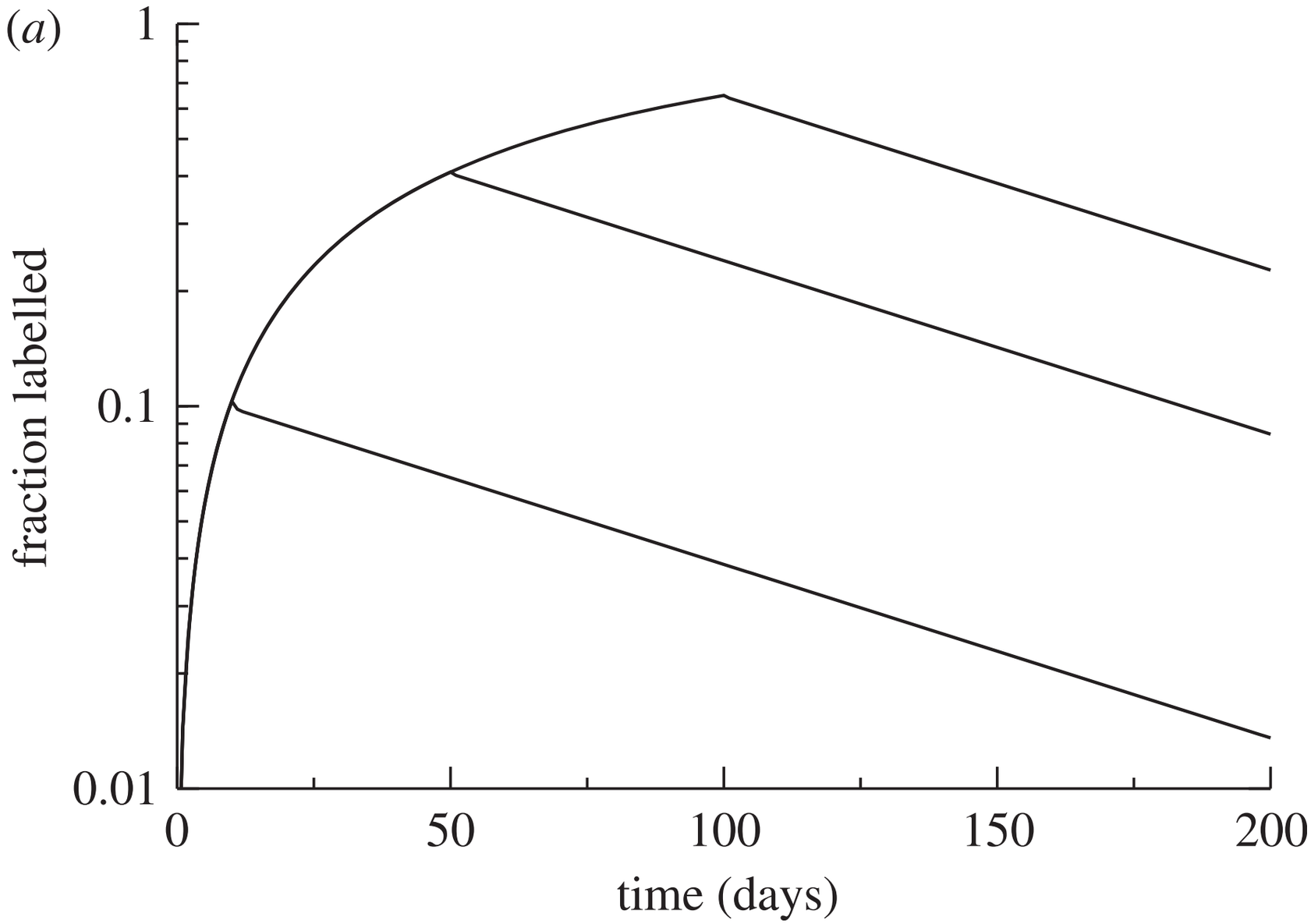
where α, e_1 and e_2 are combinations of r, a, c, d_R and d_A.

However, since this solution is very close to what we had above:

$$L(t) = \begin{cases} \sum \alpha_i (1 - \mathrm{e}^{-d_i t}) \ , & \text{if } t \le T_\mathrm{end} \ , \\ \sum \alpha_i (1 - \mathrm{e}^{-d_i T_\mathrm{end}}) \mathrm{e}^{-d_i (t - T_\mathrm{end})}, & \text{otherwise} \ , \end{cases}$$

both models fit the data equally well. Thus, one can longer interpret their parameters biologically.

Create D-water data using the LCMV parameters



$$\frac{\mathrm{d}R}{\mathrm{d}t} = rA - (a + d_\mathrm{R})R$$

$$\frac{\mathrm{d}A}{\mathrm{d}t} = caR - (r + d_\mathrm{A})A.$$

$a = 0.02\mathrm{d}^{-1}$

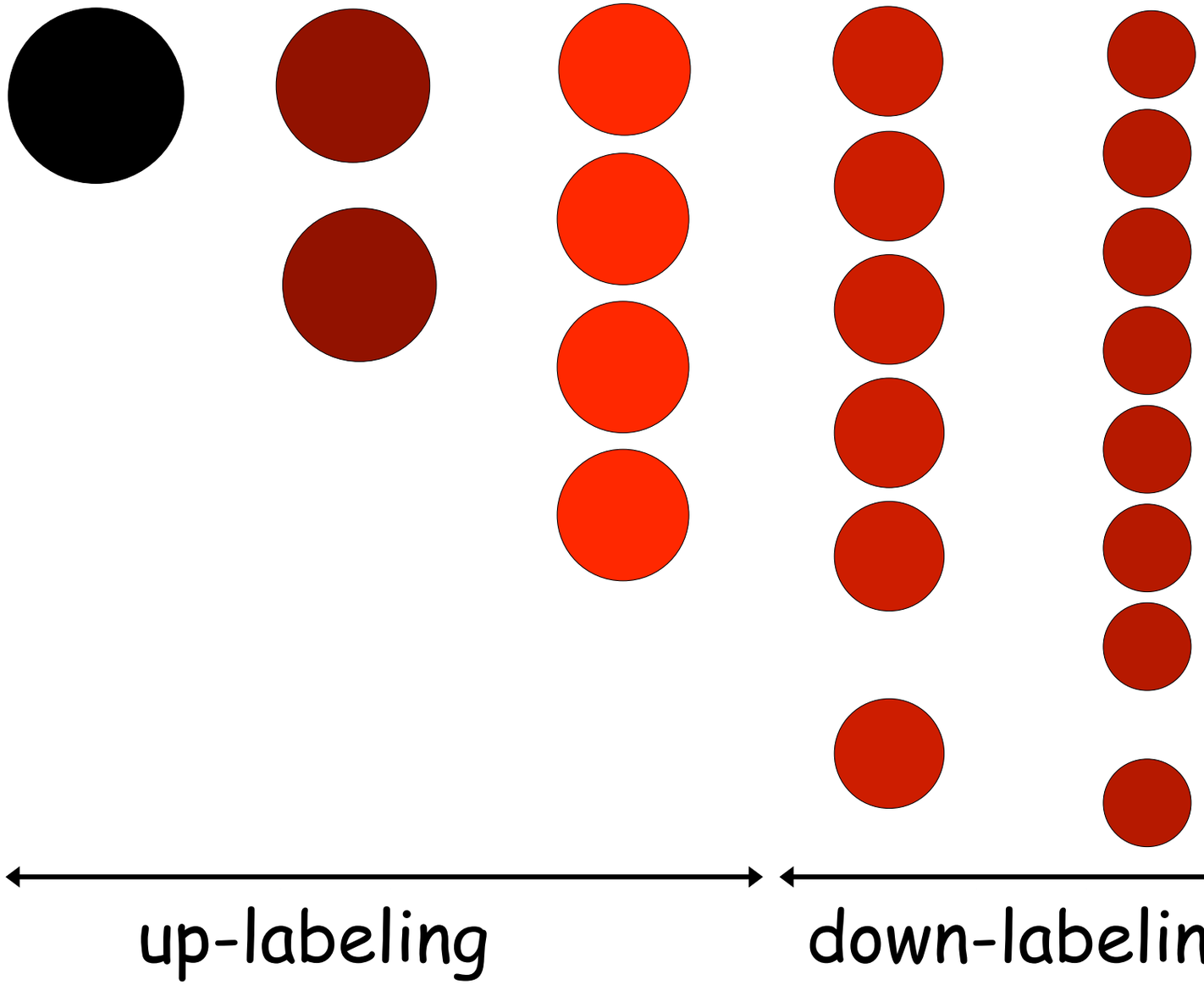
$d_R = 0.001\mathrm{d}^{-1}$

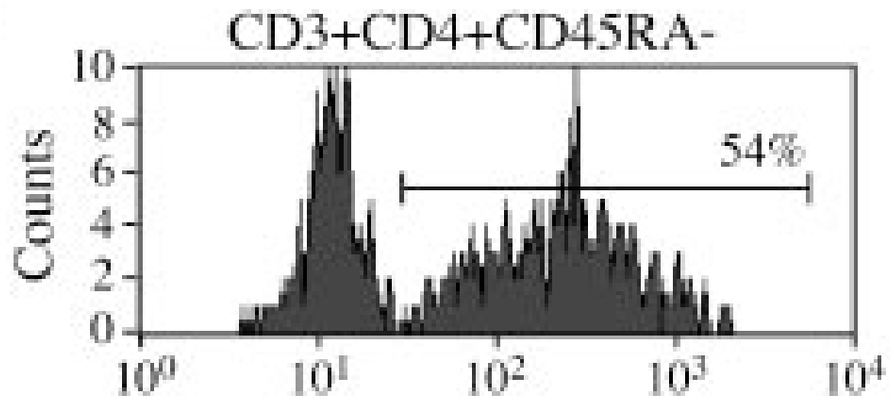
$d_A = 1\mathrm{d}^{-1}$

If this data is fitted with either of the two models,
the fit is perfect but the estimated turnover rate is 2-fold off.
This gets better when the average turnover rate is higher.

Piluygin et al., JTB 2003; De Boer et al., JRSI 2012

BrdU labeling





BrdU is a nucleoside analog incorporates into new DNA strands

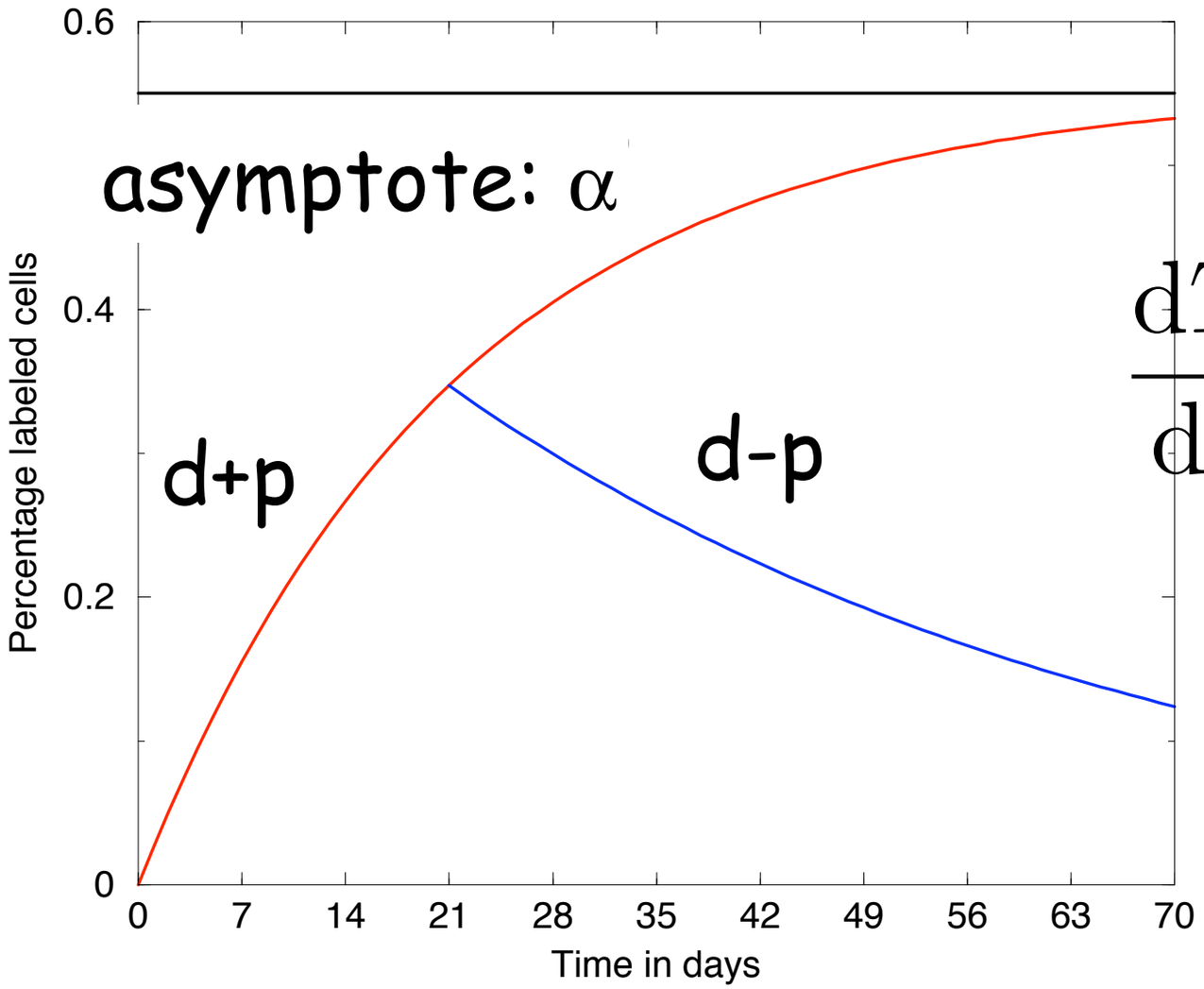
Can be detected in cells by staining cells with an antibody.

Brightness reflects fraction of BrdU labeled DNA strands

Above some "threshold brightness" cells are coined $BrdU^+$

BrdU labeling

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



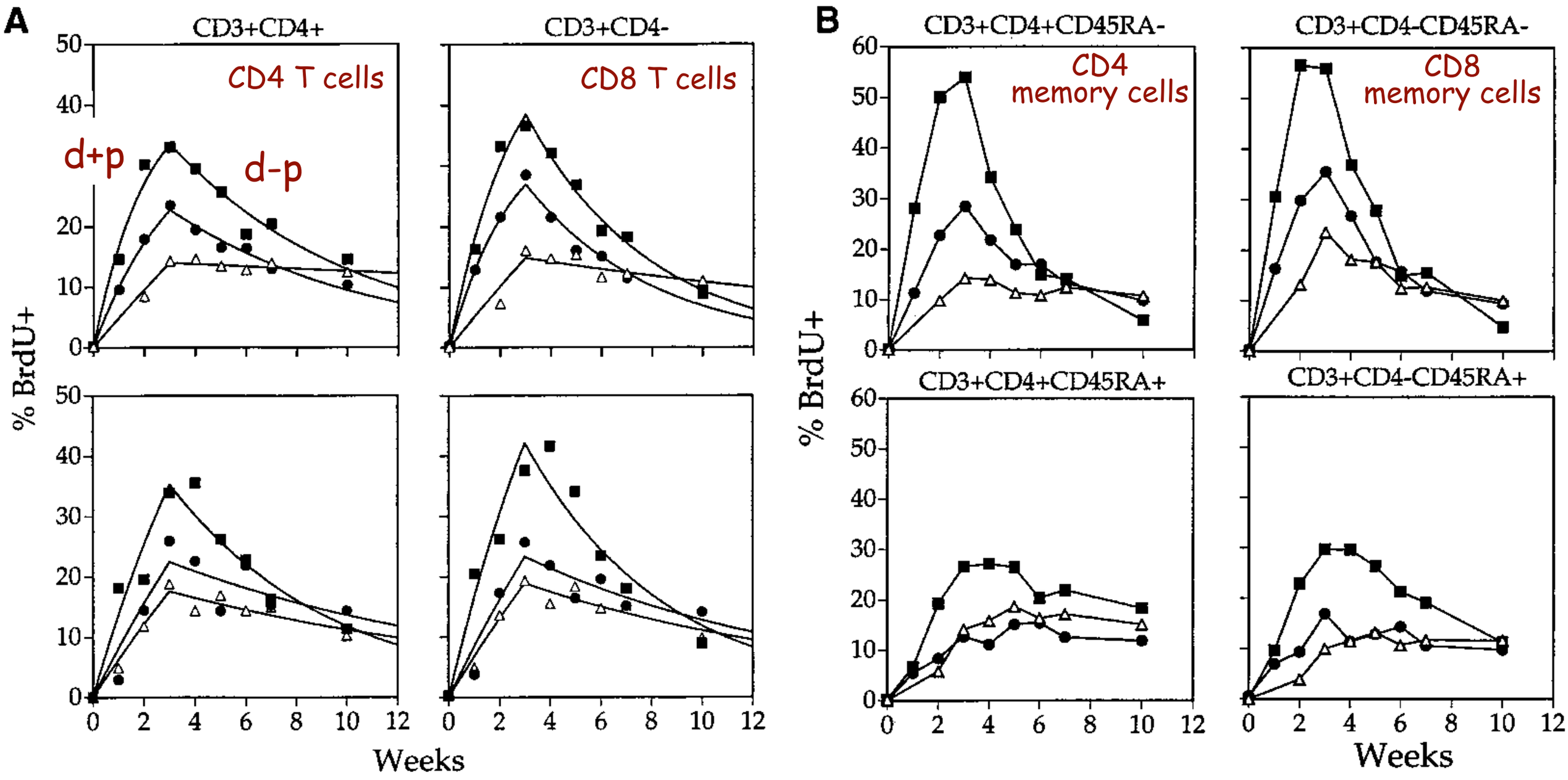
$$\frac{\mathrm{d}T}{\mathrm{d}t} = s + (p - d)T = 0$$

Labeling:
one cell -> two labeled cells

De-labeling:
one labeled cell -> two labeled cells

Self-renewing population at steady state (s=0, p=d) should have a zero down-slope.

The zero down-slope is a problem

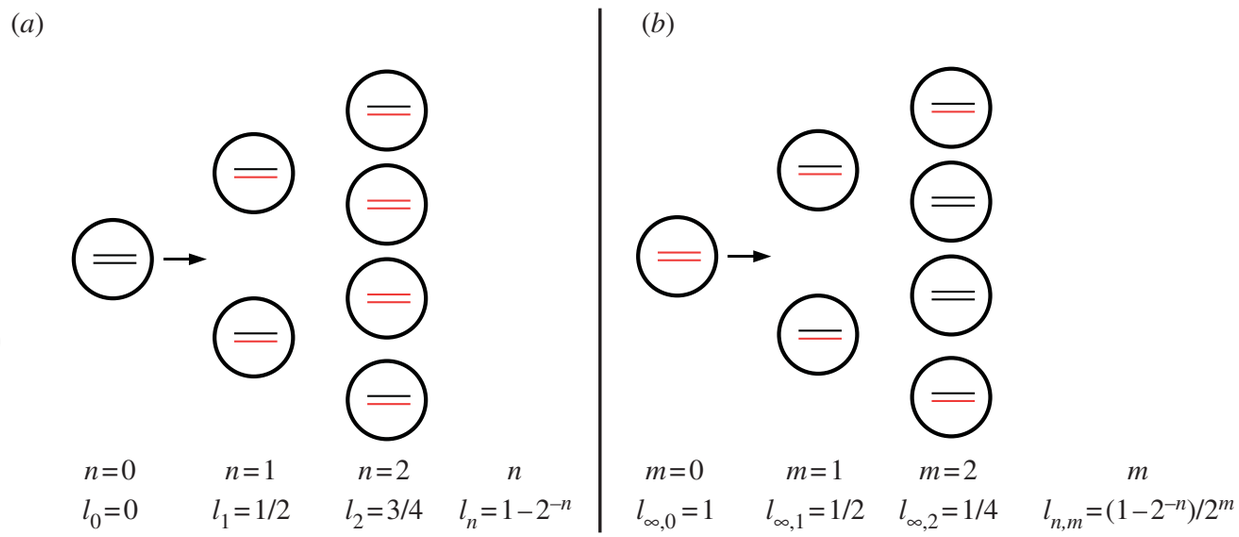


Macaques having BrdU in drinking water for 3 weeks
Uninfected (triangle) and SIV infected monkeys

Mohri, Bonhoeffer, Monard, Perelson & Ho, Science 1998

Solution is to allow for BrdU dilution

$$\frac{\mathrm{d}N_0(t)}{\mathrm{d}t} = -(p+d)N_0(t) \ ,$$
$$\frac{\mathrm{d}N_n(t)}{\mathrm{d}t} = 2pN_{n-1}(t) - (p+d)N_n(t) \ ,$$



Solution with Poisson distribution

$$N_n(t) = N(t) \times \frac{(2pt)^n}{n!} e^{-2pt} = N(t) \times f_n(t,p) \ , \quad \text{<- labeling (T days)}$$

$$N_{n,m}(t) = N(t) \times f_n(T,p) \times f_m(t-T,p) \ , \quad \text{<- delabeling}$$

Fraction of labeled cells:

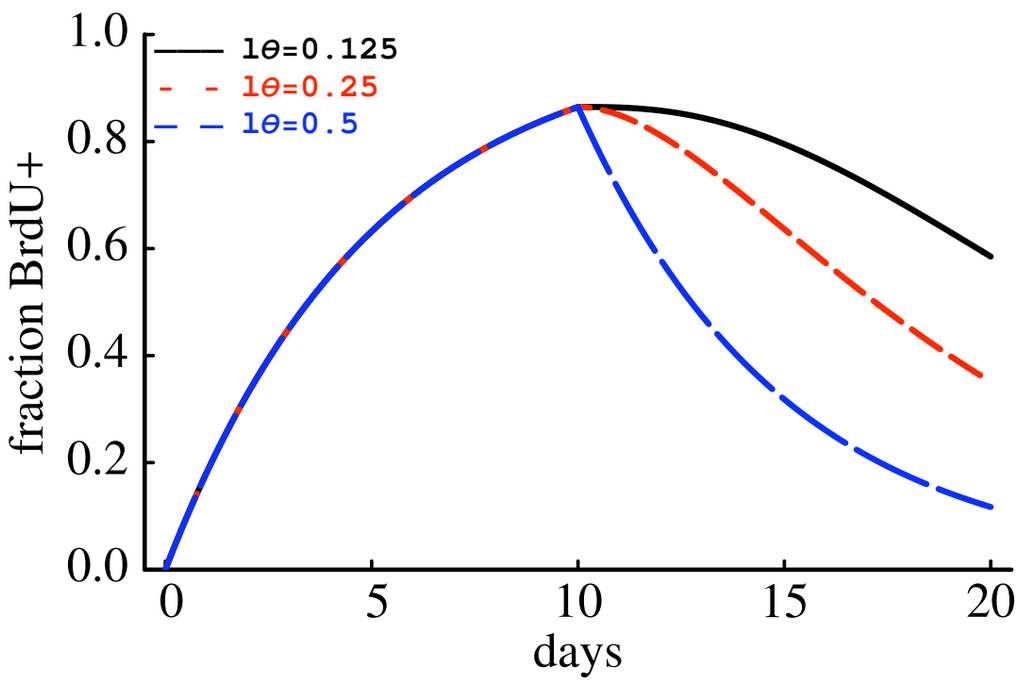
$$L(t) = \begin{cases} \sum_{n=1}^{\infty} H(l_n - l_\theta) \times f_n(t,p) \ , & \text{if } t \le T \ , \\ \sum_{n=1}^{\infty} \sum_{m=1}^{\infty} H(l_{n,m} - l_\theta) \times f_n(T,p) \times f_m(t-T,p) \ , & \text{otherwise} \ . \end{cases}$$

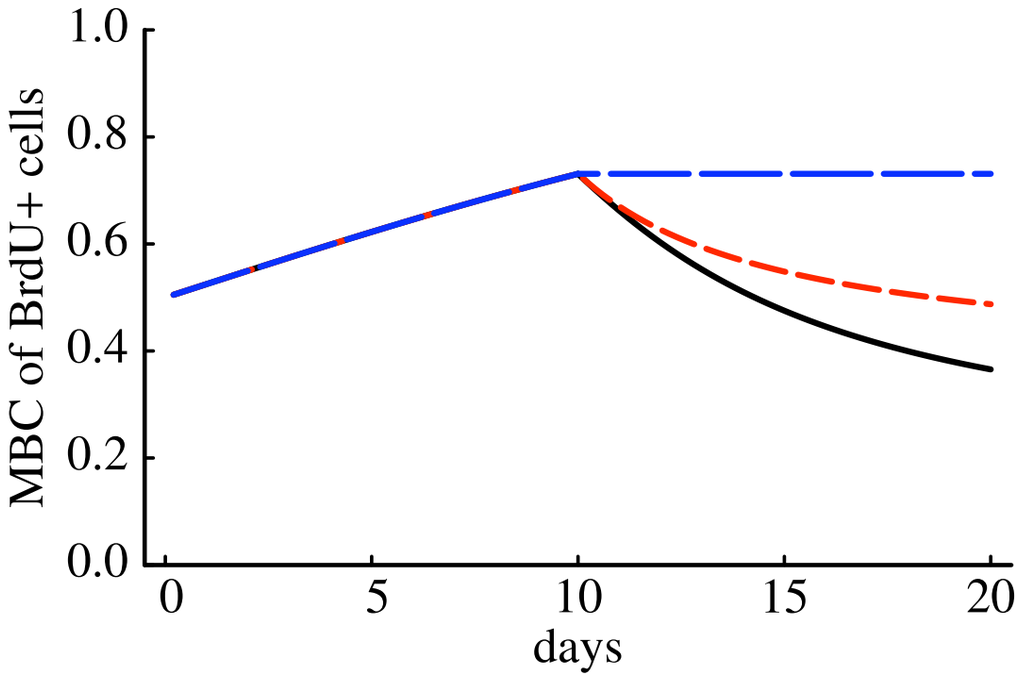
$$l_n = 1 - 2^{-n} \qquad l_{n,m} = (1-2^{-n})/2^m$$

$H(x)$ is a Heaviside function, i.e., $H(x) = 0$ whenever $x < 0$

Ganusov & De Boer, JRSI 2012

Now we obtain a down-slope when p=d





Fraction BrdU⁺ cells
for 3 detection thresholds

Model also allows one to define a "mean fluorescence intensity" (MFI).

MFI need not decline much when fraction of BrdU⁺ cells is decreasing

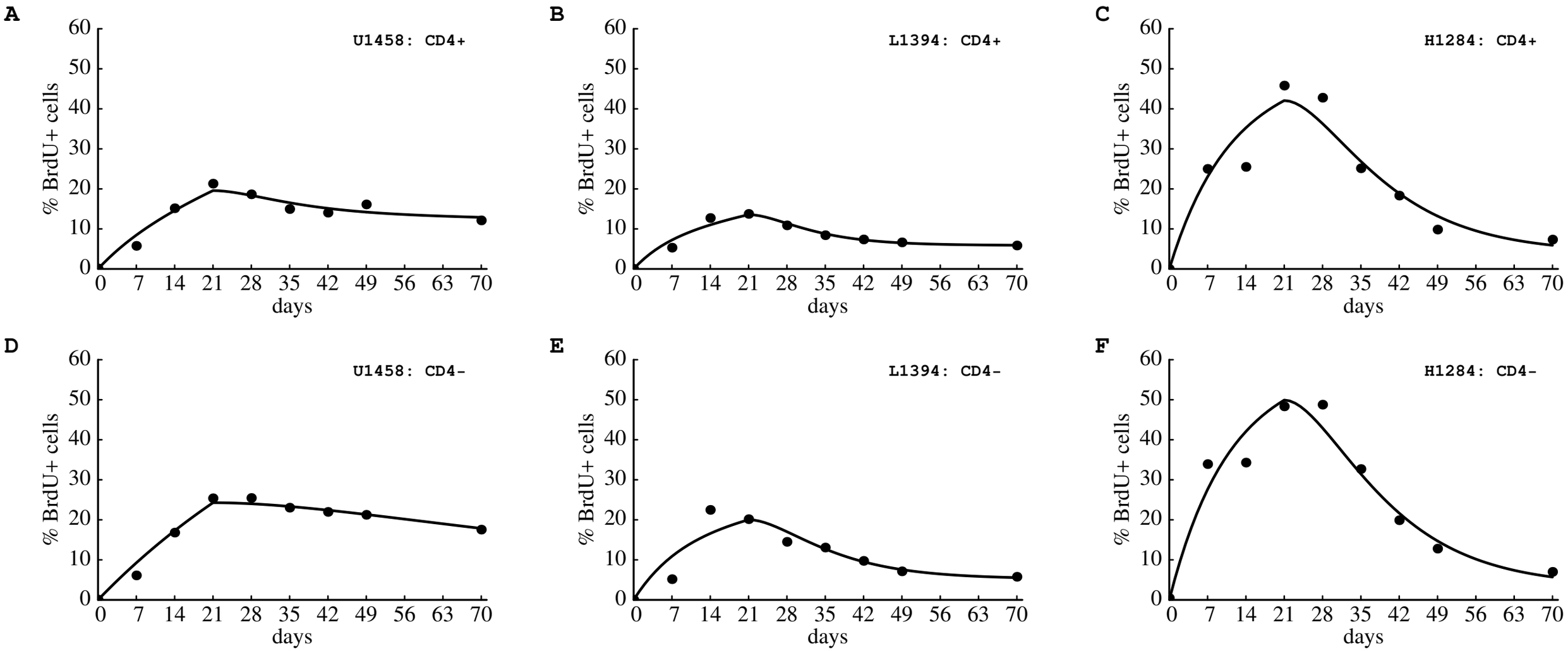
Ganusov & De Boer, JRSI 2012

(p
arretta: threshold 1θ at 0.25)

Adding on heterogeneity to the model

$$L(t) = \begin{cases} \sum_{i=1}^{k} \alpha_i \sum_{n=1}^{\infty} H(l_n - l_\theta) \times f_n(t, p_i) \ , & \text{if } t \le T \ , \\ \sum_{i=1}^{k} \alpha_i \sum_{n=1}^{\infty} \sum_{m=1}^{\infty} H(l_{n,m} - l_\theta) \times f_n(T, p_i) \times f_m(t - T, p_i), & \text{otherwise} \ , \end{cases}$$

k times
$p_i = d_i$

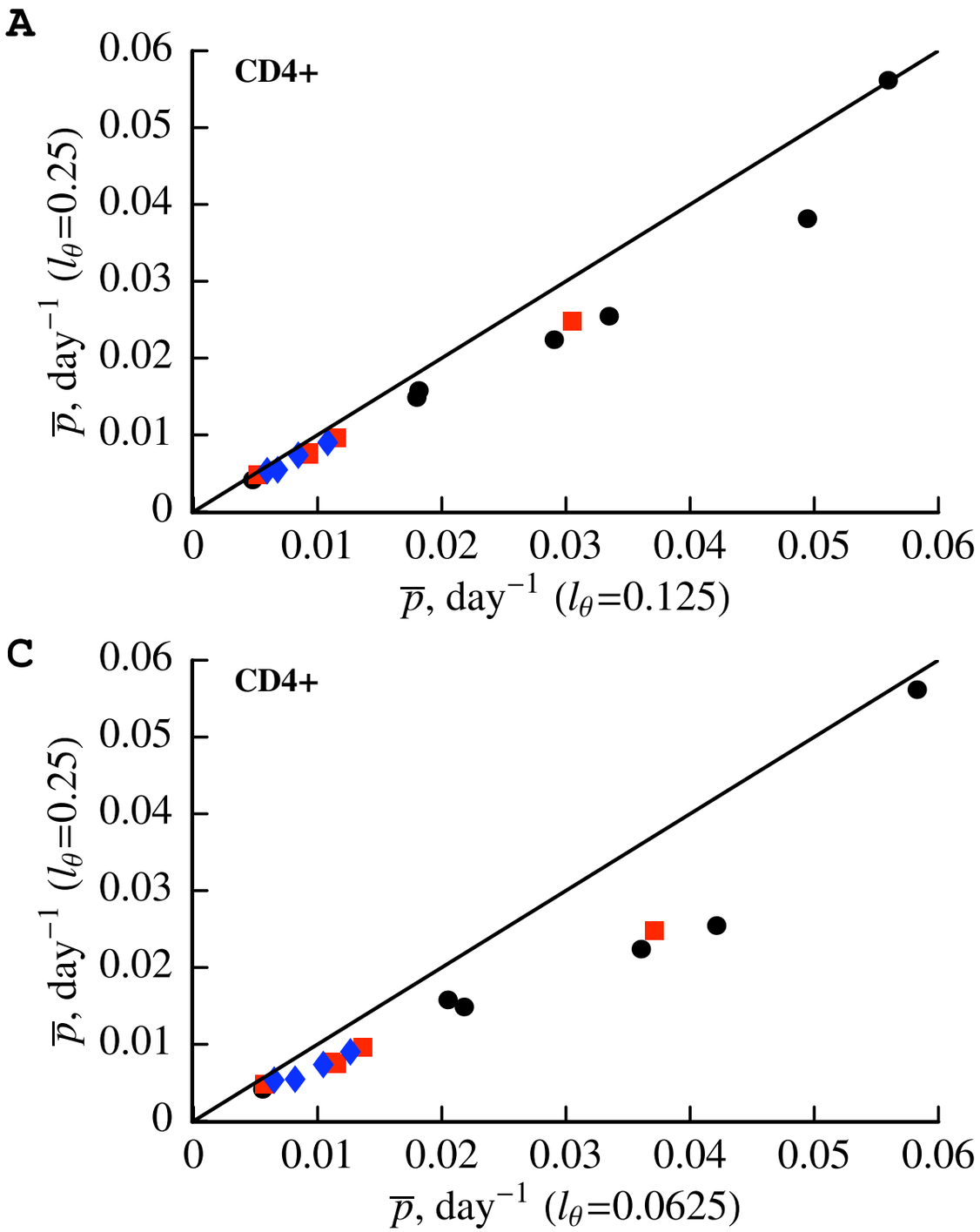


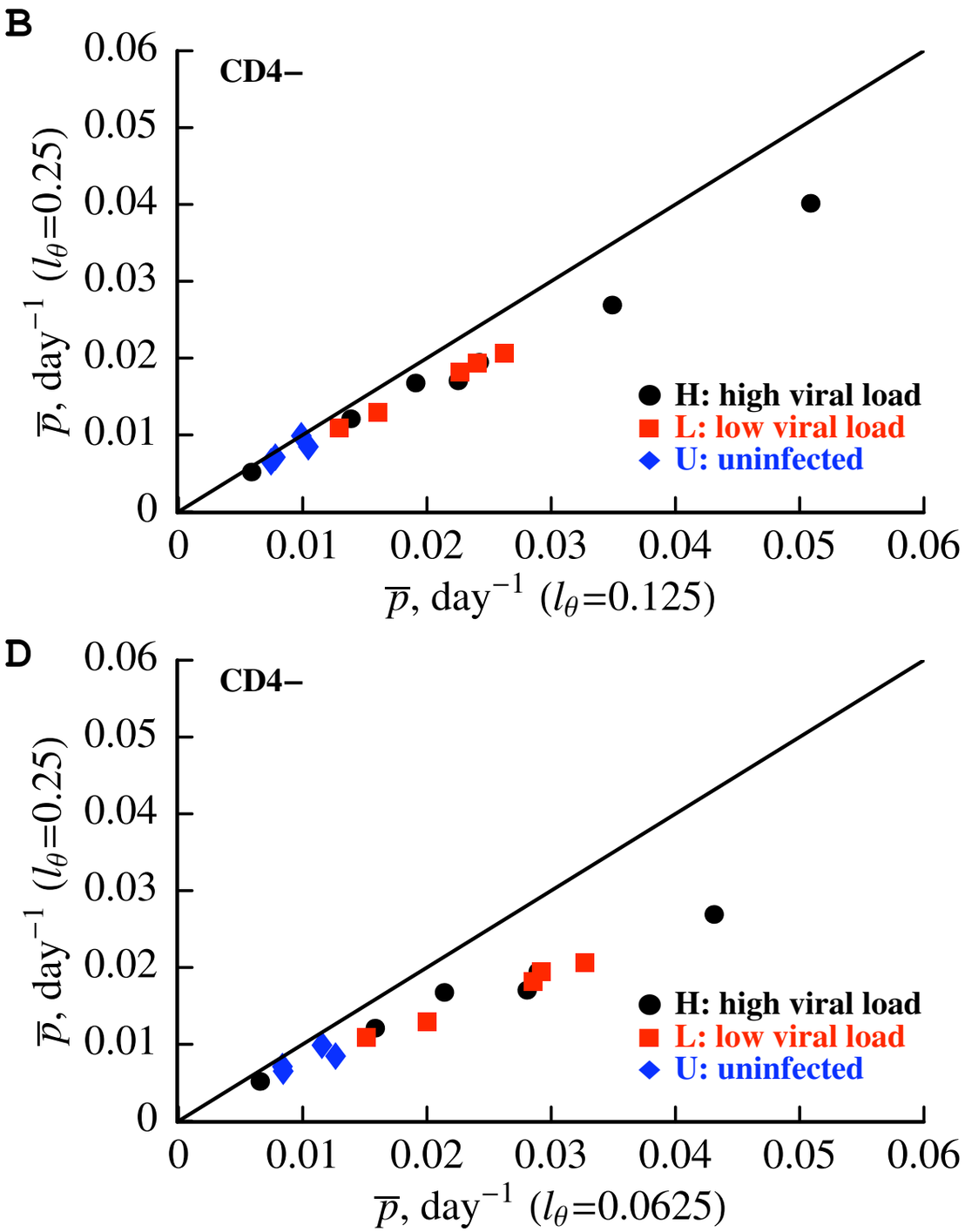
BrdU data from uninfected and SIV-infected monkeys.

We no longer need a large source.

Average turnover rate depends on the detection limit.

Estimated turnover decreases with detection limit

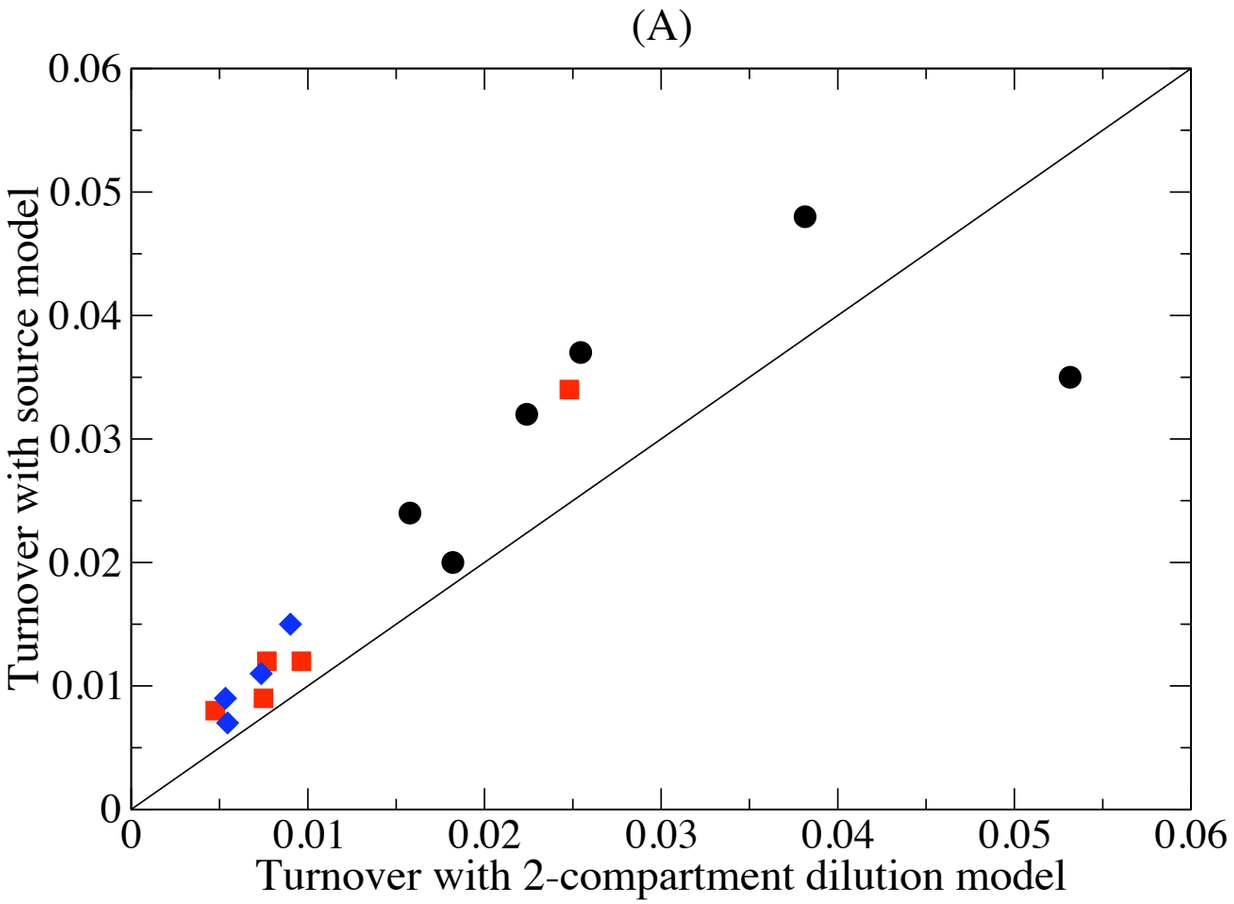


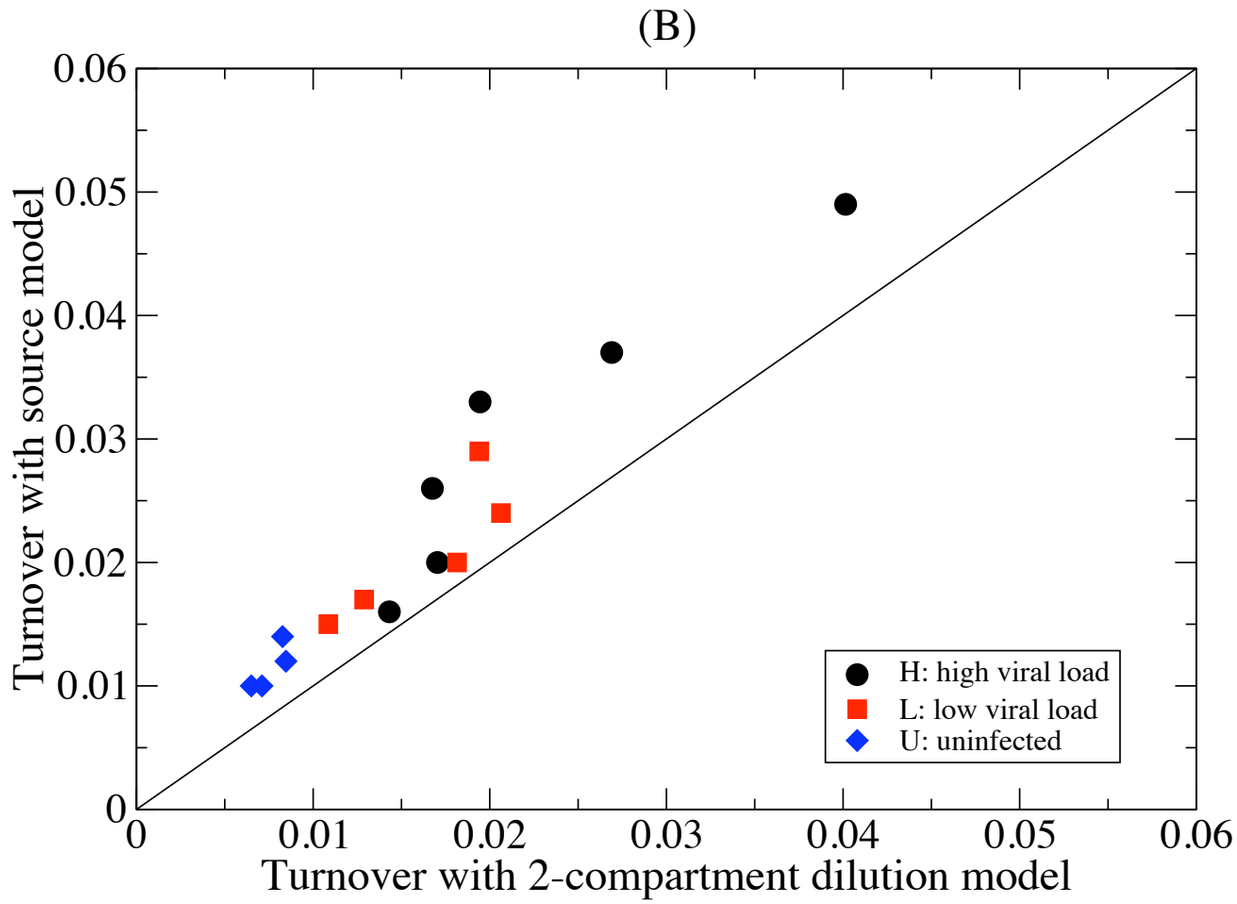


Threshold ι_θ = 0.25 fits best

but differences in quality of the fit are small

Dilution model estimates lower turnover rates





Small differences in quality of the fits

Threshold ι_θ at 0.25

Ganusov & De Boer, JRSI 2012

Difference between BrdU and deuterium

BrdU:

$$\mathrm{d}T_U/\mathrm{d}t = -(p+d)T_U\ , \quad \mathrm{d}T_L/\mathrm{d}t = \sigma + 2pT_U + (p-d)T_L \text{ during labeling, and}$$
$$\mathrm{d}T_U/\mathrm{d}t = \sigma + (p-d)T_U\ , \mathrm{d}T_L/\mathrm{d}t = (p-d)T_L \text{ during de-labeling .}$$

Fraction labeled:

$$\frac{\mathrm{d}L}{\mathrm{d}t} = [2p + s(t)](1-L) \quad \text{and} \quad \frac{\mathrm{d}L}{\mathrm{d}t} = -s(t)L \quad \text{where} \quad s(t) = \frac{\sigma}{T(t)}\ ,$$

At steady state:

$$\mathrm{d}L/\mathrm{d}t = (p+d)(1-L) \quad \text{and} \quad \mathrm{d}L/\mathrm{d}t = (p-d)L$$

Renewing (s=0): L' = 2p(1-L) but **Source** (p=0): L' = d(1-L)

Deuterium:

$$\mathrm{d}T_U/\mathrm{d}t = -dT_U\ , \quad \mathrm{d}T_L/\mathrm{d}t = \sigma + p[T_U + T_L] - dT_L \text{ during labeling, and}$$
$$\mathrm{d}T_U/\mathrm{d}t = \sigma + p[T_U + T_L] - dT_U\ , \mathrm{d}T_L/\mathrm{d}t = -dT_L \text{ during de-labeling ,}$$

Fraction labeled:

$$\frac{\mathrm{d}L}{\mathrm{d}t} = [p + s(t)](1-L) \quad \text{and} \quad \frac{\mathrm{d}L}{\mathrm{d}t} = -[p + s(t)]L \quad \text{where} \quad s(t) = \frac{\sigma}{T(t)}\ ,$$

At steady state:

$$\mathrm{d}L/\mathrm{d}t = d(1-L) \quad \text{and} \quad \mathrm{d}L/\mathrm{d}t = -dL$$

Depends on d only!

Ganusov & De Boer, JRSI 2012

Conclusions on using labeling to infer T cell population dynamics

Interpretation of deuterium data seemed so simple:
no toxic effects, no dilution, loss by death only.
Nevertheless very contradictory estimates.

Important to gather dense data having several points during early up and down-slope and fit these with an appropriate model

Naive T cells have life spans of
several years in humans and several weeks in mice.
Memory T cells live shorter than naive T cells.

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Theoretical Immunology Laboratory U Tennessee

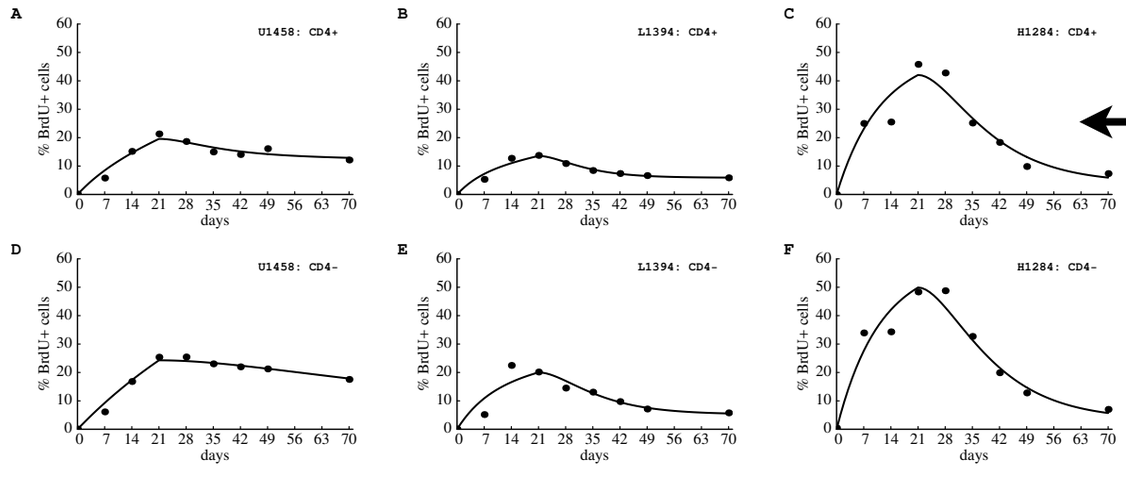
Vitaly Ganusov

Los Alamos National Laboratory

Ruy Ribeiro

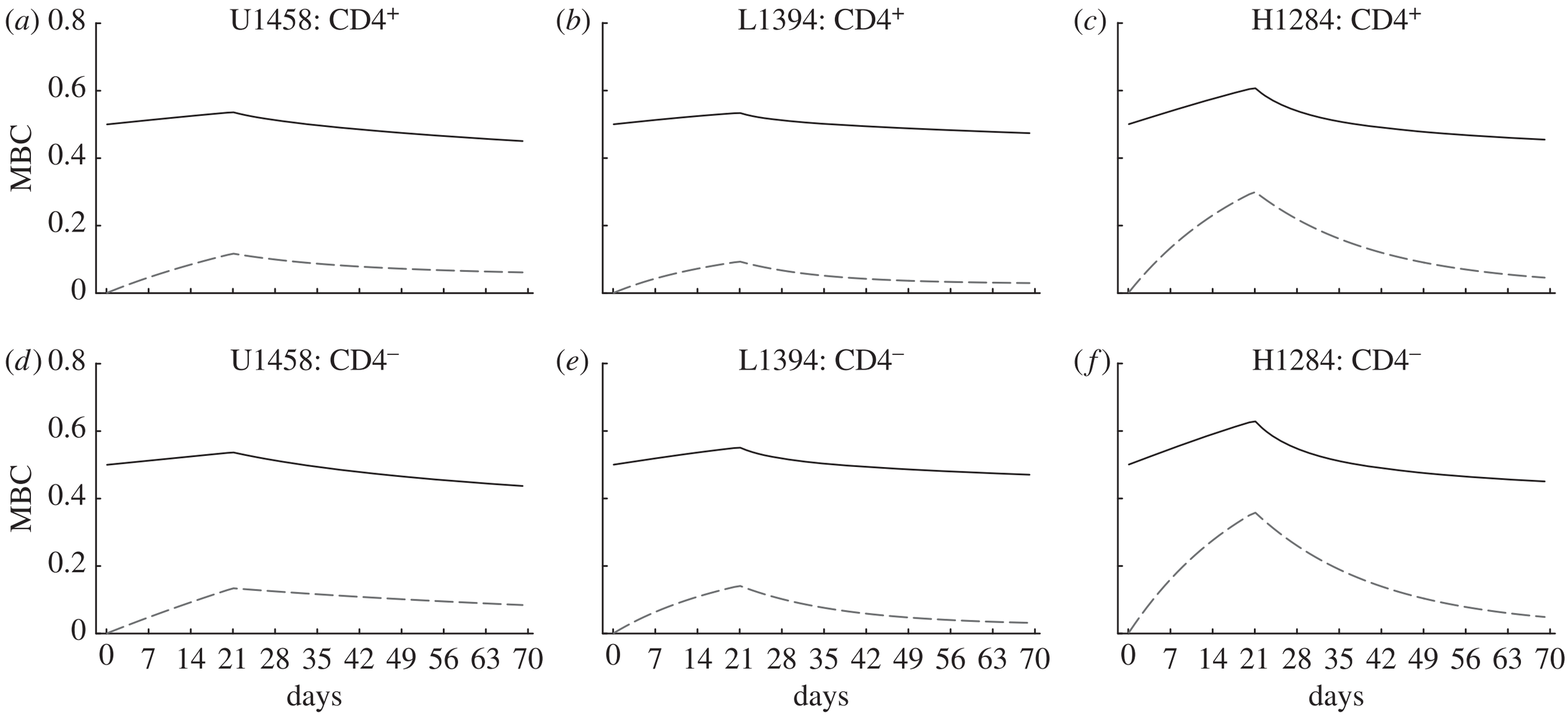
Alan Perelson

Adding on heterogeneity: MFI

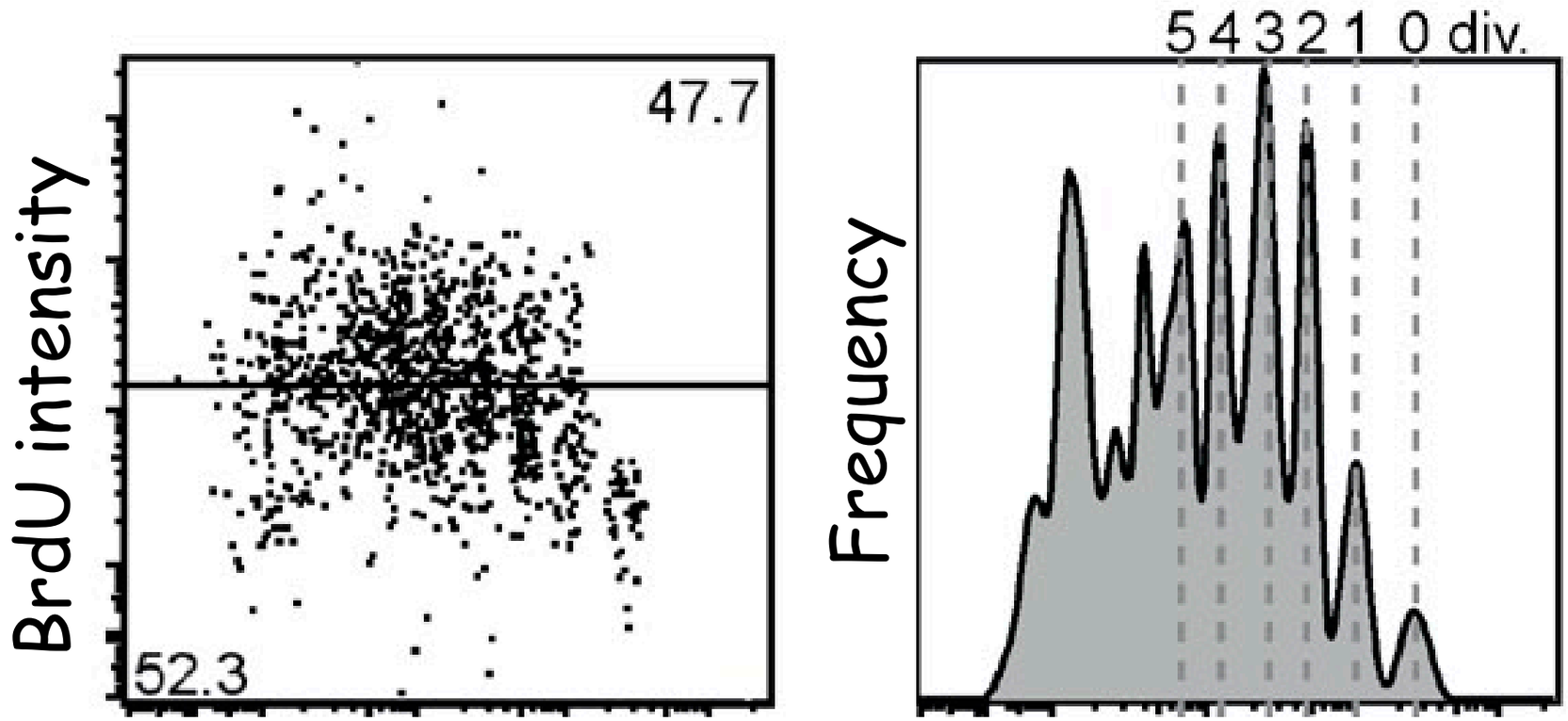


%BrdU⁺

"MFI"



Problem: is BrdU intensity reflecting #divisions?



From: Takizawa.jem11

In vivo CFSE labeled cells after 14d BrdU up-labeling

Intensity not linear in number of divisions

(we expect b, b+1/2, b+3/4, b+7/8, ...; scale is log)

Could help to explain absence of variation in BrdU intensity profiles and/or MFI