How to model a viral infection: HCV

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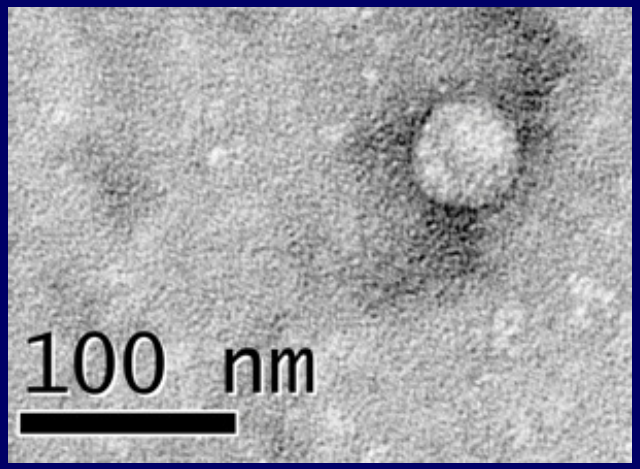
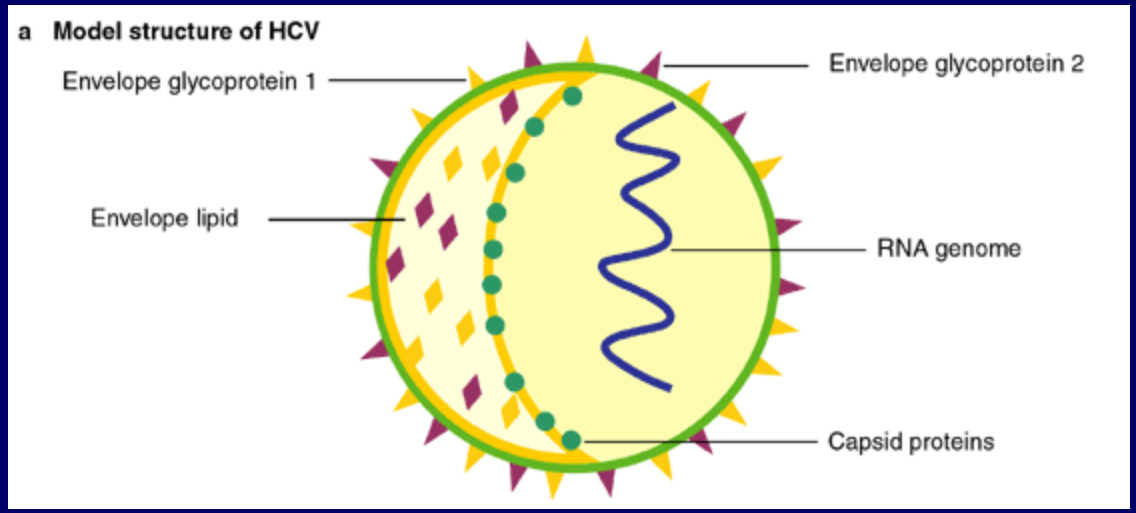
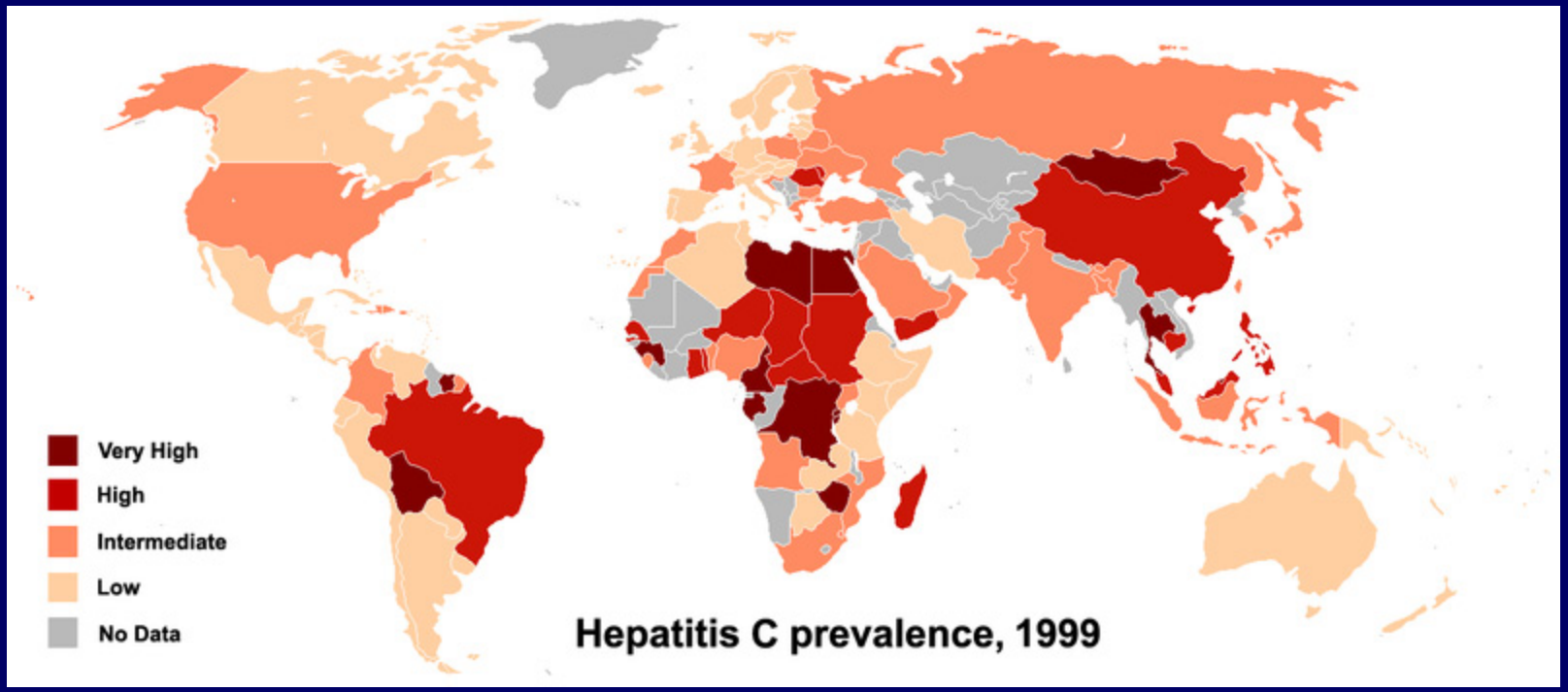
Basic HCV Virology

- 9,600 nt single stranded (+) sense RNA virus in the *Flaviviridae* family.
- Viral particles are enveloped, about 60 nm in size, and associates with lipid particles.
- No vaccine and therapy cures about 50% of people treated.



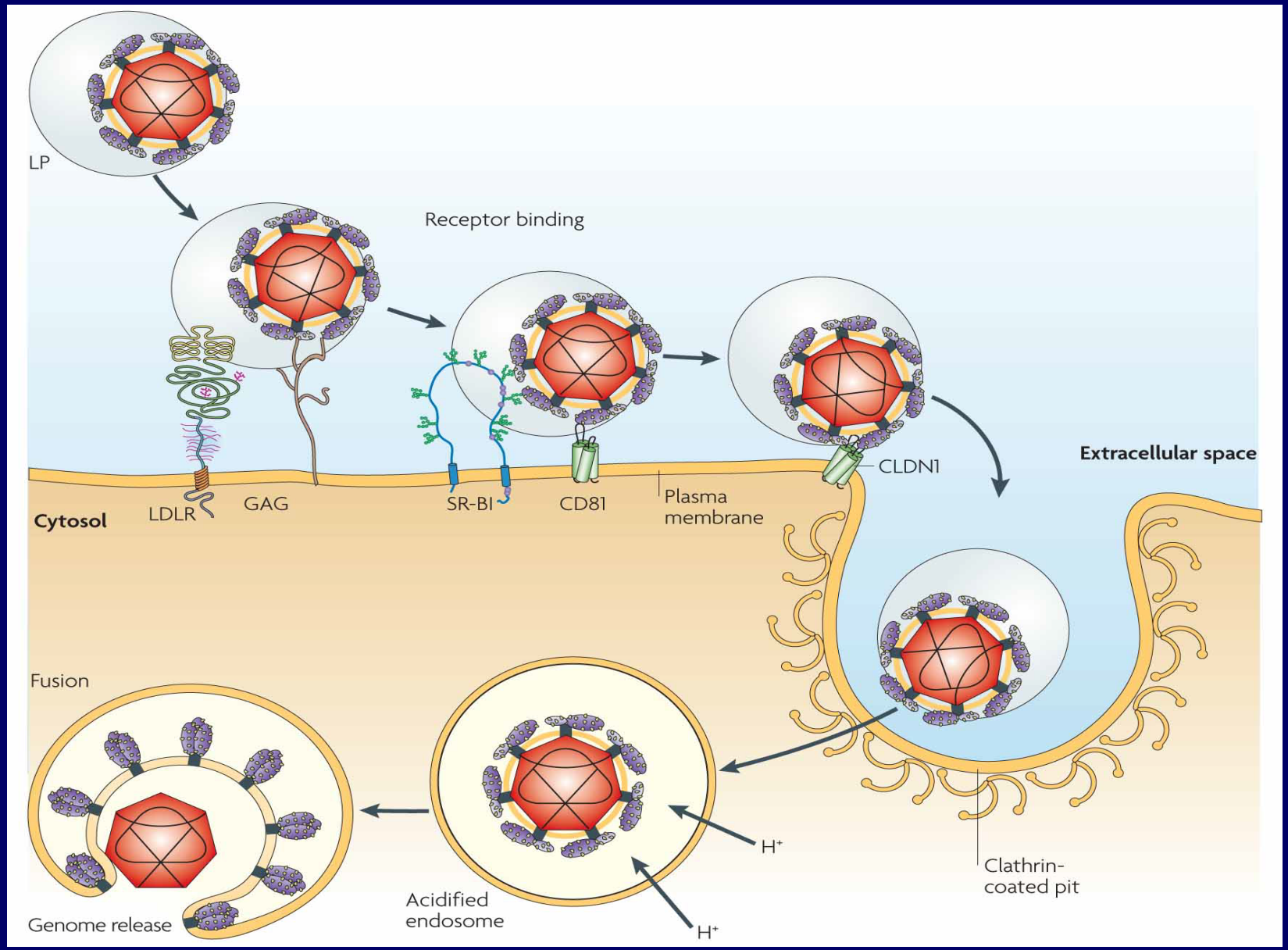


Epidemiology of HCV

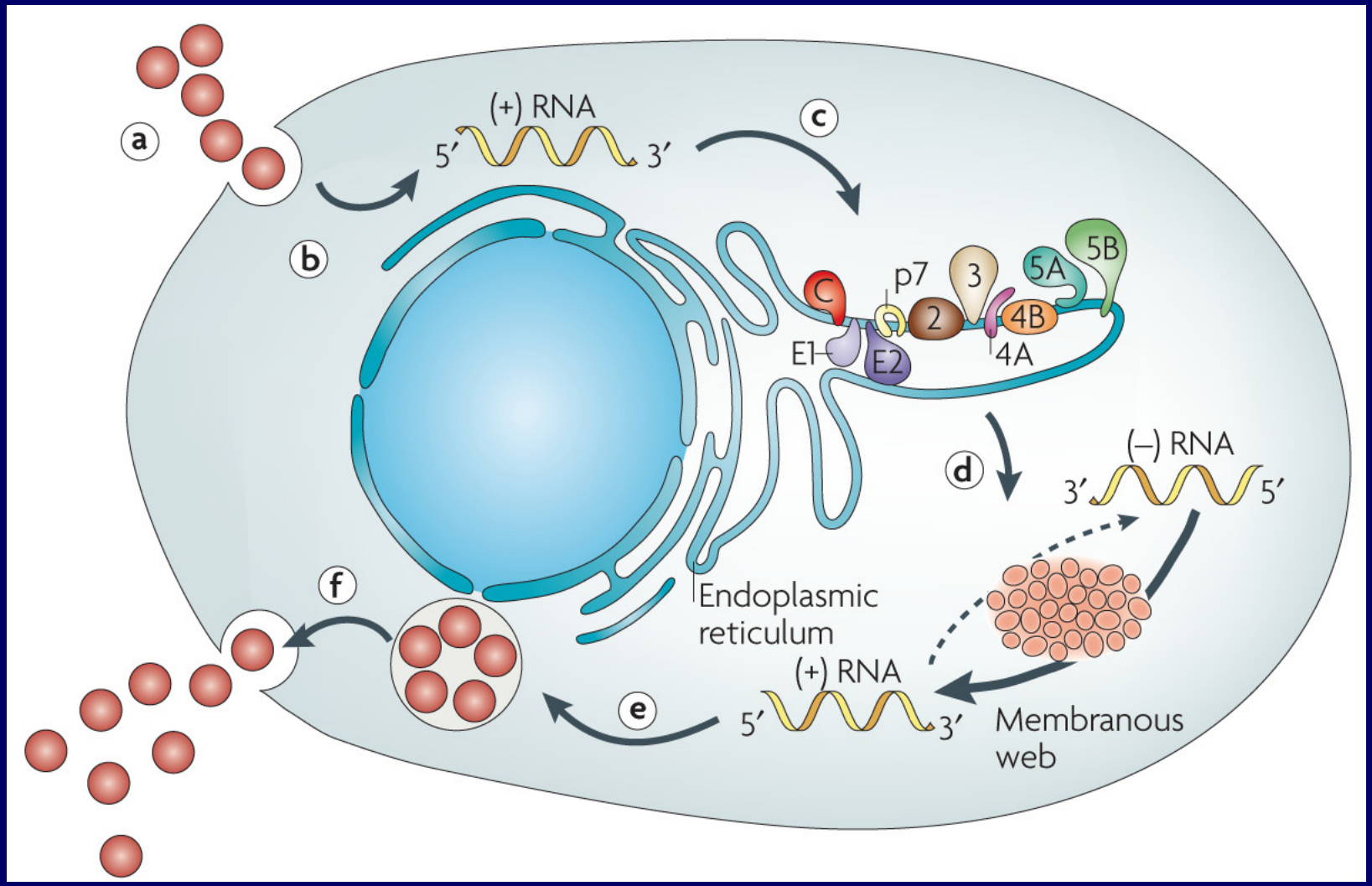


WHO: Hepatitis C is comparable to a 'viral time bomb'. WHO estimates that about 200 million people, 3% of the world's population, are infected with HCV and 3 to 4 million persons are newly infected each year. Infected patients are at risk of developing liver cirrhosis, liver cancer and other morbidities.

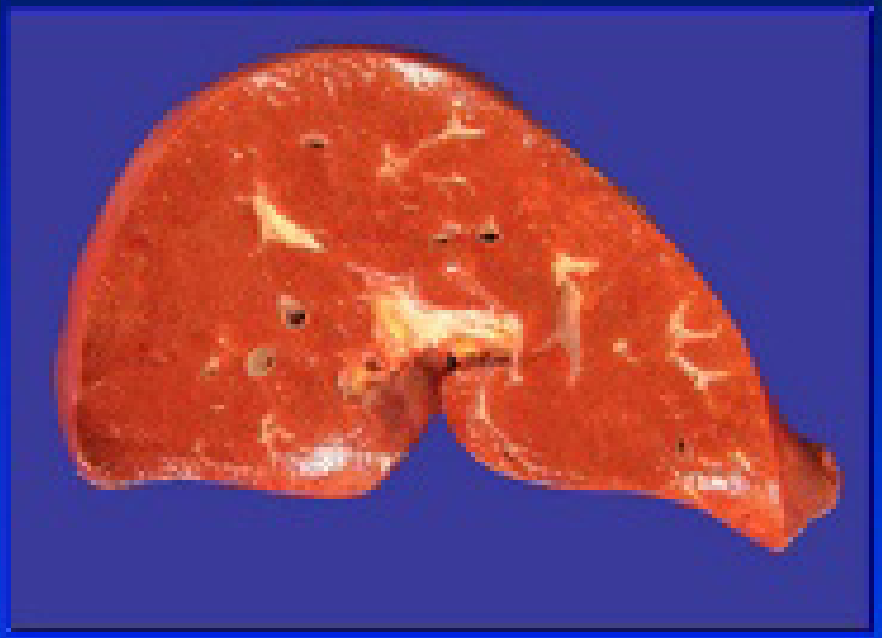
Model for Hepatitis C Virus (HCV) Entry



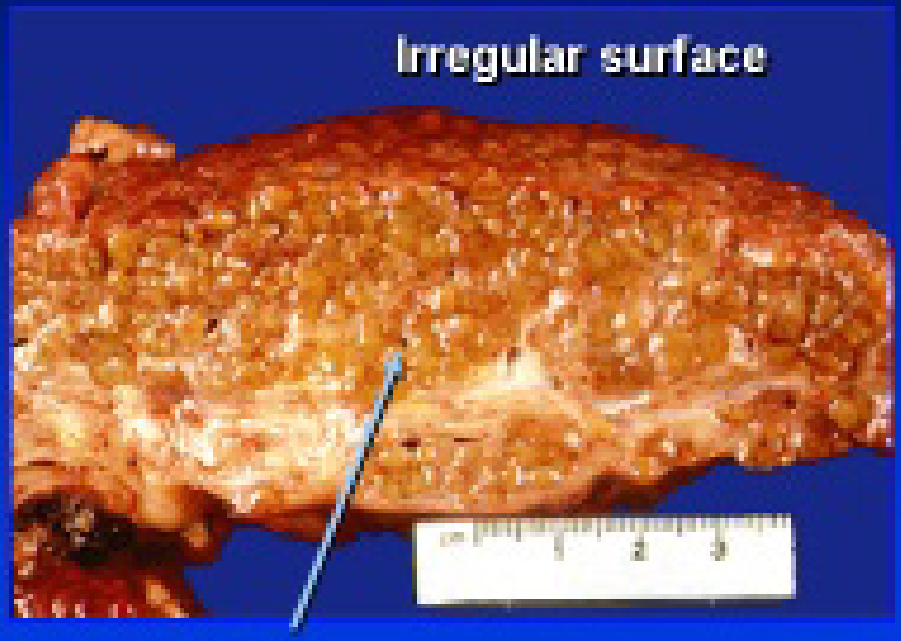
Lifecycle of Hepatitis C Virus



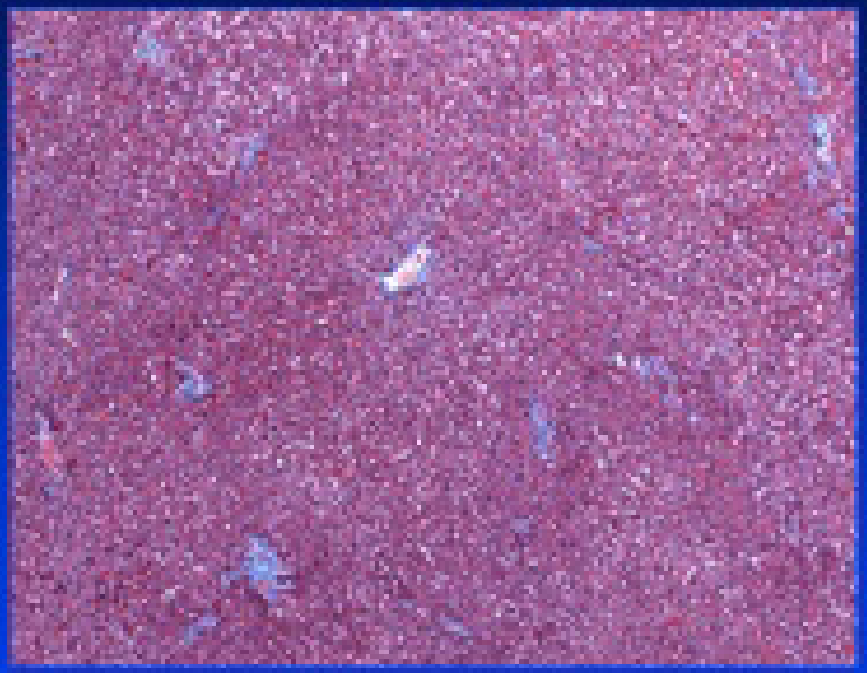
Normal



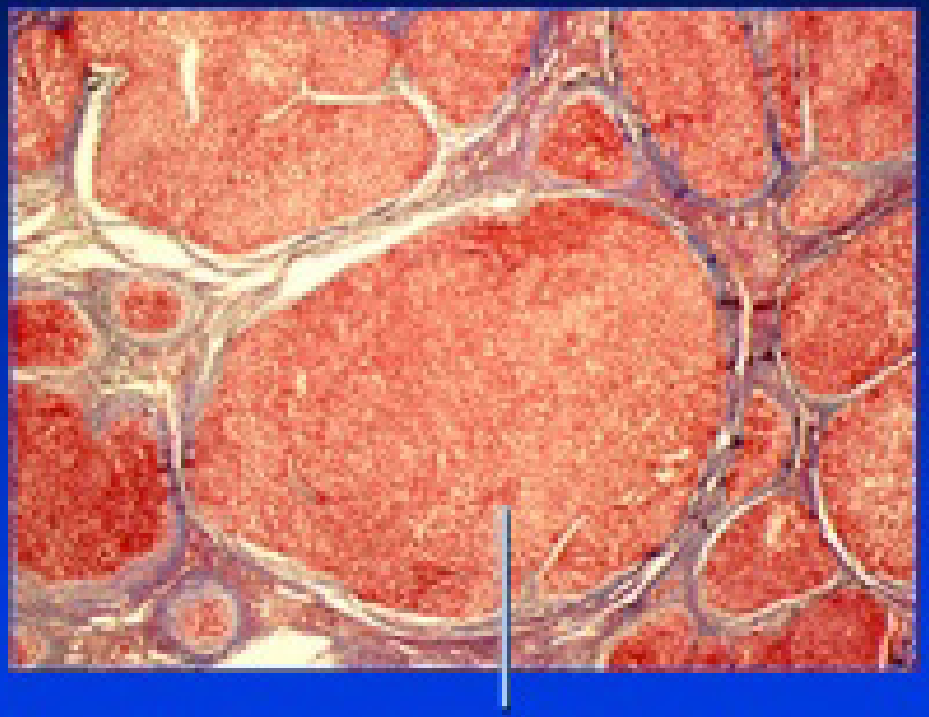
Cirrhosis



Normal



Cirrhosis

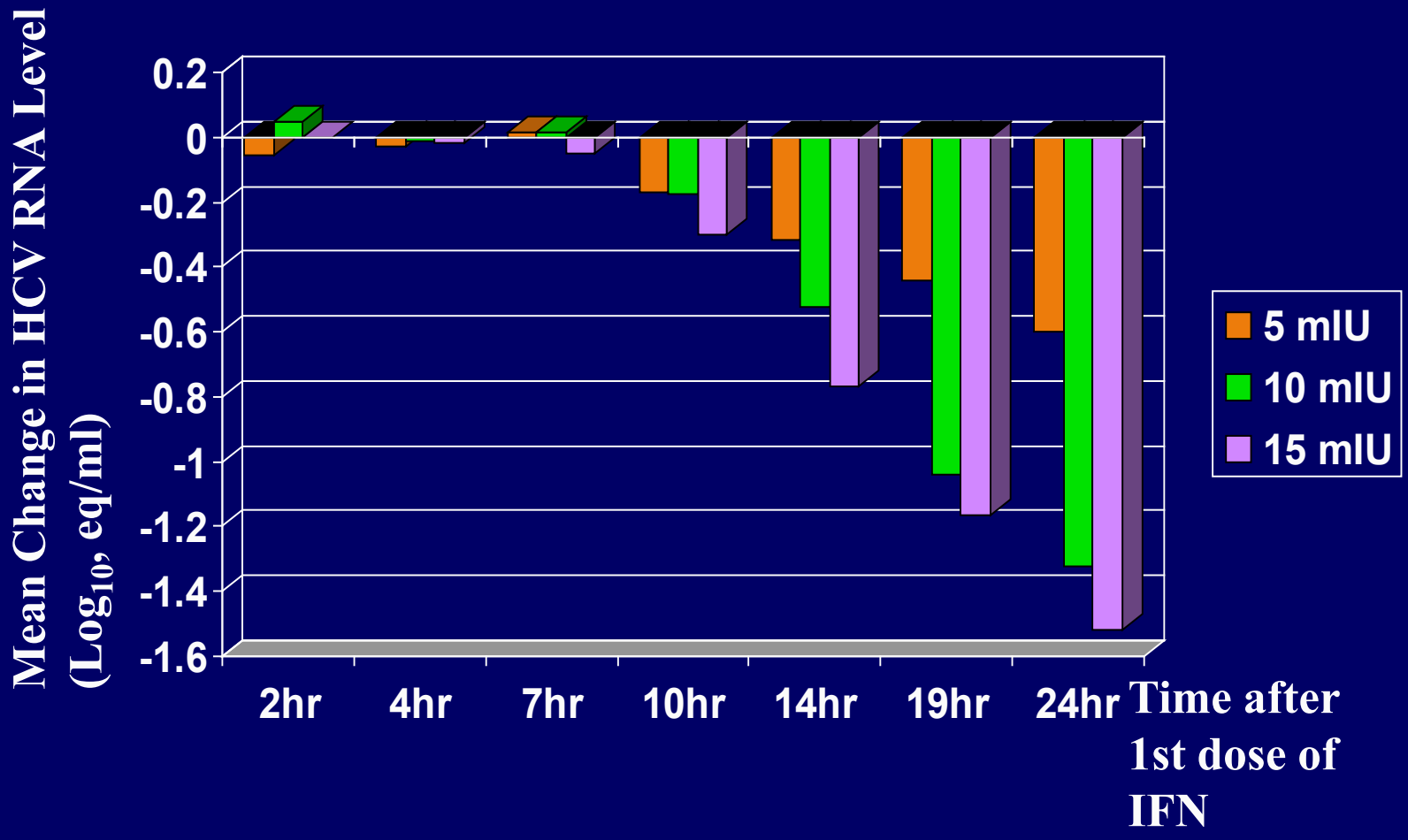


Nodules surrounded by fibrous tissue

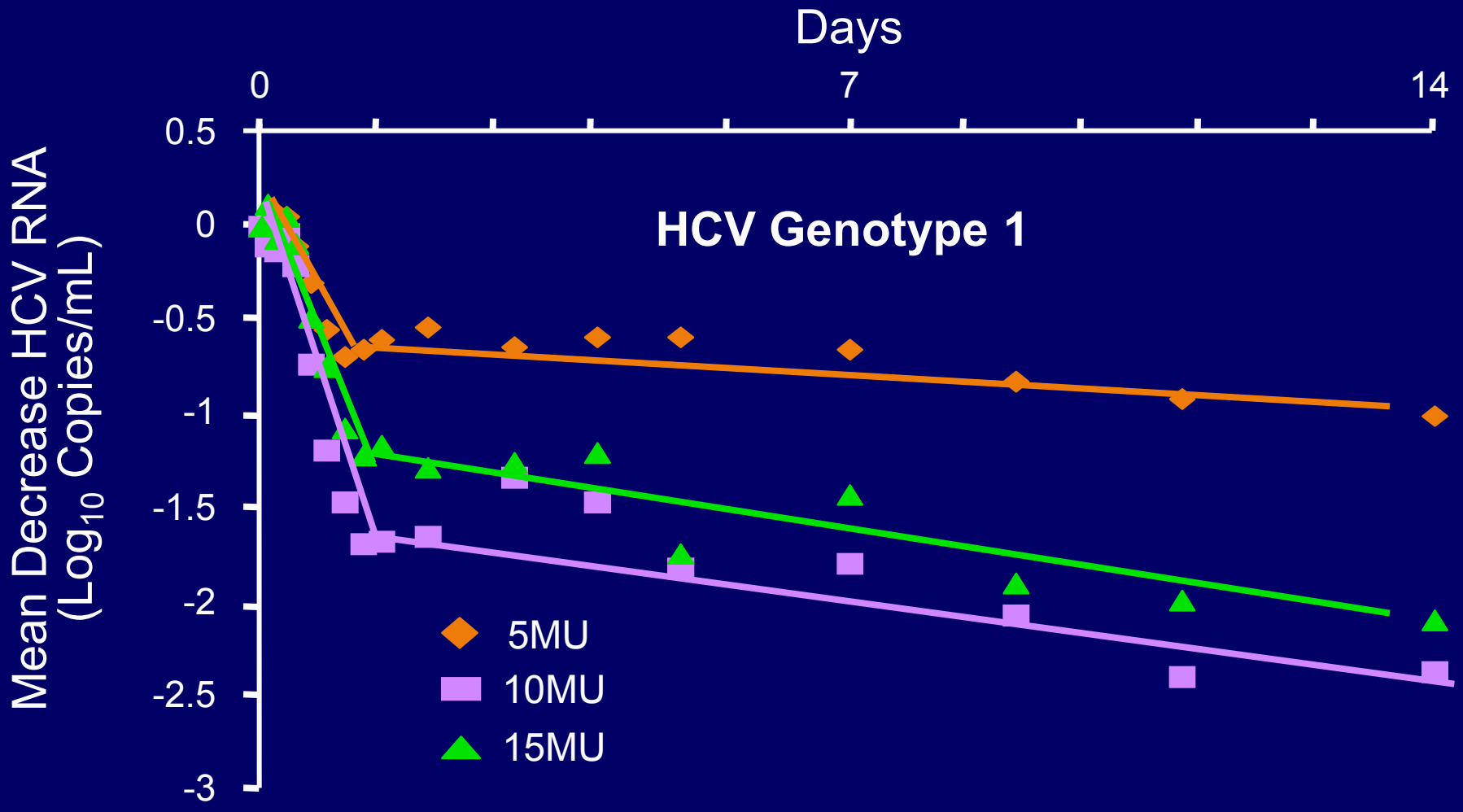
Treatment of HCV

- Two drugs are currently used to treat HCV infection
  - Interferon – α (IFN), which is naturally made cytokine involved in protection against viral infections.
  - Ribavirin (RBV), which is a nucleoside analog of guanosine. Its mechanism of action is controversial but it may act as a mutagen.
- New direct acting antiviral agents, eg HCV protease and polymerase inhibitors are in clinical trials.

Acute Changes in HCV RNA Level Following First Dose of IFN-α



Mean Decrease in HCV RNA Levels Over First 14 Days of QD IFN-α Treatment

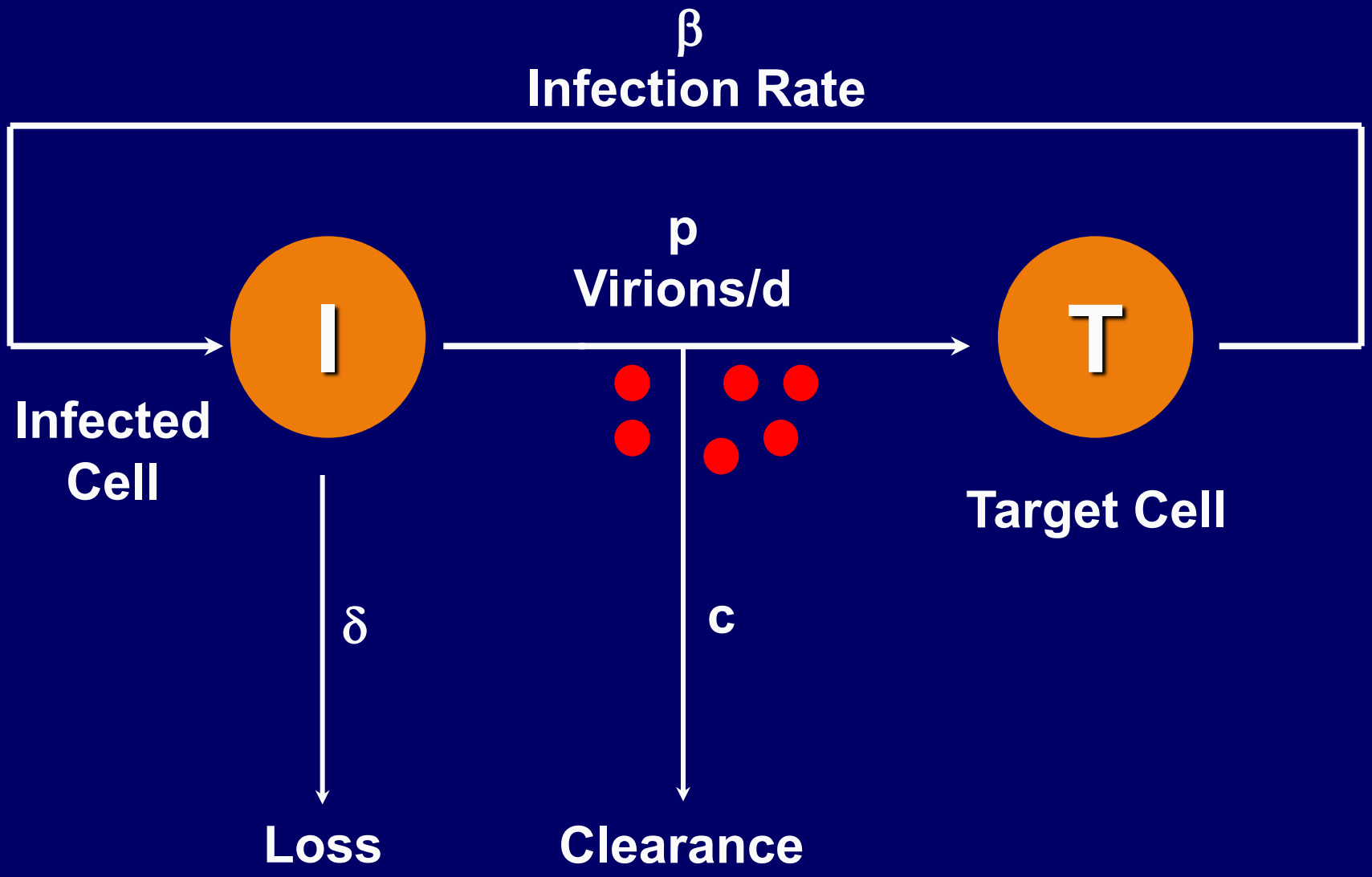


Lam N. DDW. 1998 (abstract L0346).

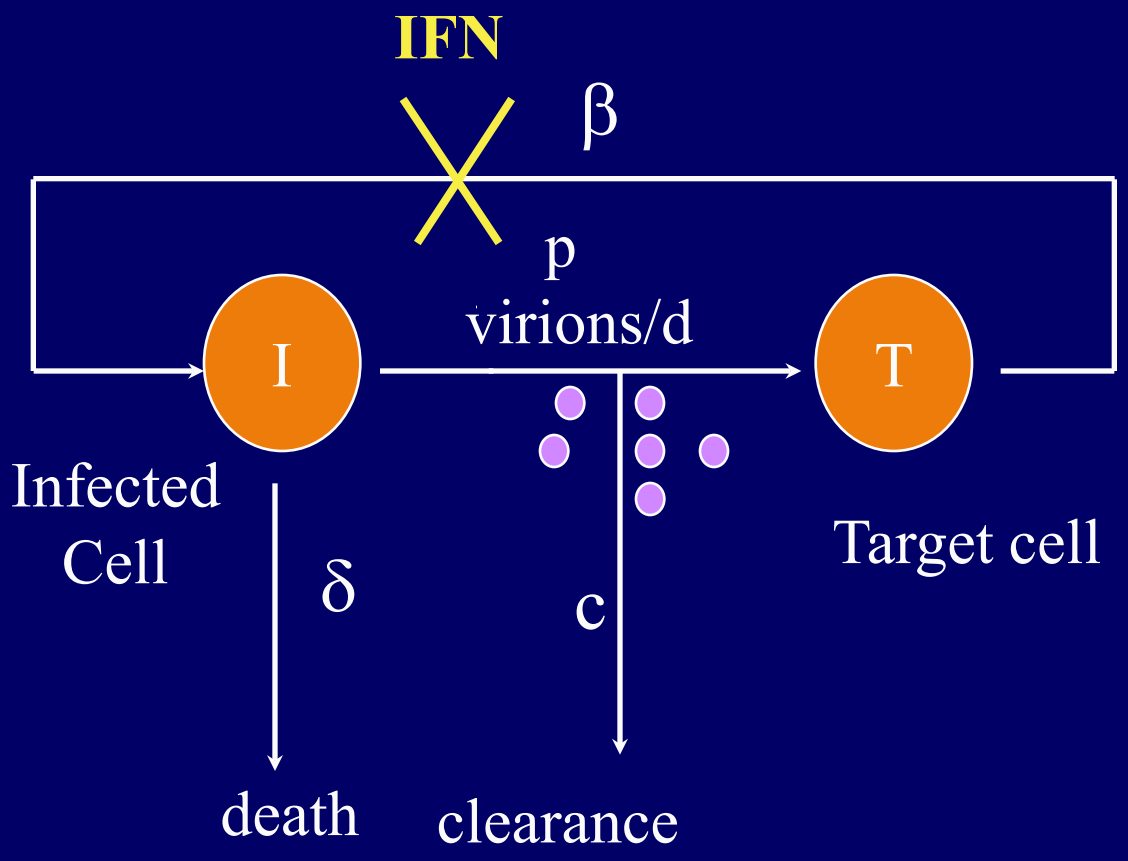
Biphasic Decline

- Why is there a biphasic decline?
- Why is the first phase about 10x faster than in HIV?
- What can we learn about HCV from this observation?

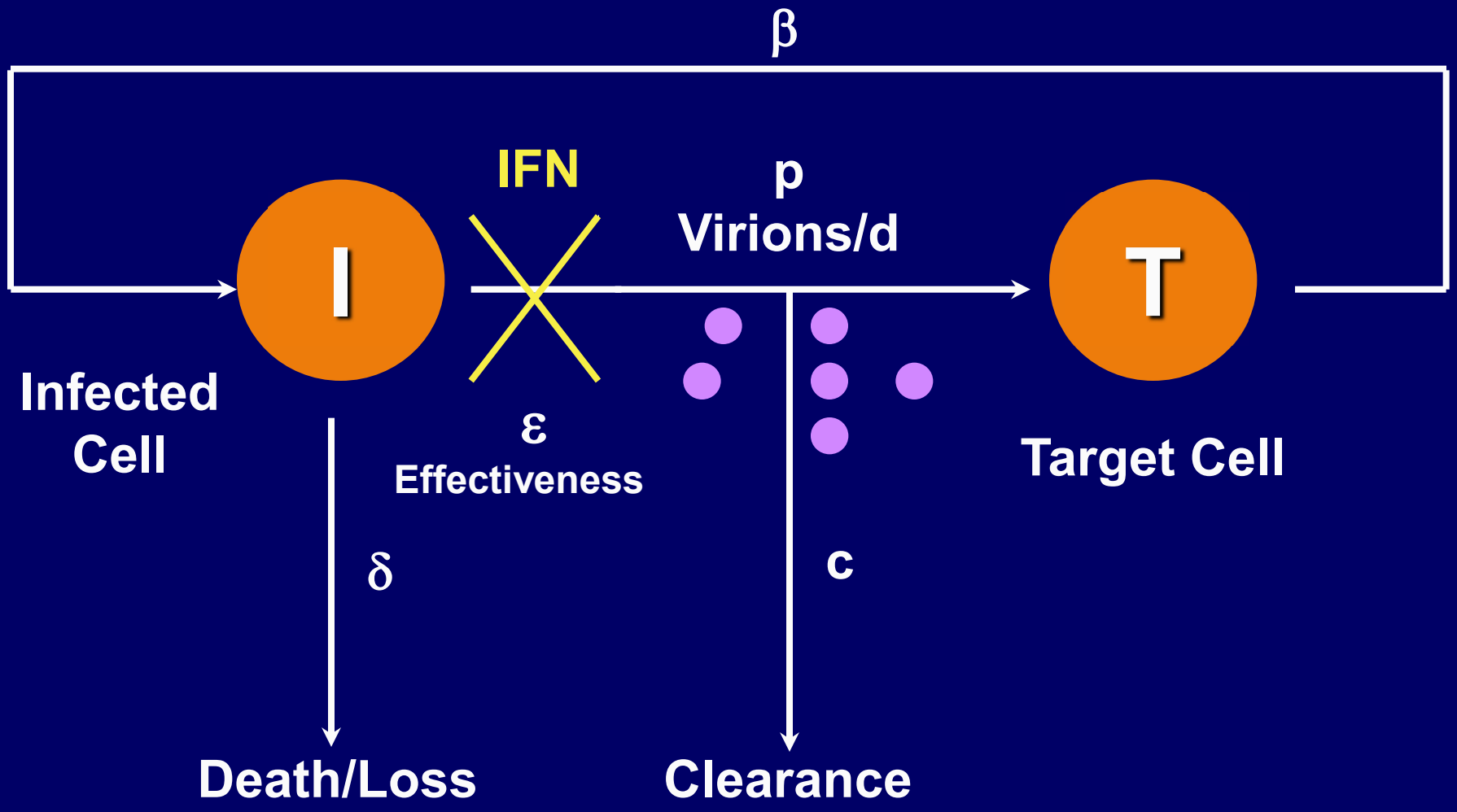
Model of HCV Infection



What if IFN blocks infection?



IFN
Blocks Production of Virus



IFN Effectiveness in Blocking Production

- Let ε = *effectiveness* of IFN in blocking production of virus
  - $\varepsilon = 1$ is 100% effectiveness
  - $\varepsilon = 0$ is 0% effectiveness
- $dV/dt = (1 - \varepsilon)pI - cV$

Early Kinetic Analysis

- Before therapy, assume steady state so that $pI_0 = cV_0$. Also, assume at short times, $I = \text{constant} = I_0$, so that $pI = cV_0$

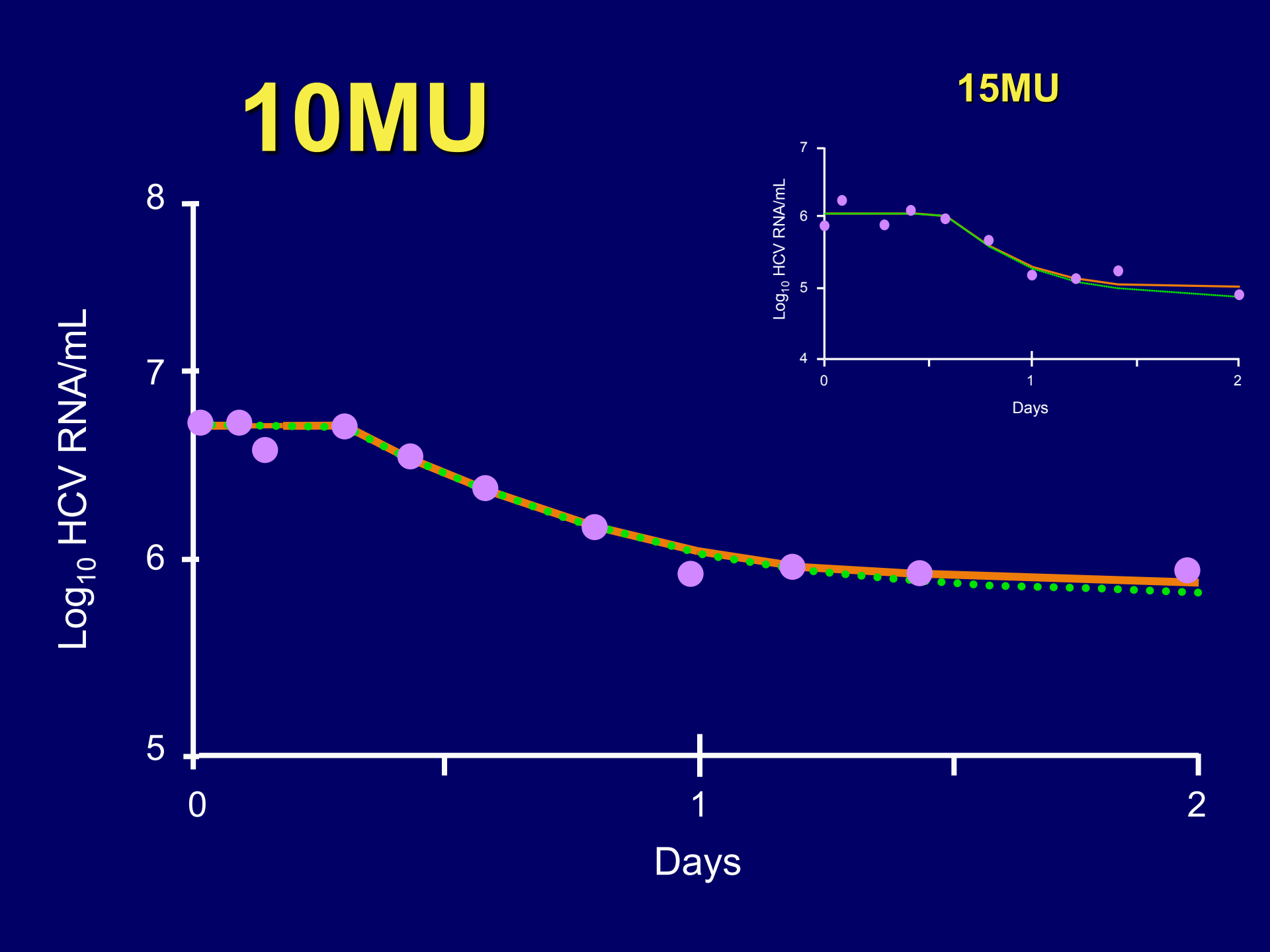
$$dV/dt = (1-\varepsilon)pI - cV = (1-\varepsilon)cV_0 - cV, \; V(0) = V_0$$

- Model predicts that after therapy is initiated, the viral load will initially change according to:

$$\mathbf{V(t) = V_0[1 - \varepsilon + \varepsilon \exp(-ct)]}$$

- This equation can be fit to data and **c** and **ε** estimated.

- Thus drug effectiveness can be determined within the first few days!


10MU
15MU
Log10 HCV RNA/mL
8
7
6
5
0
1
2
Days
Log10 HCV RNA/mL
7
6
5
4
0
1
2
Days

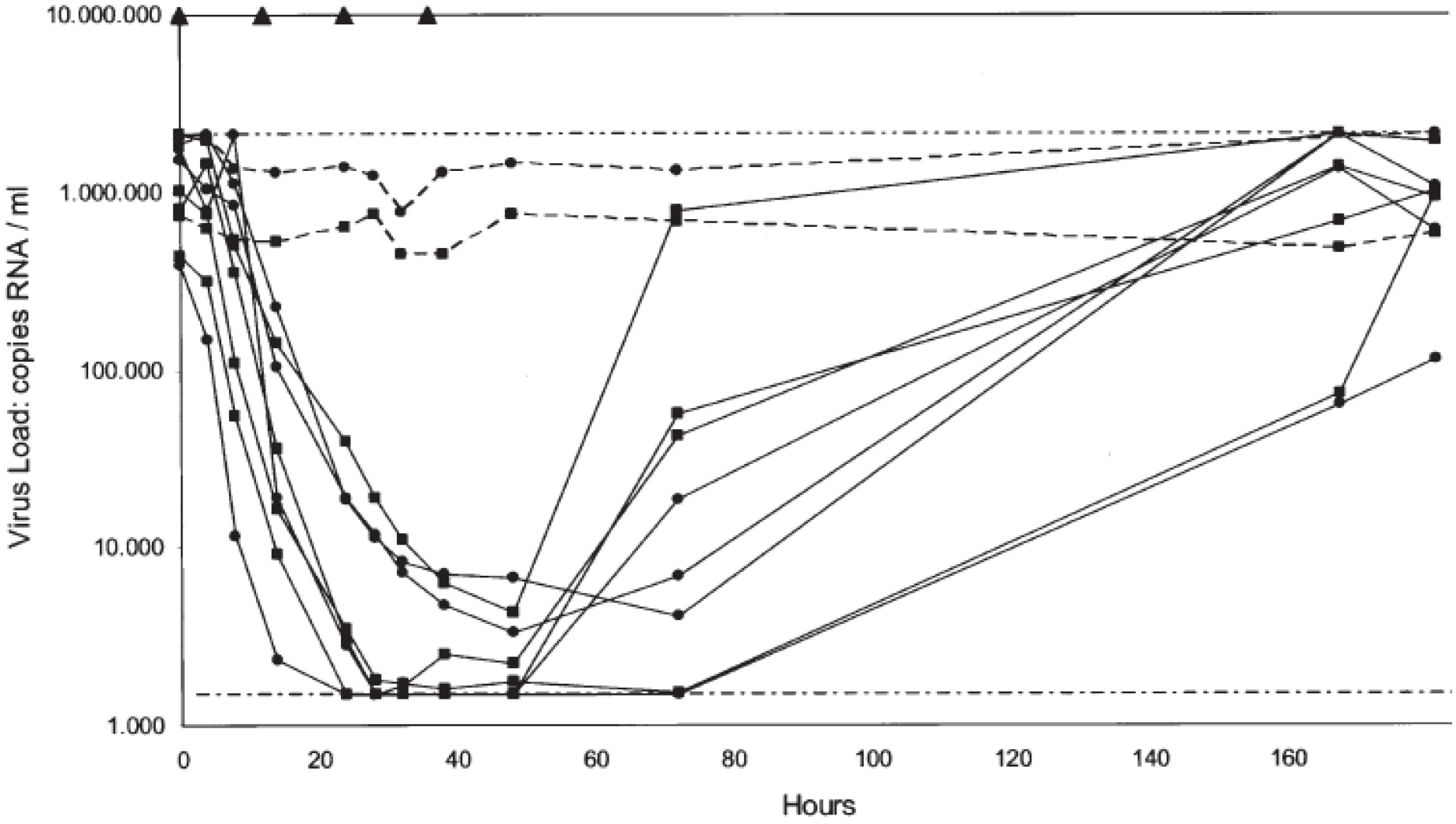
Viral Kinetics of HCV Genotype 1

	Drug Efficacy	Viral Clearance Constant (1/d)	Half-life of Virions (Hours)	Production & Clearance Rates (10^{12} Virions/d)
5MU	81 ± 4%	6.2 ± 0.8	2.7	0.4 ± 0.2
10MU	95 ± 4%	6.3 ± 2.4	2.6	2.3 ± 4
15MU	96 ± 4%	6.1 ± 1.9	2.7	0.6 ± 0.8

$t_{1/2}$ estimates independently validated for 2 HIV/HCV co-infected patients (Ramratnam et al. Lancet 1999)

The first 2 day clinical trial (BILN 2061)

Hinrichsen et al. Gastro. 127: 1347 (2004)



Longer Times: Second Phase

- Cells with reduced HCV RNA production are ultimately lost, either through death or further cessation of viral production.
- From the “second phase” decay slope we can estimate the rate of infected cell loss, δ (more precisely $\varepsilon\delta$)
- SVR (or cure) probably corresponds to loss of all infected cells.

Standard Model of HCV Dynamics

Equations

$$\frac{dT}{dt} = \lambda - dT - \beta VT$$

$$\frac{dI}{dt} = \beta VT - \delta I$$

$$\frac{dV}{dt} = (1-\varepsilon)pI - cV$$

Parameters

- λ Supply of target cells
- d Net loss rate of target cells
- β Infectivity rate constant
- δ Infected cell death rate
- ε Drug efficacy
- p Virion production rate
- c Virion clearance rate constant

Variables

- T Target Cell Density
- I Infected Cell Density
- V Virus Concentration

Initial Conditions

$T(0) = T_0$ $\quad V(0) = V_0$

$I(0) = I_0$

Solution: Change in Viral Load

- Assuming $T = T_0$ =constant,

$$V(t) = \frac{1}{2} V_0 [(1 - \frac{c + \delta - 2\varepsilon c}{\theta}) e^{-\lambda_1 (t - t_0)} + (1 + \frac{c + \delta - 2\varepsilon c}{\theta}) e^{-\lambda_2 (t - t_0)}]$$

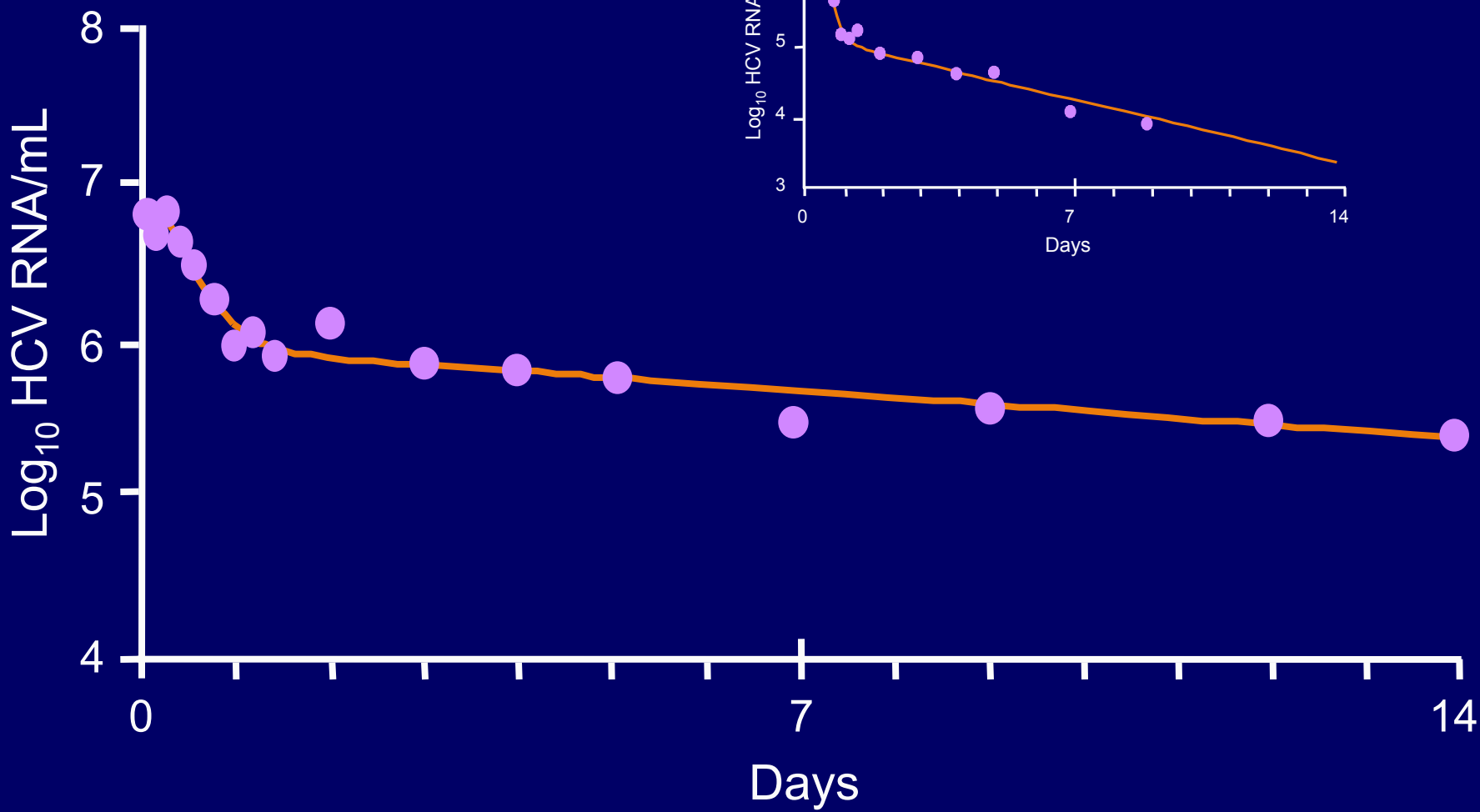
where

$$\lambda_1 = \frac{1}{2}(c + \delta + \theta) \qquad \lambda_2 = \frac{1}{2}(c + \delta - \theta) \qquad \theta = \sqrt{(c - \delta)^2 + 4(1 - \varepsilon)c\delta}$$

t_0 = delay between treatment commencement and onset of effect

- When c>>δ, $\lambda_1 \approx c$ and $\lambda_2 \approx \varepsilon\delta$

10MU



15MU

Neumann Perelson Science 1998

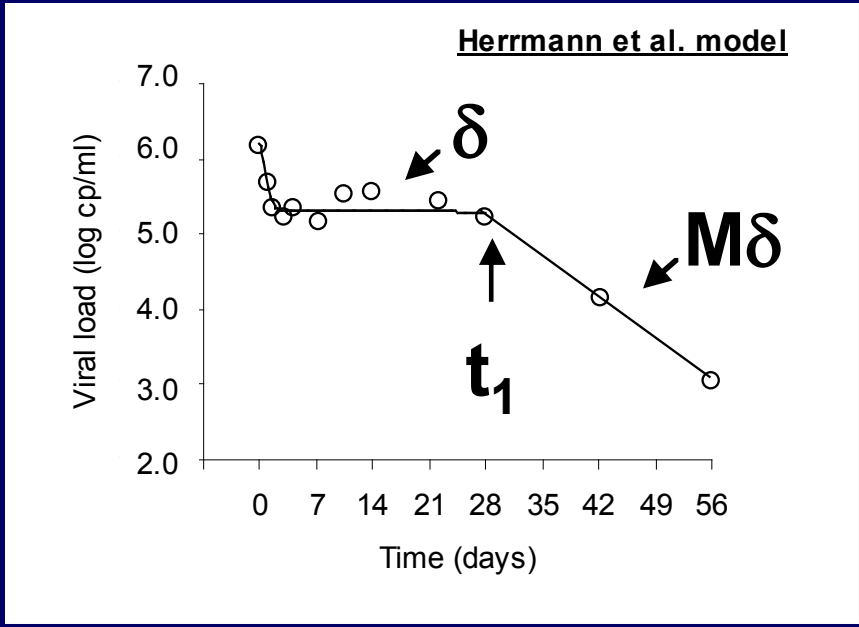
Viral Kinetics of HCV Genotype 1

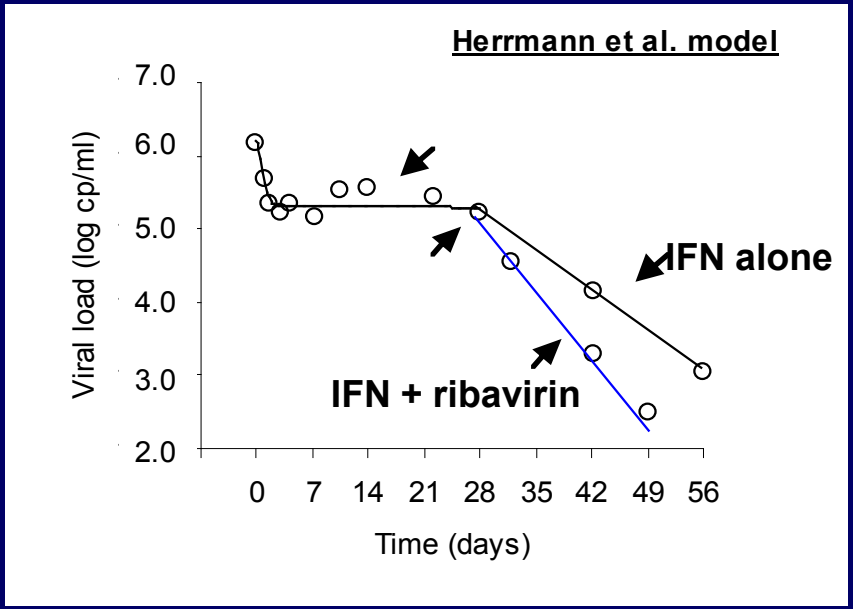
	Drug Efficacy	Second Phase Decay Constant, δ (1/d)	Half-life of Infected Cells (Days)
5MU	81 ± 4%	0.09 ± 0.14	2.2–69.3
10MU	95 ± 4%	0.10 ± 0.05	4.3–17.3
15MU	96 ± 4%	0.24 ± 0.15	1.7–6.3

Everything looked neat and theory seemed to fit all available data

- However, unlike HIV there were no cell culture systems and confirming predicted parameter estimates was difficult.
- Discrepancies with theory started arising.

Triphasic Decay

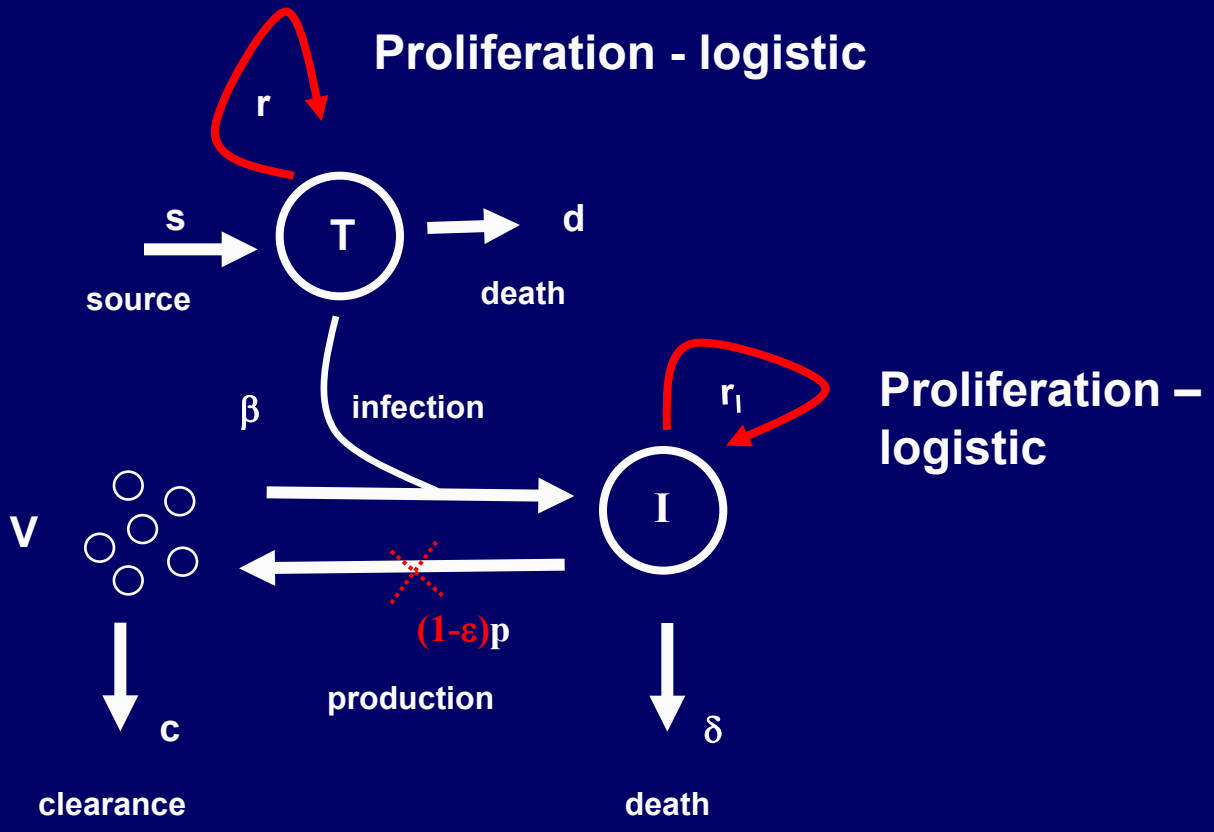




Some patients seem to have a flat second phase followed by a 3rd phase of decay. For these patients the estimate of $\delta \sim 0$ may be incorrect

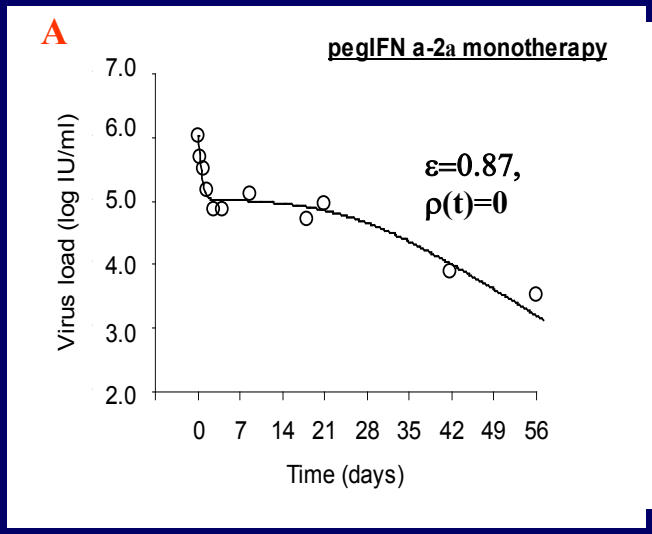
Herrmann et al. Hepatol 2003

Extended Model: Proliferation

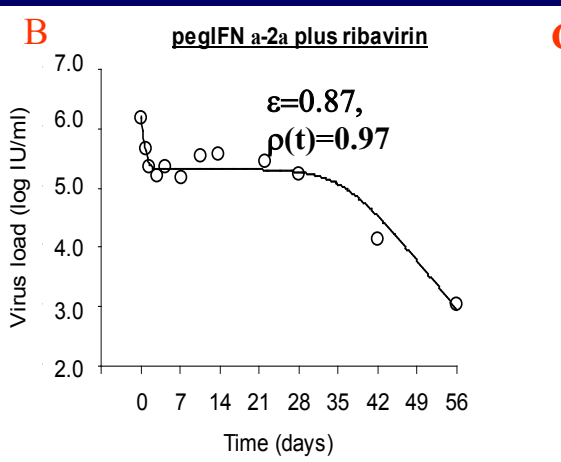


Dahari et al., Hepatology 2007; JTB 2007
Reluga et al SIAM J Appl Math 2009

Extended model: Fits to data



Herrmann et al.
(Hepatology 2003)



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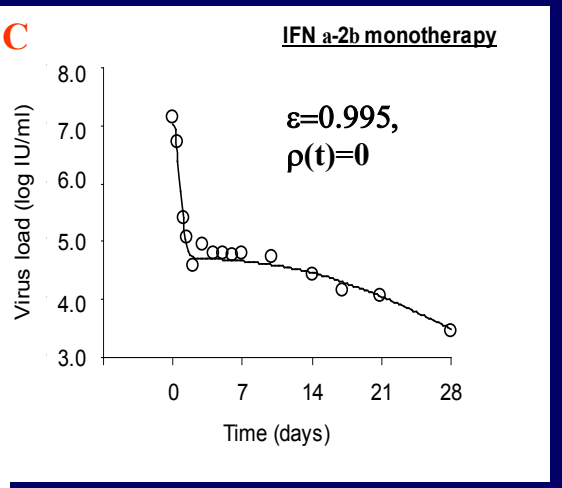


Bekkering et al.
(BMC Gastro 2001)

We fit the extended model to data from patients treated with pegylated interferon α-2a alone (A) or in combination with ribavirin (B), and with daily therapy with interferon α-2b alone (C). ρ = RBV efficacy

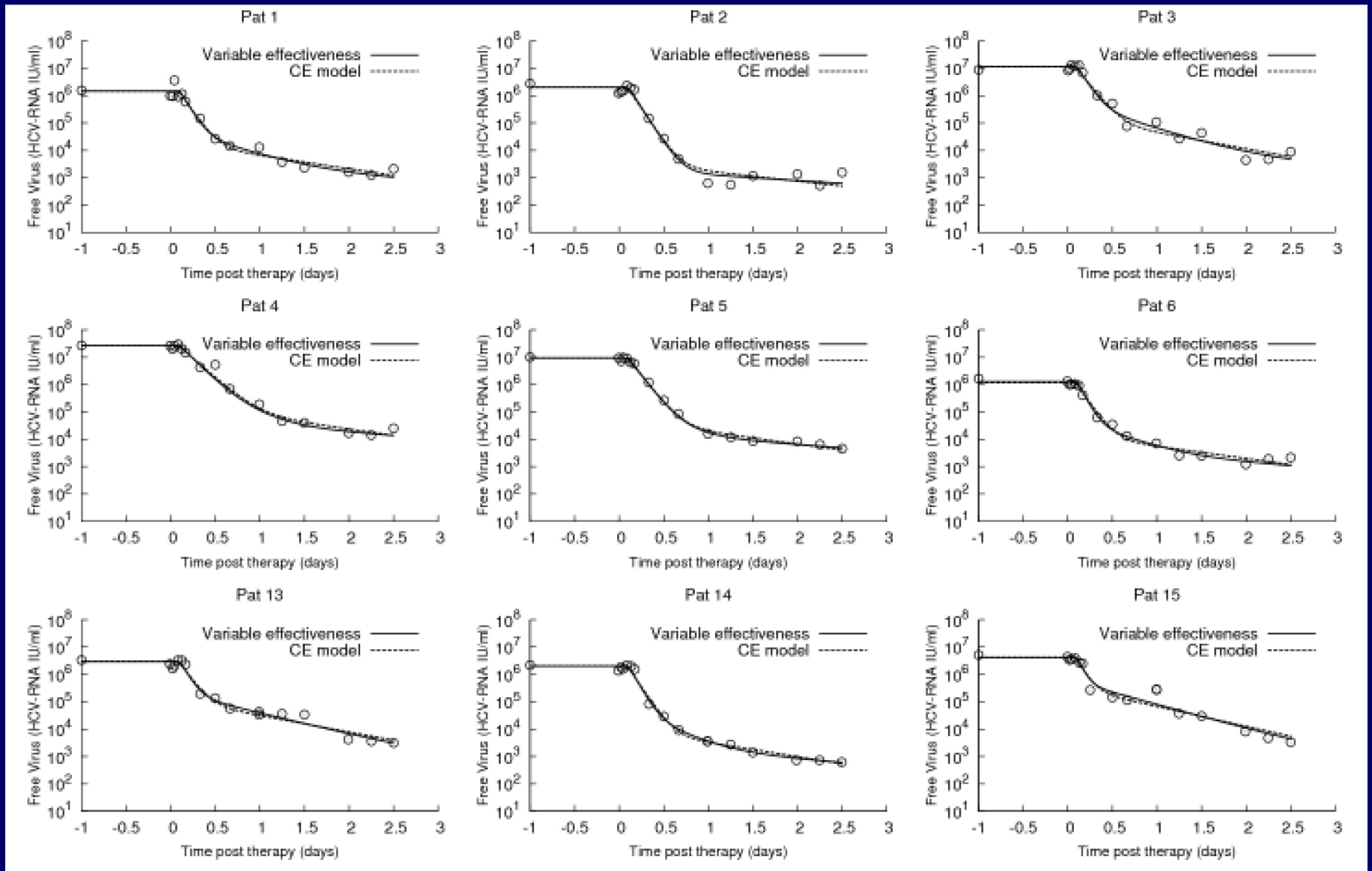
The flat second phase is due to proliferation of infected cells and de novo infection roughly matching their rate of loss.

Problem: no independent measures of in vivo proliferation rate

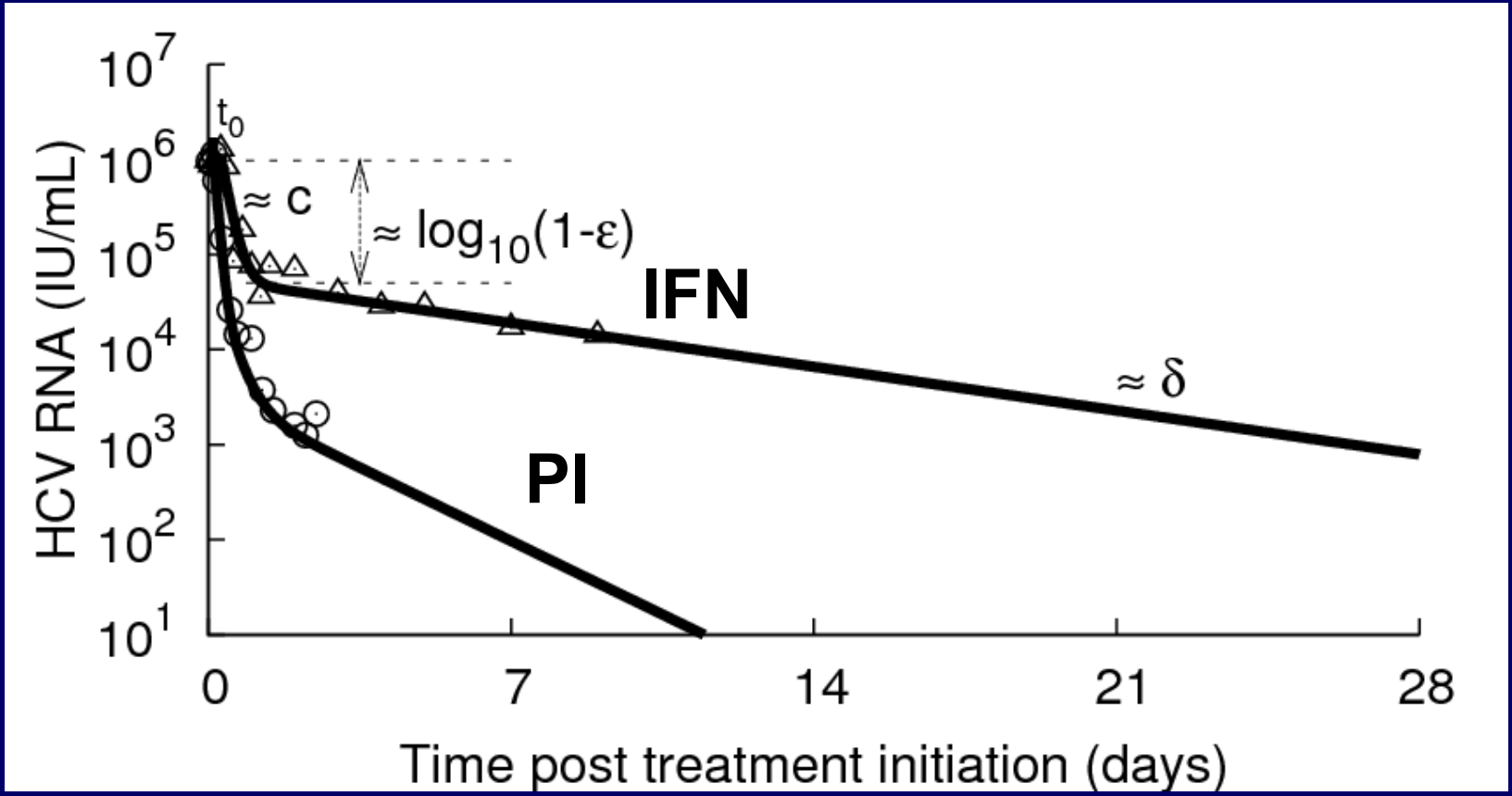
New Therapies

- Use direct acting antivirals – protease inhibitors, polymerase inhibitors, entry inhibitors,…
- Very potent compared to IFN
- Fewer side effects

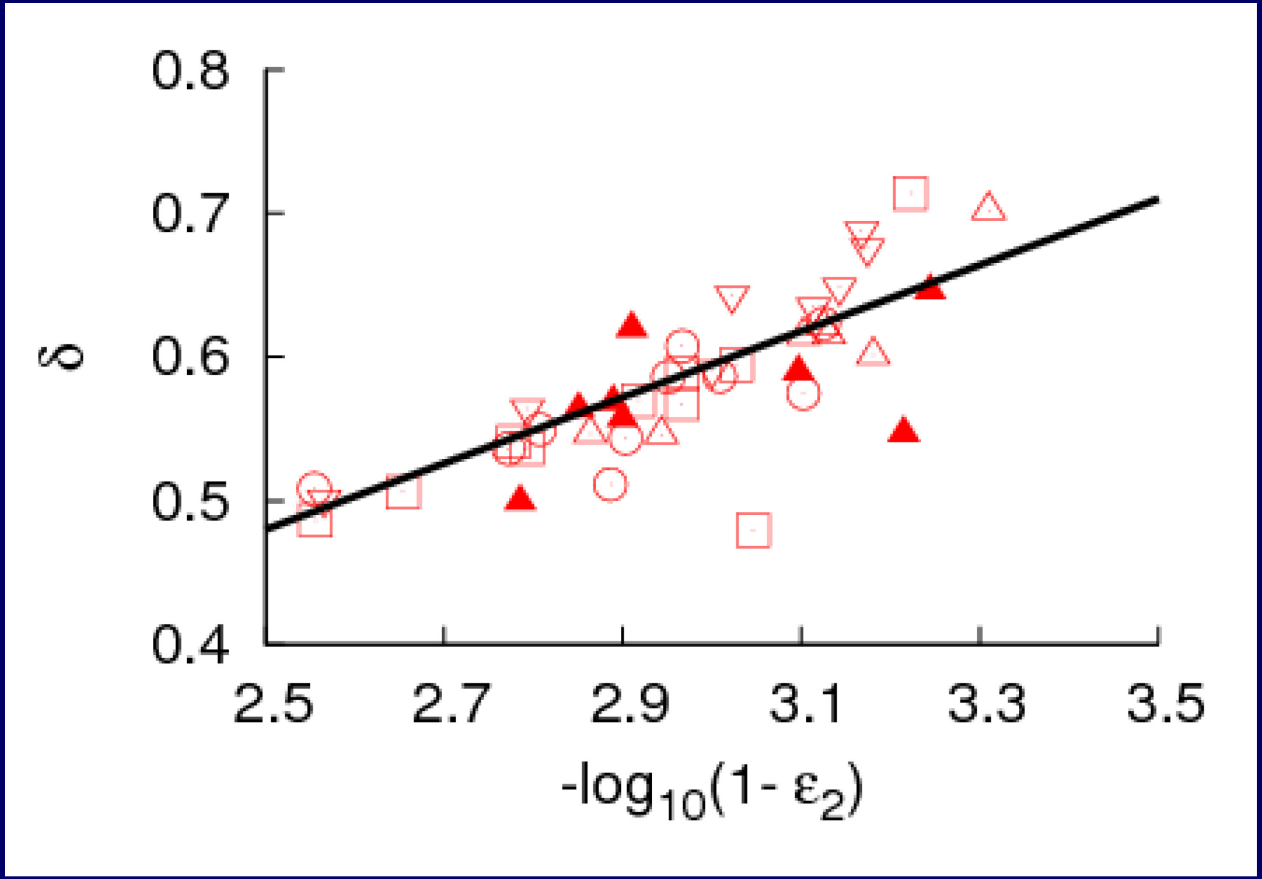
Model fits to data (n=44) from telaprevir clinical trial



IFN vs HCV protease inhibitor (telaprevir)

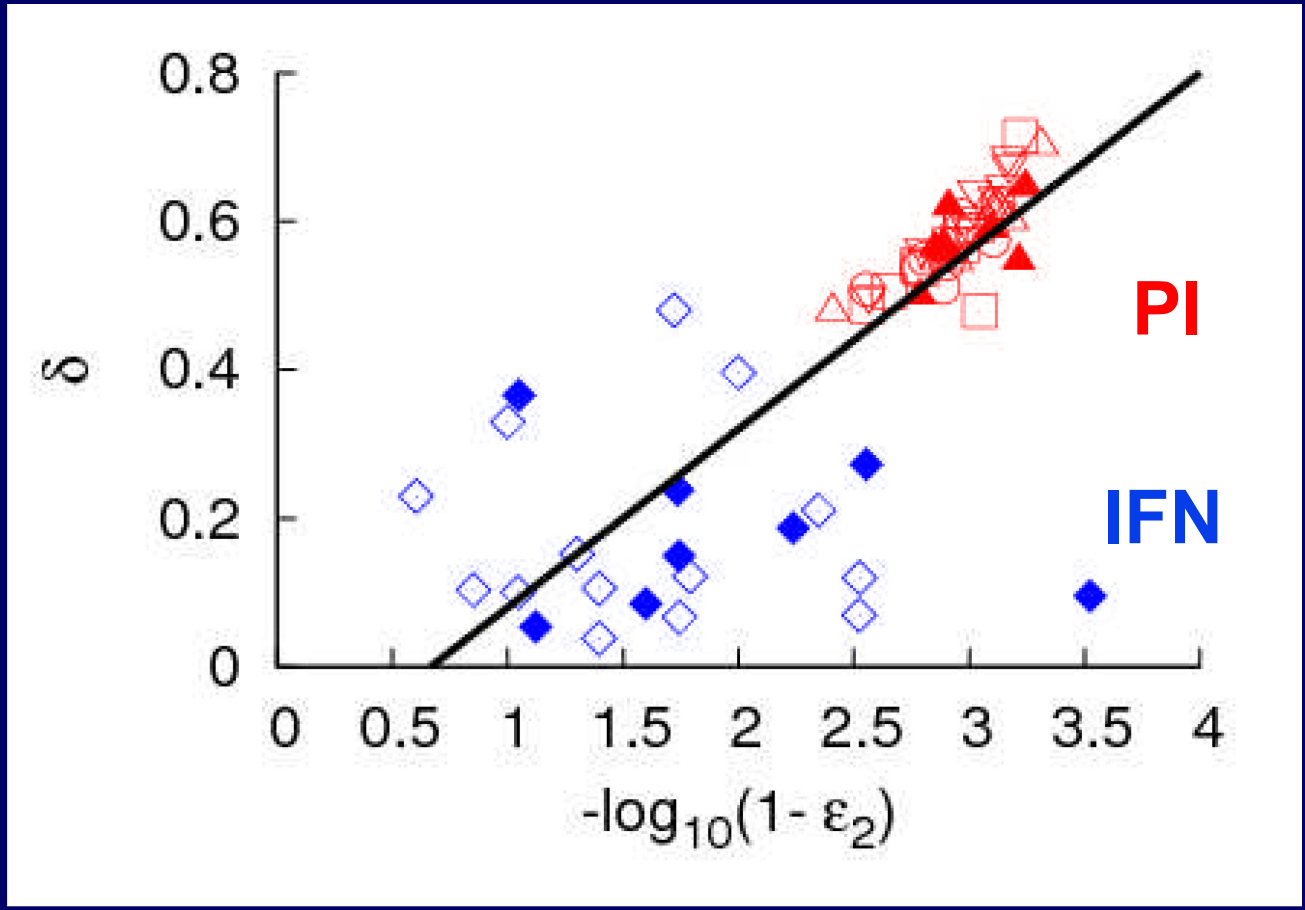


δ correlated with ε

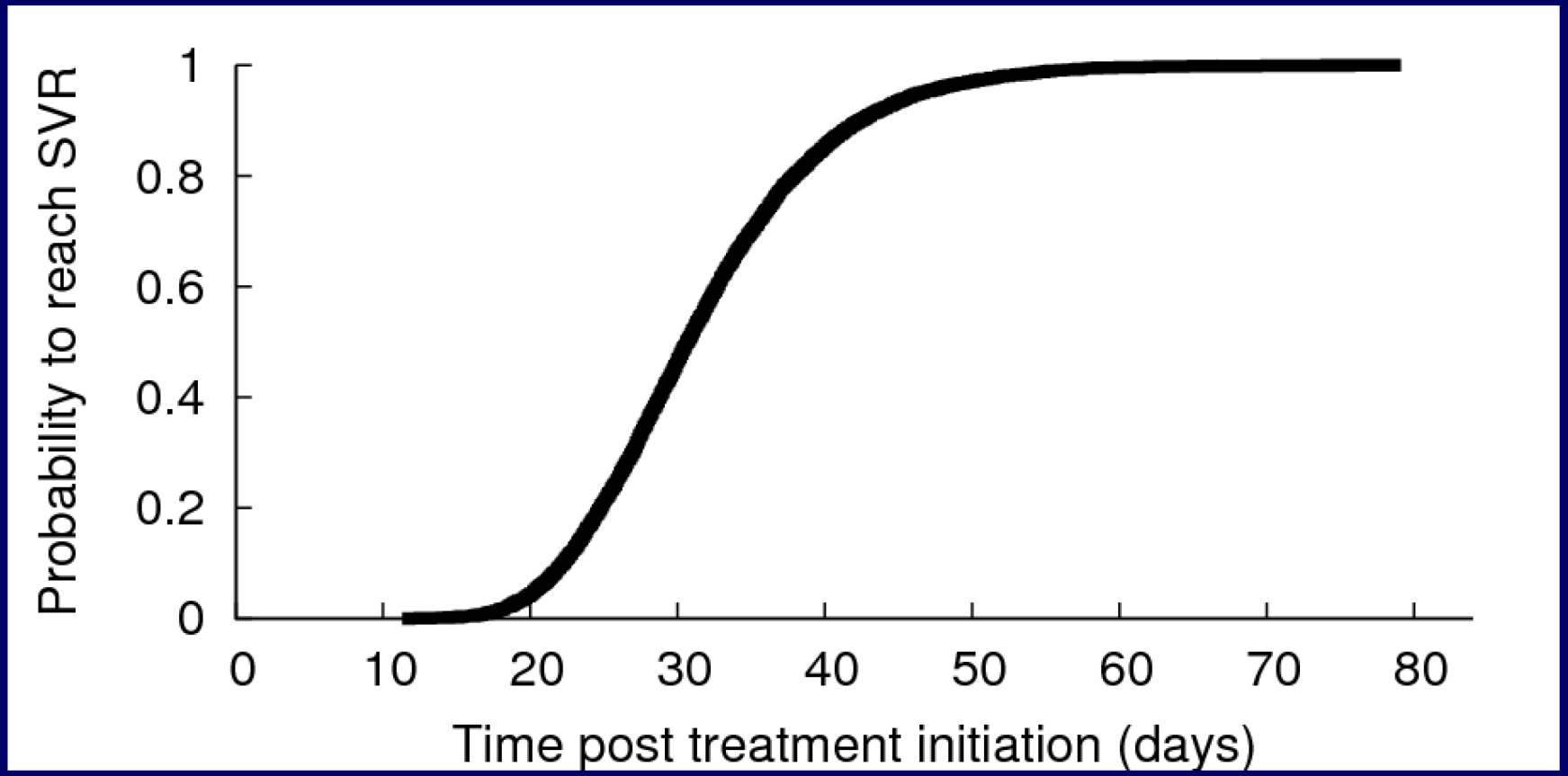


r=0.79, p< 0.001

Correlation extends to IFN



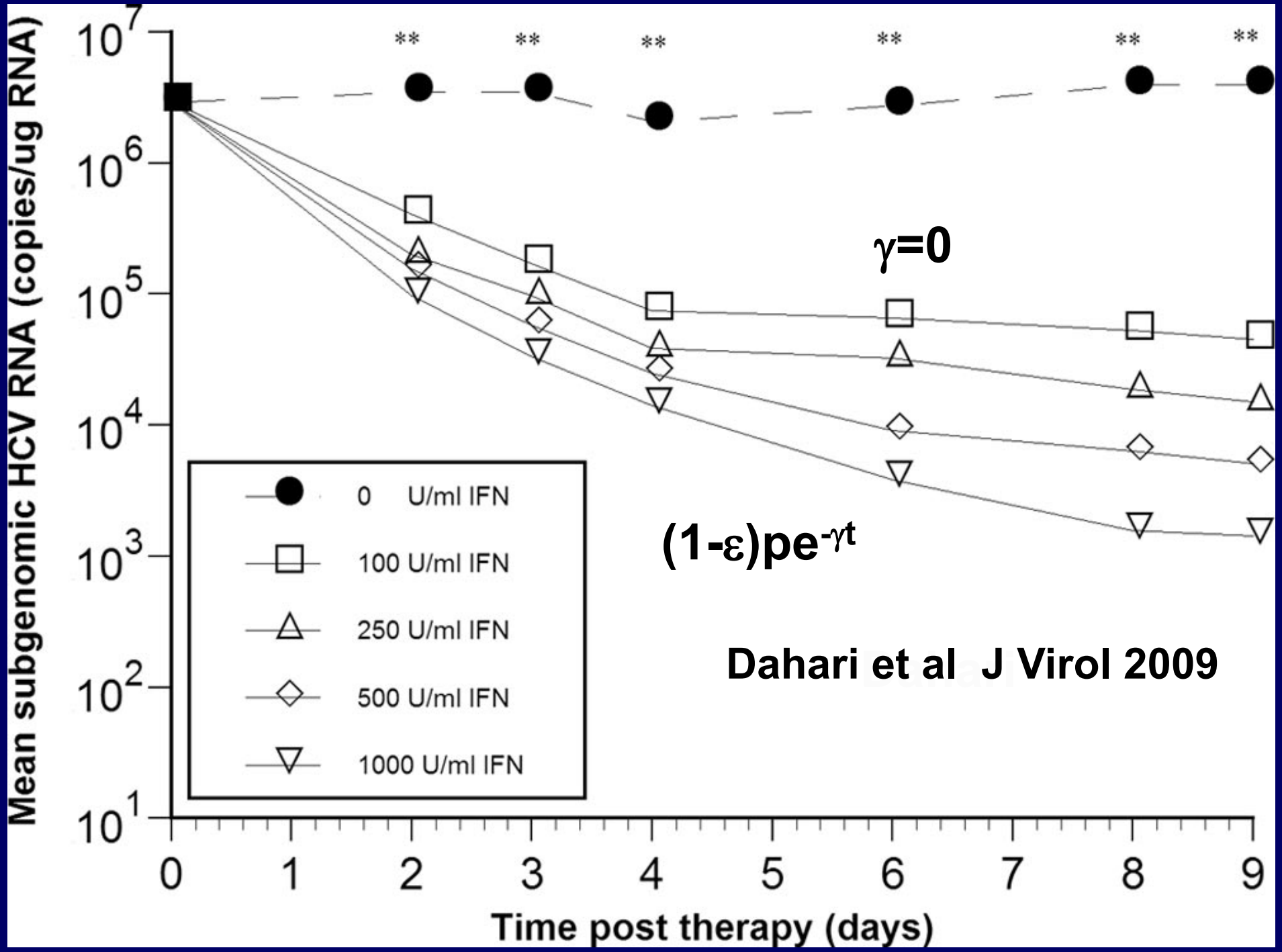
r=0.78, p< 0.001



Should be able to cure 98% of people in 60 days if no drug resistance and all drug taken

Why does δ increase with ε?

- Maybe infected cells can be "cured" and the rate or prob of cure depends on ε. Cure has been shown to occur in HBV infection.
- Drug may not only quickly reduce viral production, p -> $(1-\varepsilon)p(t)$, but residual production may also decrease with time on therapy say at rate γ. Net rate of viral decline then $\delta+\gamma$ (Guedj and Neumann, 2010)

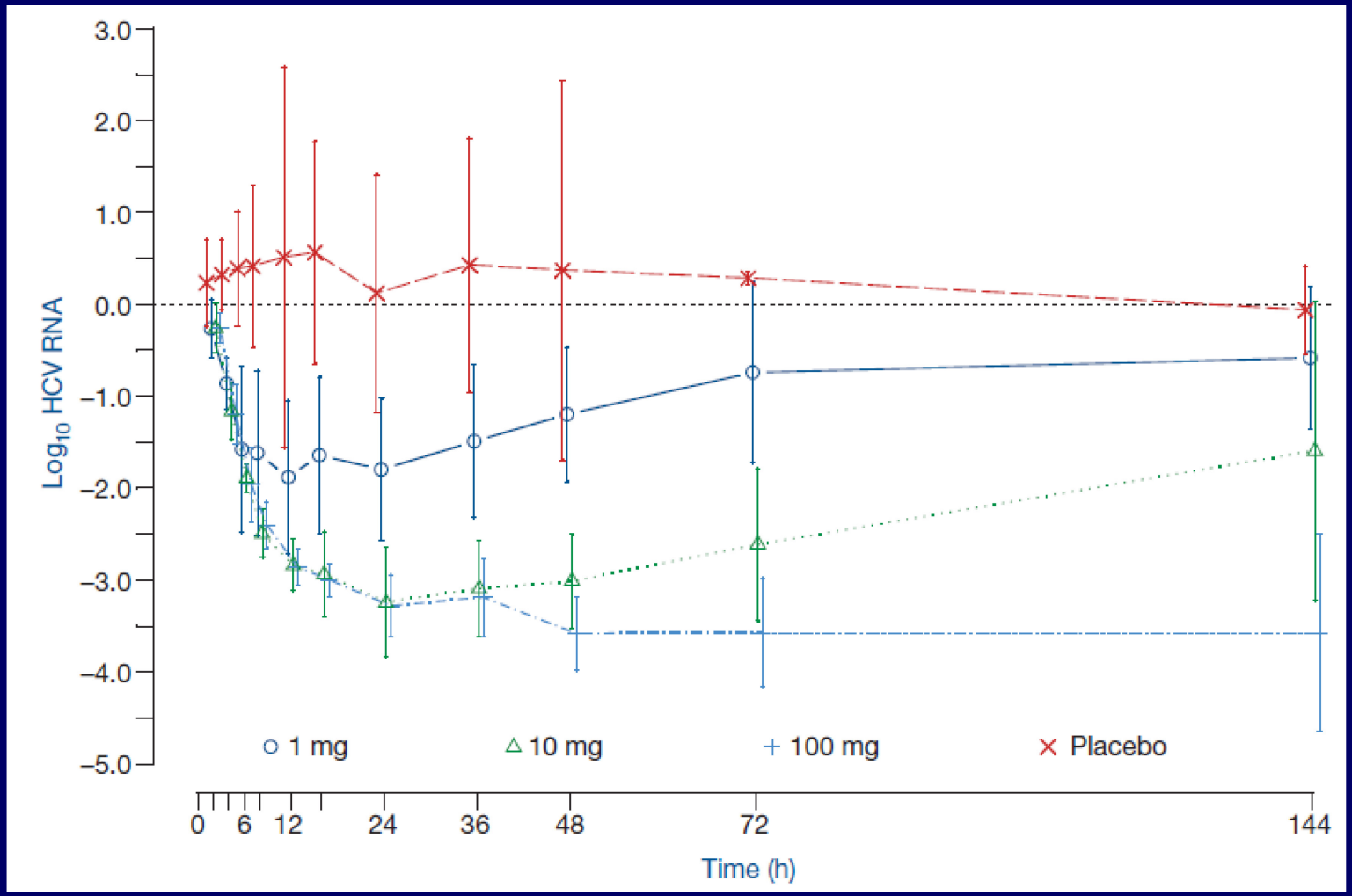

Mean subgenomic HCV RNA (copies/ug RNA)
10^7
10^6
10^5
10^4
10^3
10^2
10^1
**
**
**
**
**
**
γ=0
$(1-\varepsilon)pe^{-\gamma t}$
0 U/ml IFN
100 U/ml IFN
250 U/ml IFN
500 U/ml IFN
1000 U/ml IFN
Dahari et al J Virol 2009
0
1
2
3
4
5
6
7
8
9
Time post therapy (days)

Estimated rate of virus clearance, c, may change with drug

- IFN-therapy (n=31) $c \sim 8\ d^{-1}$, $t_{1/2} = 2.7$ hr
- Telaprevir (n=36) $c \sim 12\ d^{-1}$, $t_{1/2} = 1.4$ hr
- BMS-790052 (n=9) $c \sim 23\ d^{-1}$, $t_{1/2} = 0.7$ hr

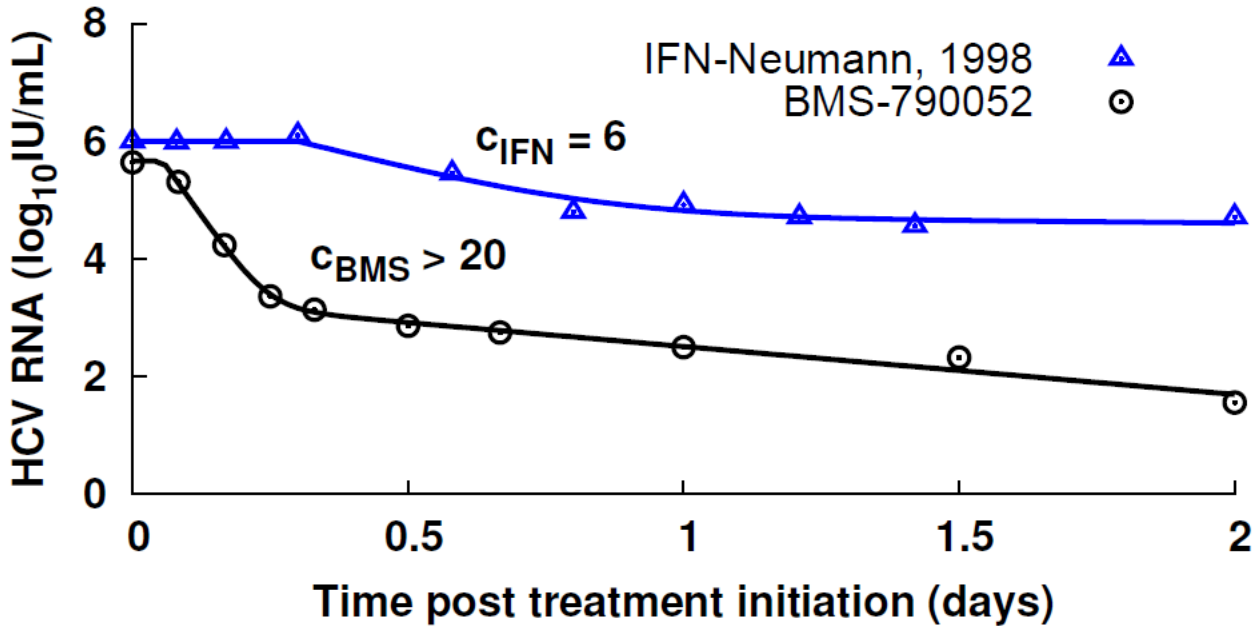
Standard models can not account for this.

NS5A inhibitor; c>20 d^{-1}



Gao et al, Nature May 2010

BMS-790052 vs IFN 1st phase kinetics



- The initial rate of viral decline is much more rapid
- Tends to be biphasic in most patients

Age-structured Multiscale Model

$$\frac{dT}{dt} = s - dT - \beta VT$$

$$\frac{\partial I}{\partial t} + \frac{\partial I}{\partial a}\frac{da}{dt} = -\delta(a)I(a,t)$$ a=age of infection

$$I(0,t) = \beta VT, \quad I(a,0) = I_0(a)$$

$$\frac{\partial R}{\partial t} + \frac{\partial R}{\partial a}\frac{da}{dt} = \alpha - (\rho + \mu)R$$ viral RNA

$$R(0,t) = 1, \quad R(a,0) = R_0(a)$$

$$\frac{dV}{dt} = \rho\int_0^\infty R(a,t)I(a,t)d\,a - cV$$

Effects of Treatment

$$\frac{dT}{dt} = s - dT - \beta VT$$

$$\frac{\partial I}{\partial t} + \frac{\partial I}{\partial a}\frac{da}{dt} = -\delta(a)I(a,t)$$

$$I(0,t) = \beta VT, \quad I(a,0) = I_0(a)$$

$$\frac{\partial R}{\partial t} + \frac{\partial R}{\partial a}\frac{da}{dt} = (1-\varepsilon_p)\alpha - ((1-\varepsilon_s)\rho + \kappa\mu)R$$

$$R(0,t) = 1, \quad R(a,0) = R_0(a)$$

$$\frac{dV}{dt} = (1-\varepsilon_s)\rho\int_0^\infty R(a,t)I(a,t)da - cV$$

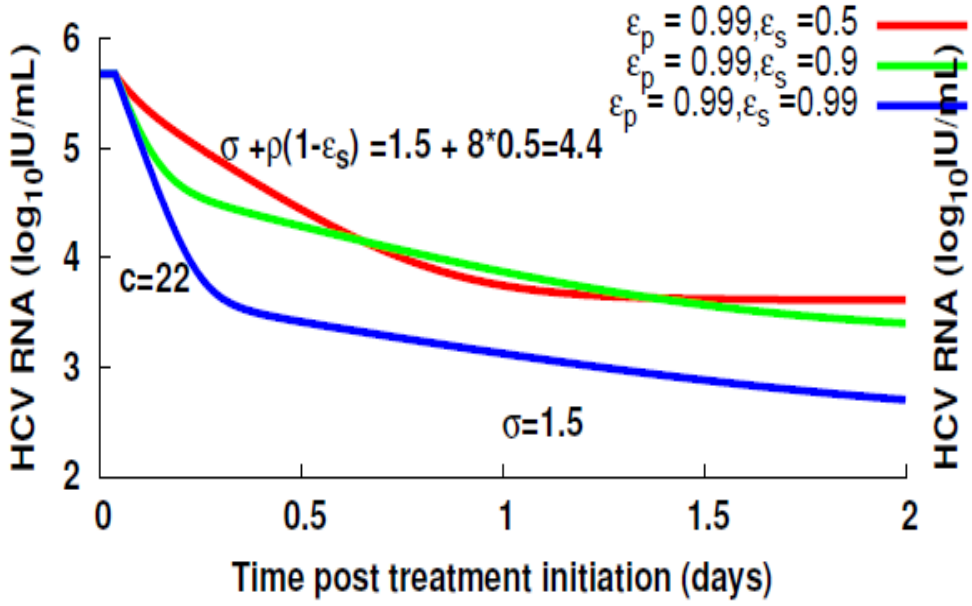
steady state before therapy with stable age distribution for I and R
Drug effects, ε and κ, should be functions of drug concentration

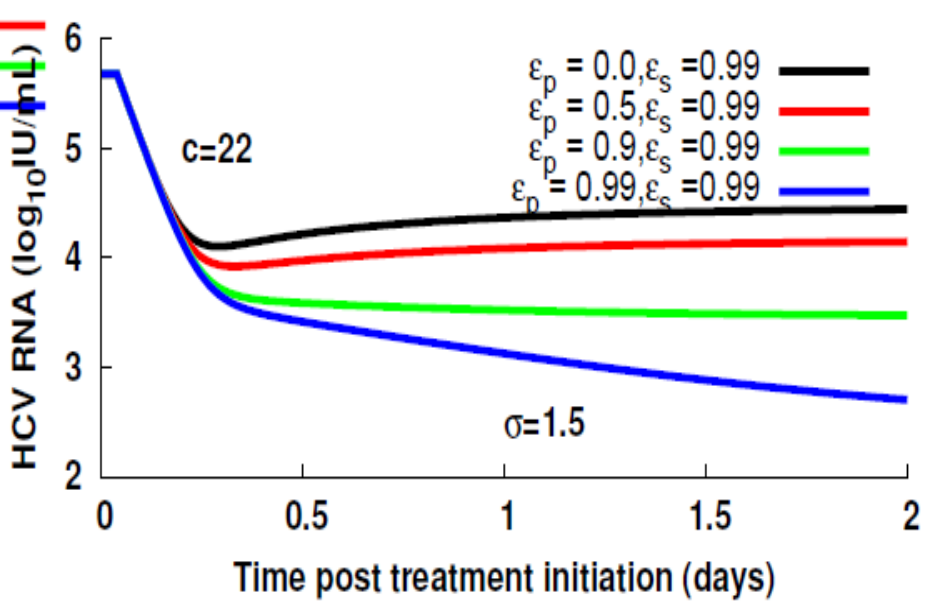
Model can be solved-
this is short-term approx

$$V(t) = V_0 e^{-ct} +$$

$$(1-\varepsilon_s)\rho[A + Be^{-ct} + Ce^{-((1-\varepsilon_s)\rho+\kappa\mu+\delta)t}]$$

A, B, C **are constants defined in terms of the intracellular and other parameters, eg** $(1-\varepsilon_p)$

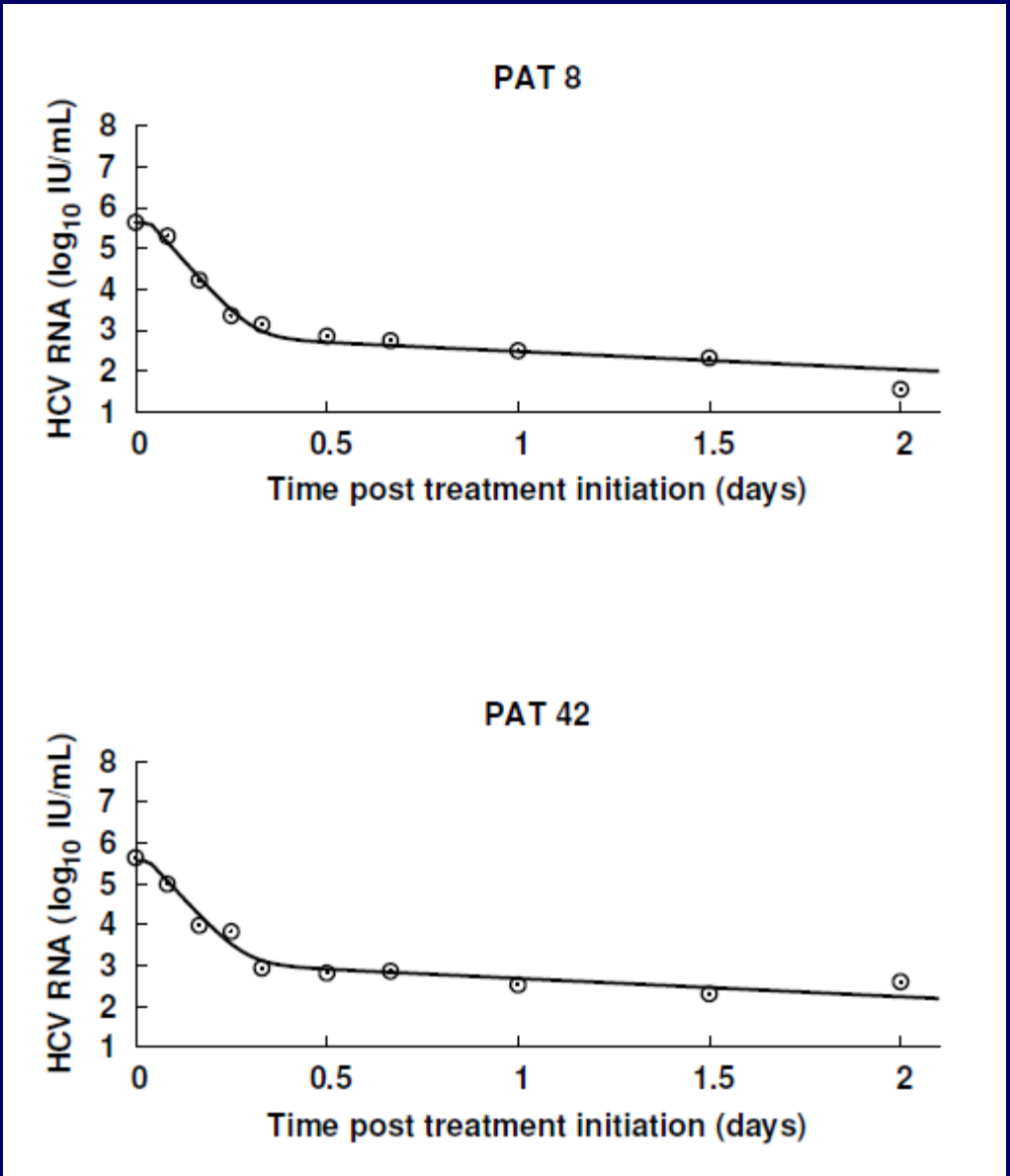




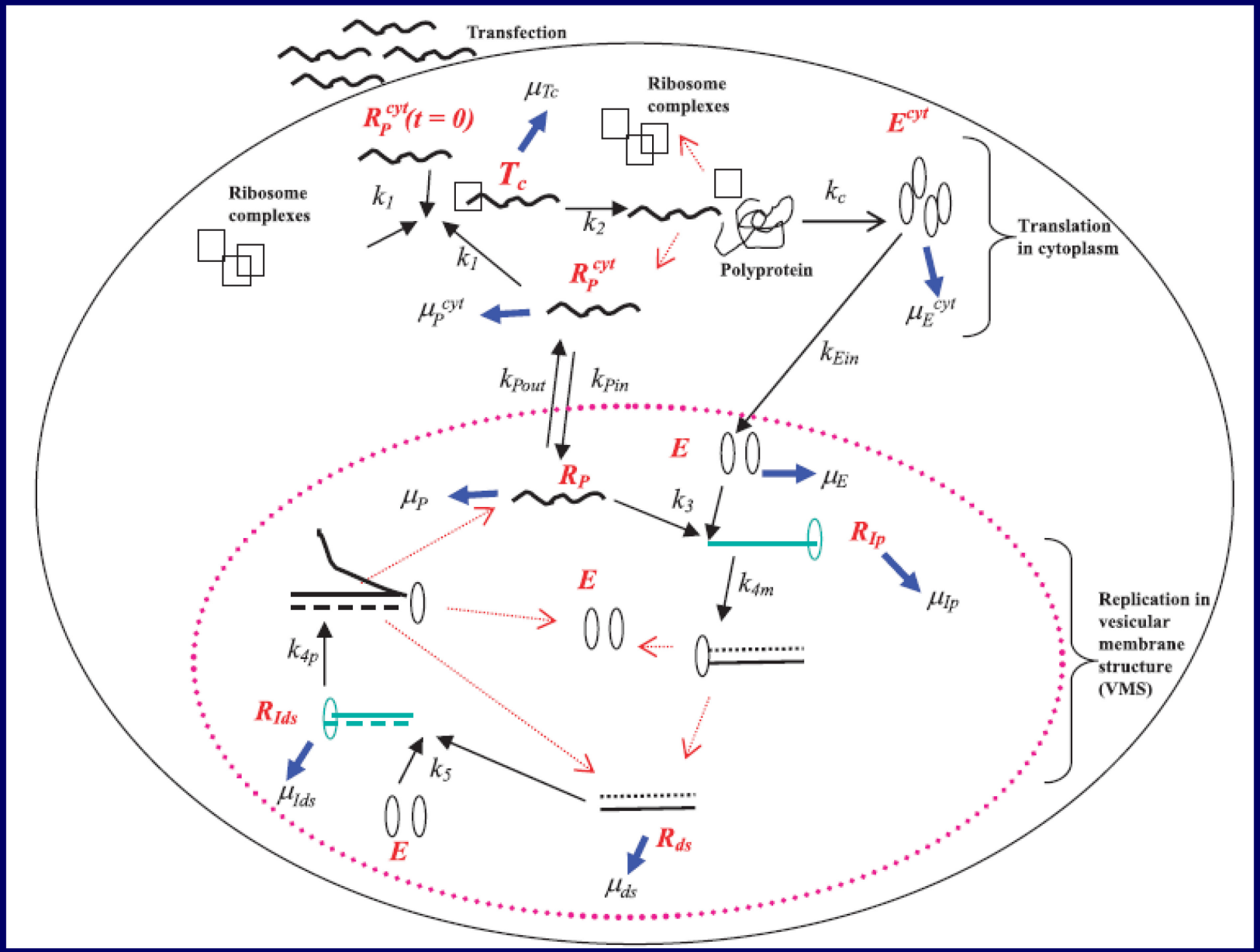
Inhibit vRNA replication **Inhibit virion secretion**

IFN mainly inhibits vRNA replication
BMS inhibits both secretion and vRNA replication

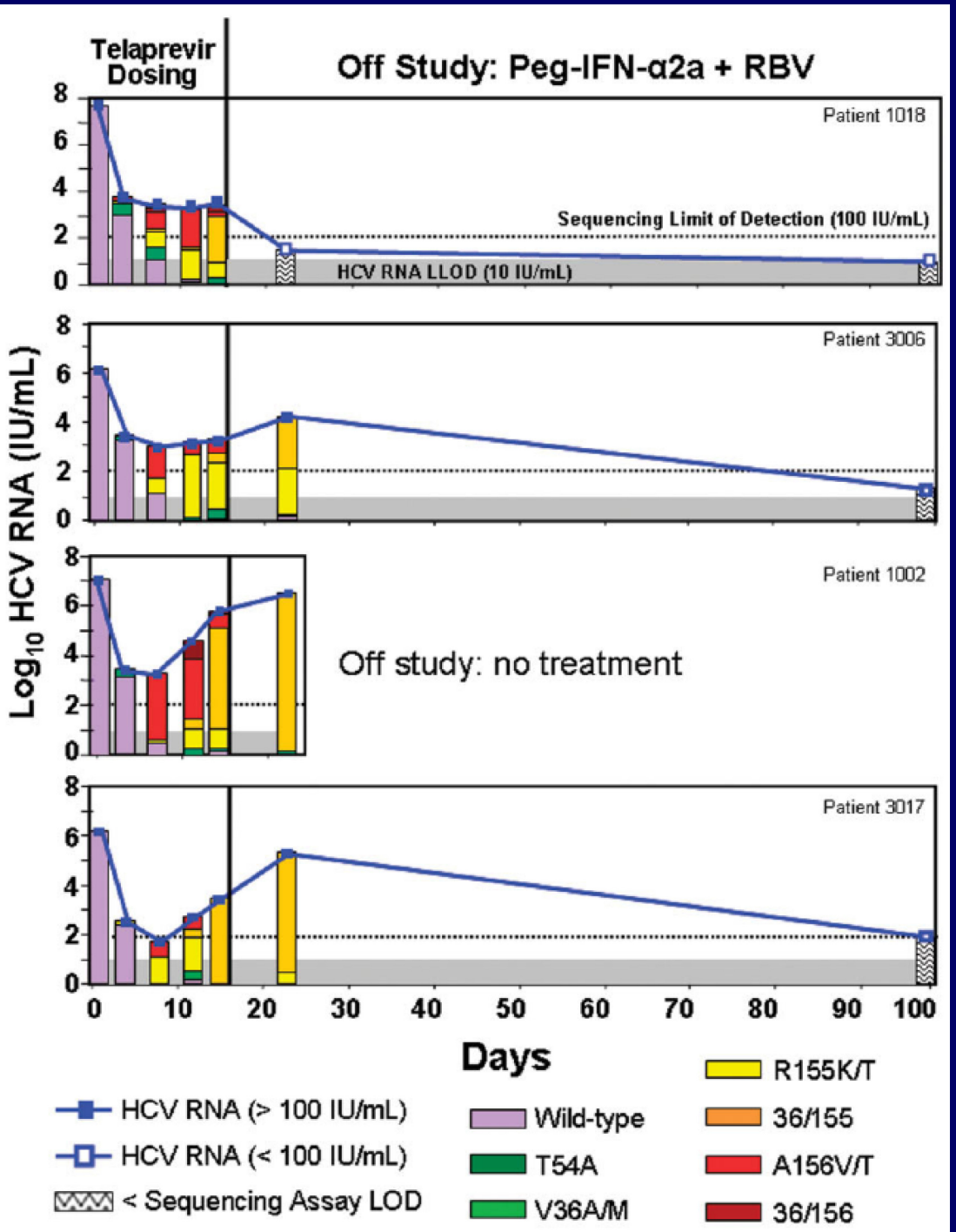
Fit of model to data



Model of HCV RNA replication



New antivirals lead to drug resistance



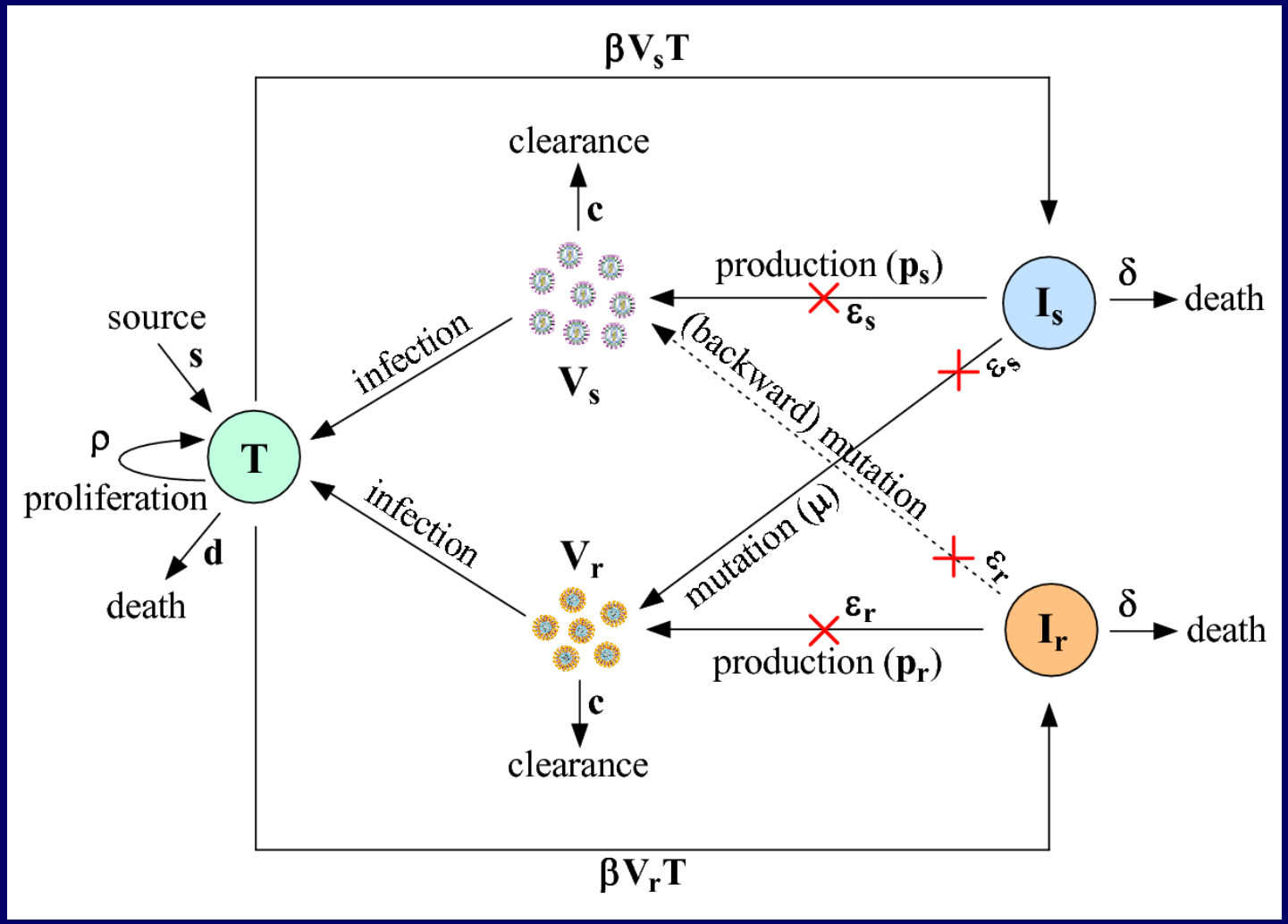
By day 2, 5-20% of virus is drug resistant

Kieffer et al. Hepatol 2007 genotype 1a pts

Baseline generation of mutants/day (using mutation rate of 10^{-5} per base copied)

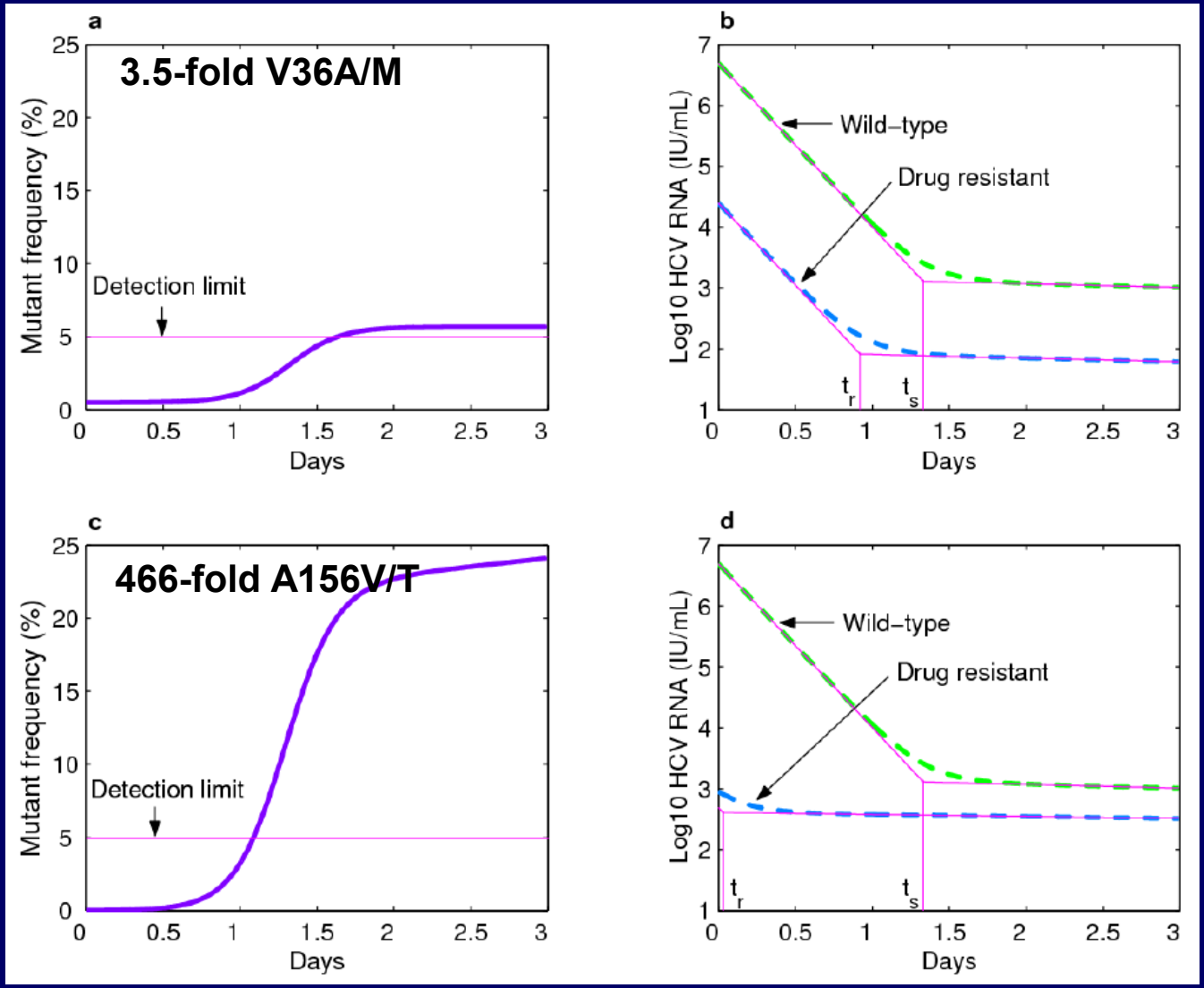
Base changes	Expected number (10^{12}/day) HCV RNA	# possible variants	% produced/ day
1 (9%)	9×10^{10}	3×10^{4}	100%
2 (0.45%)	4.5×10^{9}	4.5×10^{8}	100%
3 (0.015%)	1.5×10^{10}	4.5×10^{12}	.003%

Two-strain model

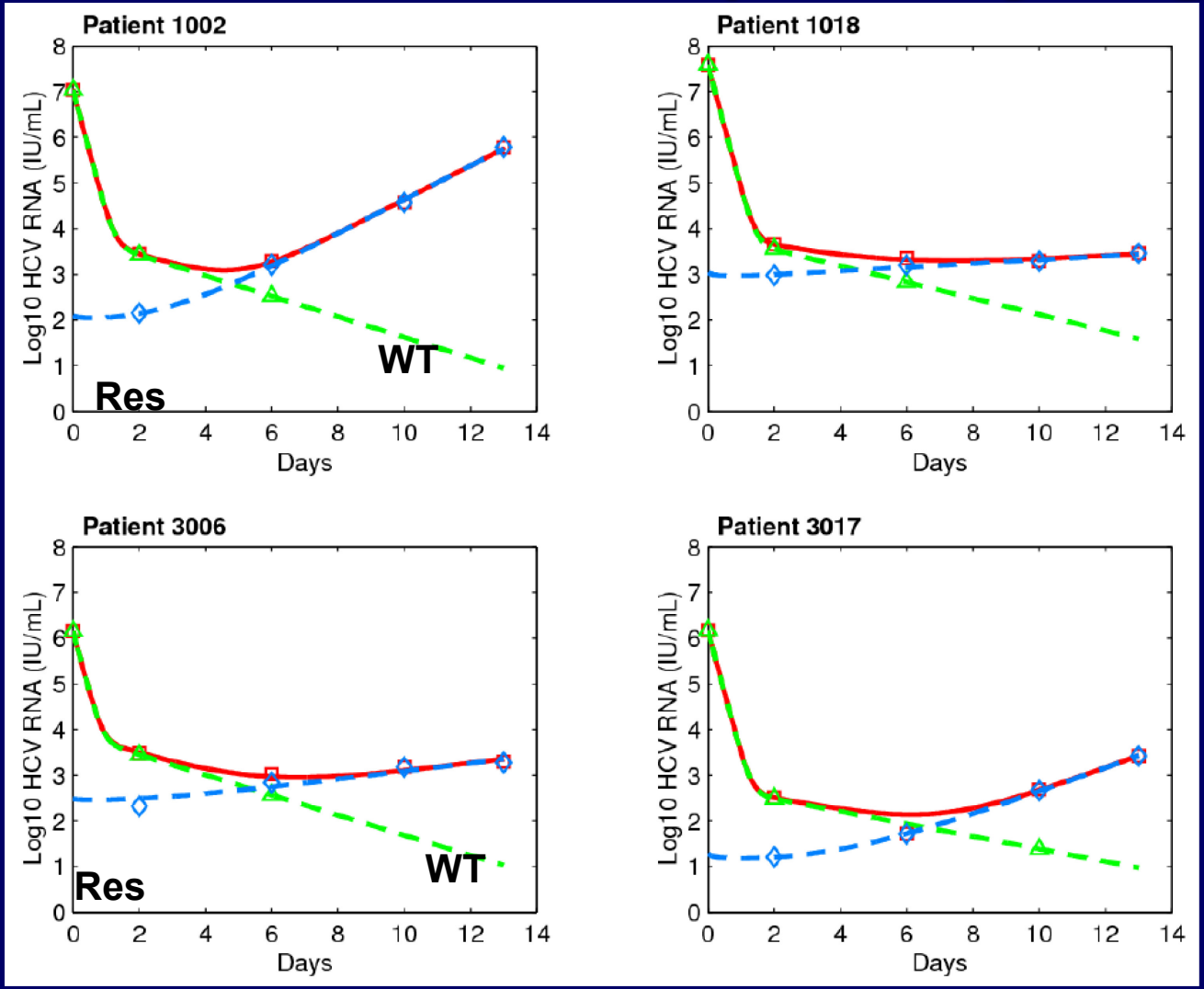


V_s =drug sensitive, V_r= drug resistant

Mutant frequency (T=const) (3 log drug)



Viral rebound (T varying)



Rong et al., Science Trans Med 2010

To get growth of resistant virus need “replication space:

- Could be loss of infected cells and replacement by new target cells by proliferation
- Could be loss of interferon induced “antiviral state” and generation of new targets without proliferation
- Could be due to superinfection – resistant virus infects already infected cells and causes them to produce resistant virus
- Could be intracellular competition and takeover of infected cells by de novo arising resistant variant

Future

- Multiscale models
  - Incorporate intracellular replication of virus
  - Incorporate host factors – eg host defense such as interferon response
- Better incorporation of PK/PD, intracellular drug conc. and mode of action of drug (Systems biology)
- Spatial models
  - Infection is in a solid tissue and viral spread may be cell-to-cell or local
- Incorporate immune response
  - T cells enter liver and cause pathology

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