Modeling HCV Infection, Treatment and Resistance to Direct-Acting Antiviral Compounds for HCV

Alan S. Perelson, PhD

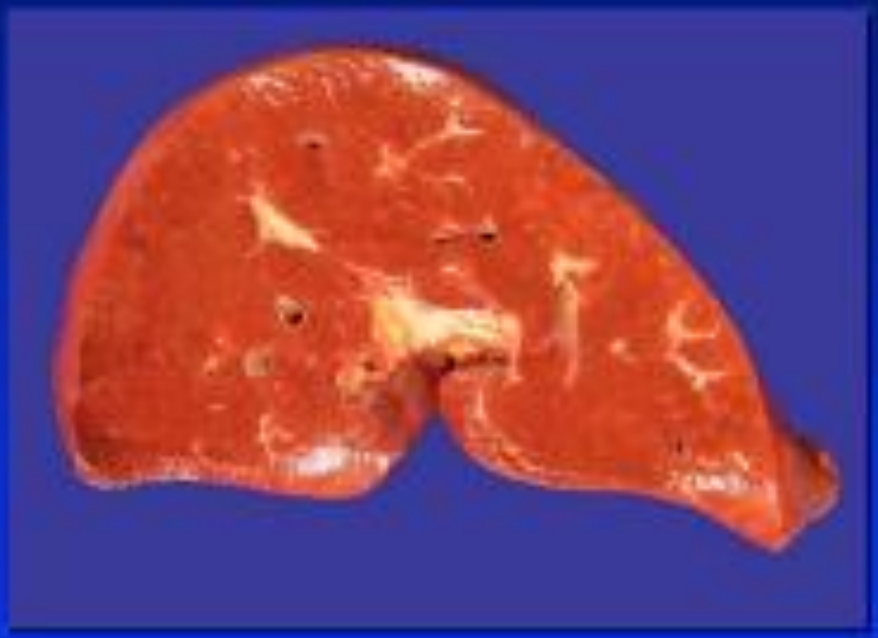
Theoretical Biology & Biophysics
Los Alamos National Laboratory
Los Alamos, NM

asp@lanl.gov

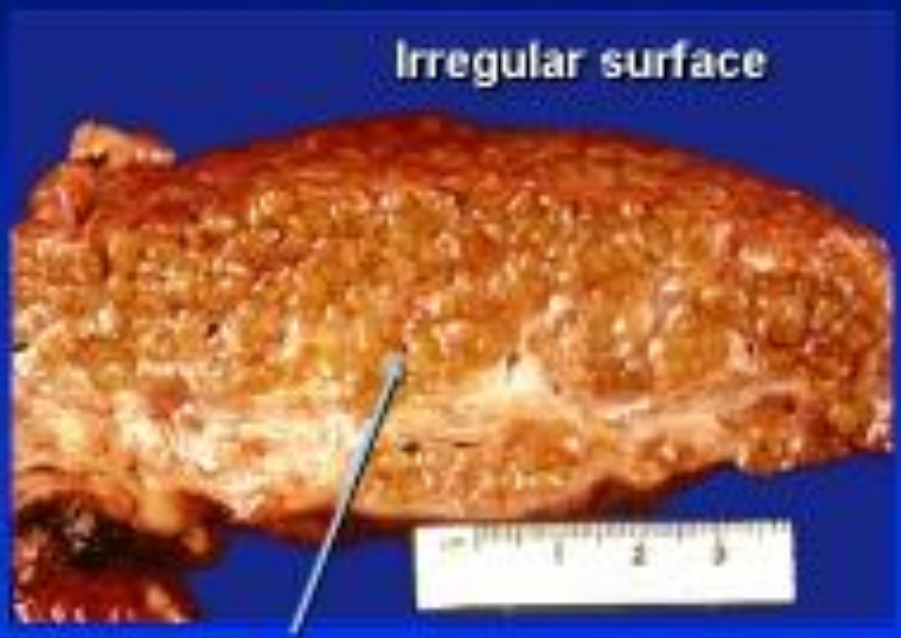
Hepatitis C Virus Infection

- HCV is a positive strand RNA virus that infects the liver; 9.6 kb; error prone RdRp
- It can lead to cirrhosis and liver cancer with a varying time course, from a few years (fulminant hepatitis) to > 30 years
- ~ 4 million infected in the US
- Can be treated but some people fail to respond to best available therapy.
- No vaccine available.

Normal

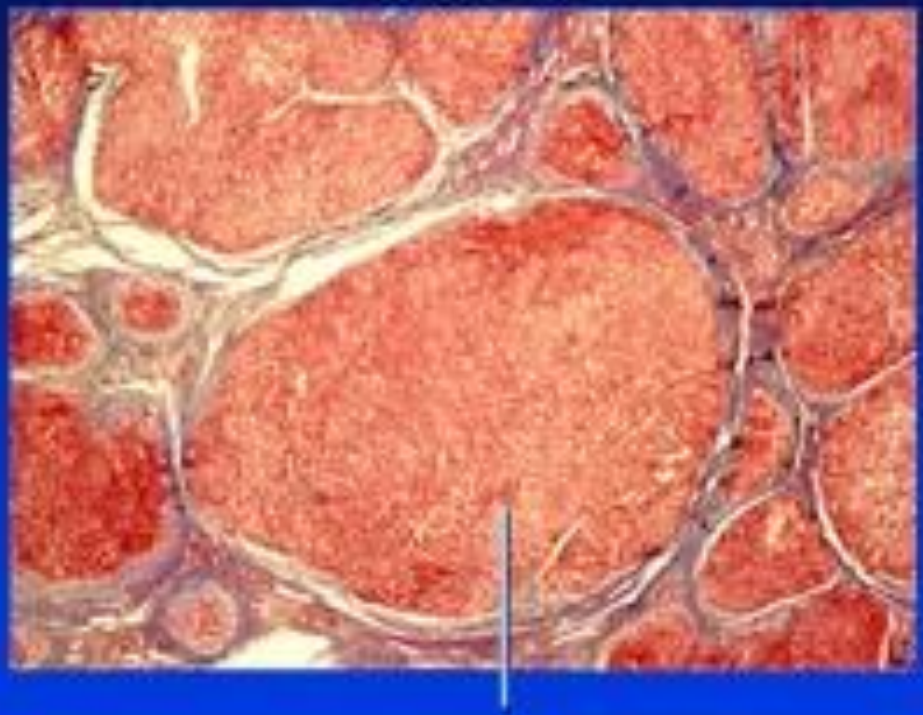


Cirrhosis



Normal

Cirrhosis



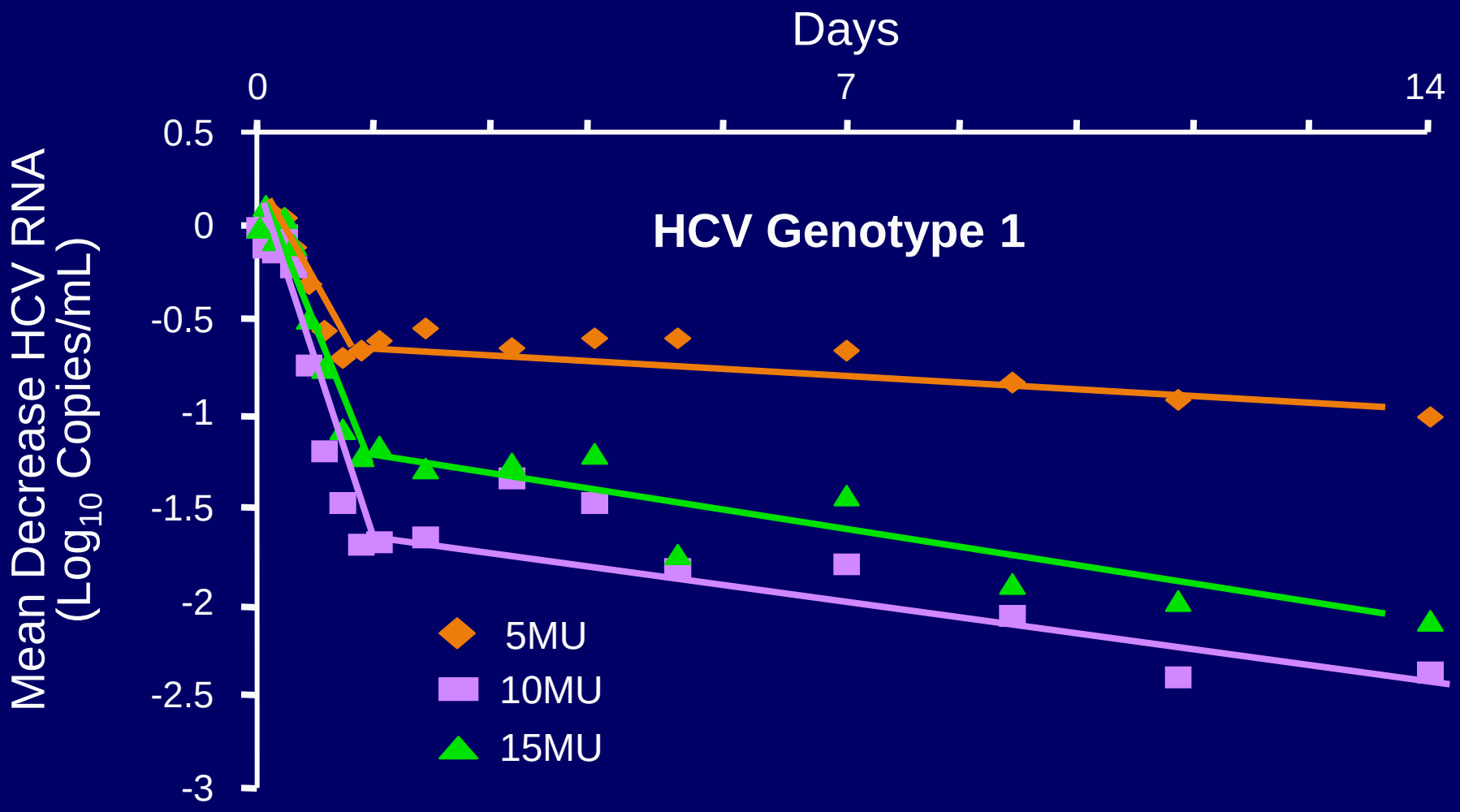
Nodules surrounded by fibrous tissue

Treatment of HCV

- Prior to May 2011 two drugs were 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.

- Since then three HCV protease inhibitors (telaprevir, boceprevir and simeprevir) and one polymerase inhibitor (sofosbuvir) have been approved for use in combination with IFN and RBV. Other drugs are in clinical trials.

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

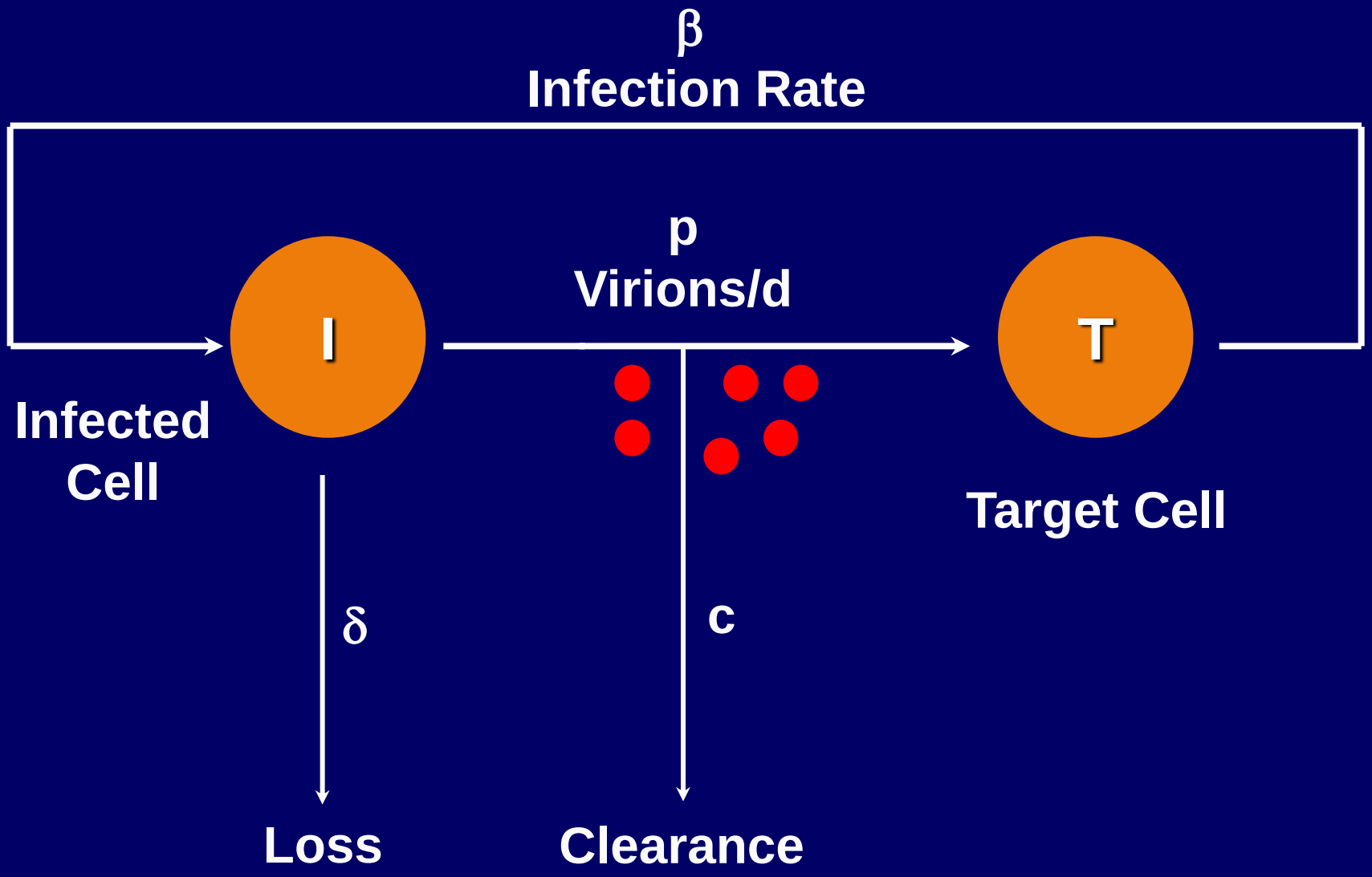


Lam Perelson et al. DDW. 1998 (abstract L0346).

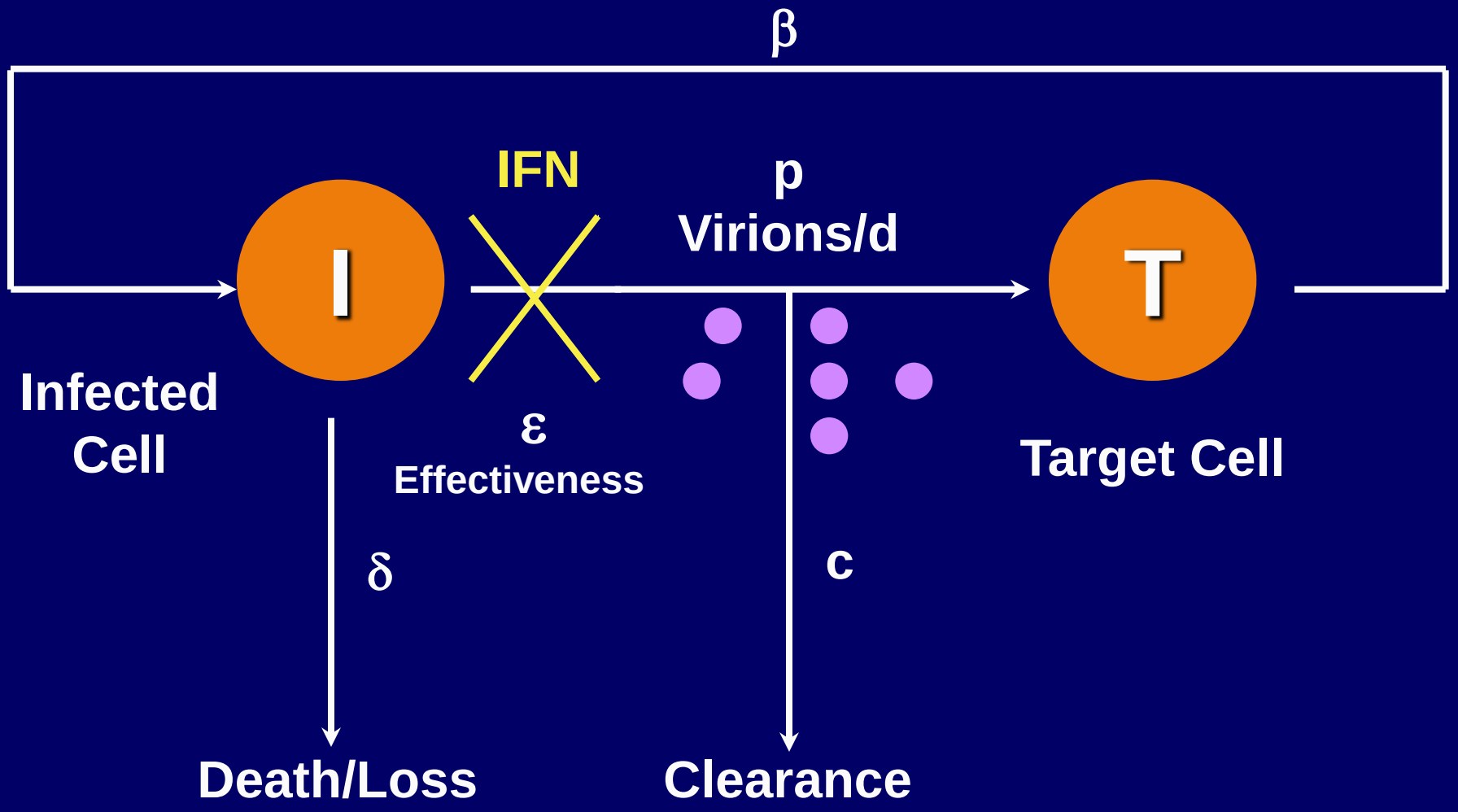
Biphasic Decline

- Why is there a biphasic decline?
- What can we learn about HCV from this observation?

Model of HCV Infection



IFN Partially 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 at t=0, assume steady state so that $pI_0 = cV_0$. Also, assume at short times, $I = \text{constant} = I_0$, so that

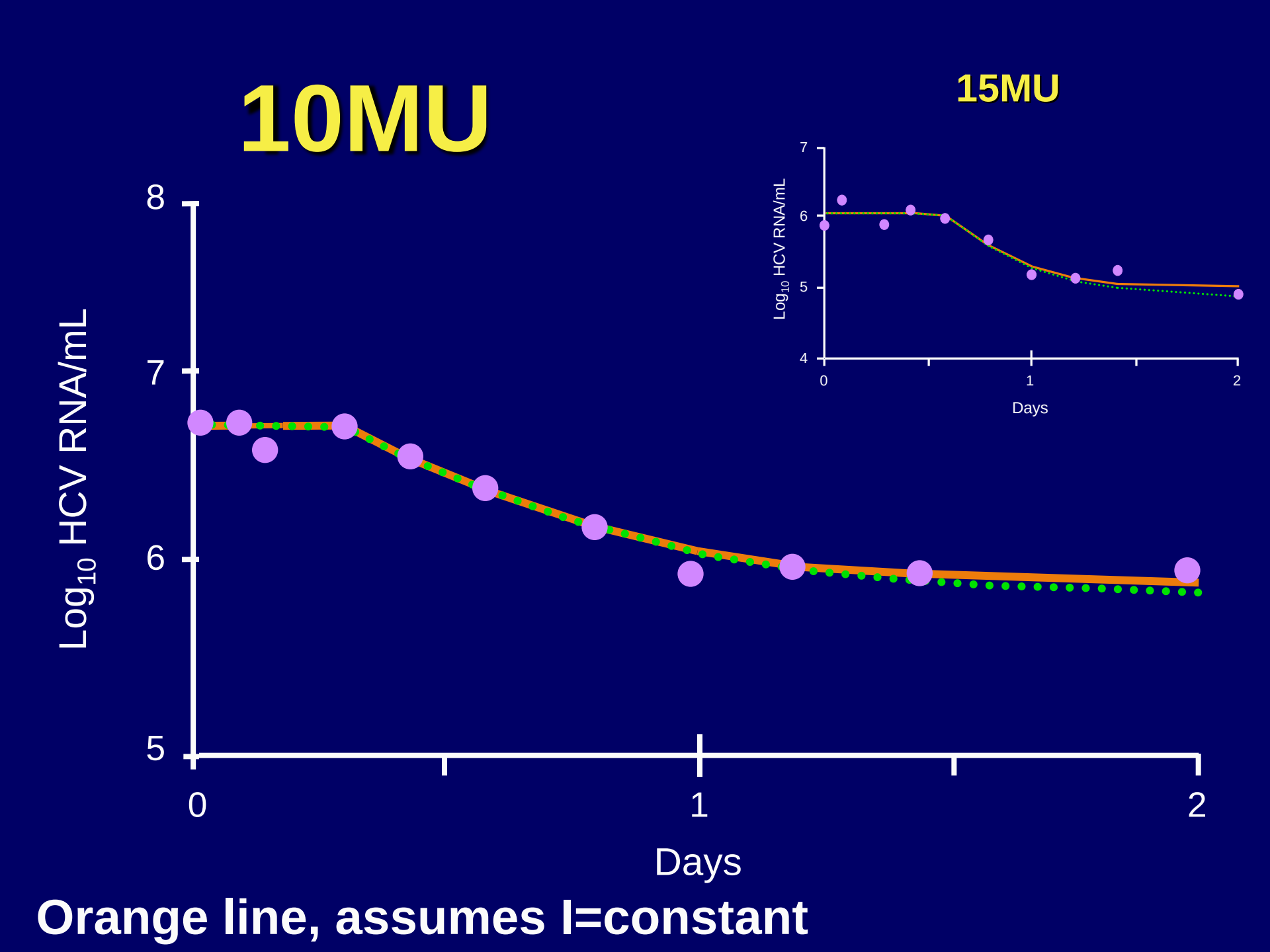
$$dV/dt = (1-\varepsilon)pI - cV = (1-\varepsilon)cV_0 - cV, \; V(0) = V_0$$

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

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

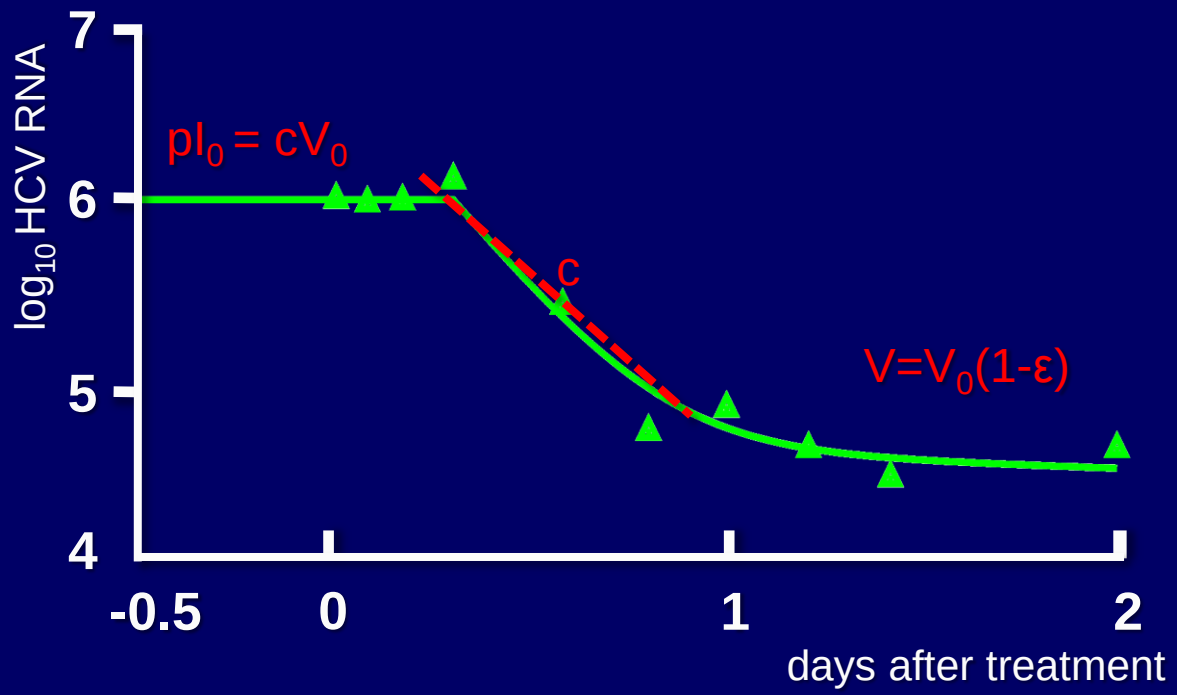
- This equation can be fit to data and **c** and **ε** estimated.

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



Orange line, assumes I=constant

Model predicts rapid viral decline. Can predict drug effectiveness from magnitude of decline



$V(t) = V_0[1 - \varepsilon + \varepsilon \exp(-ct)]$;
Can estimate c and drug effectiveness ε in a very short clinical trial

Neumann Perelson et al Science 1998

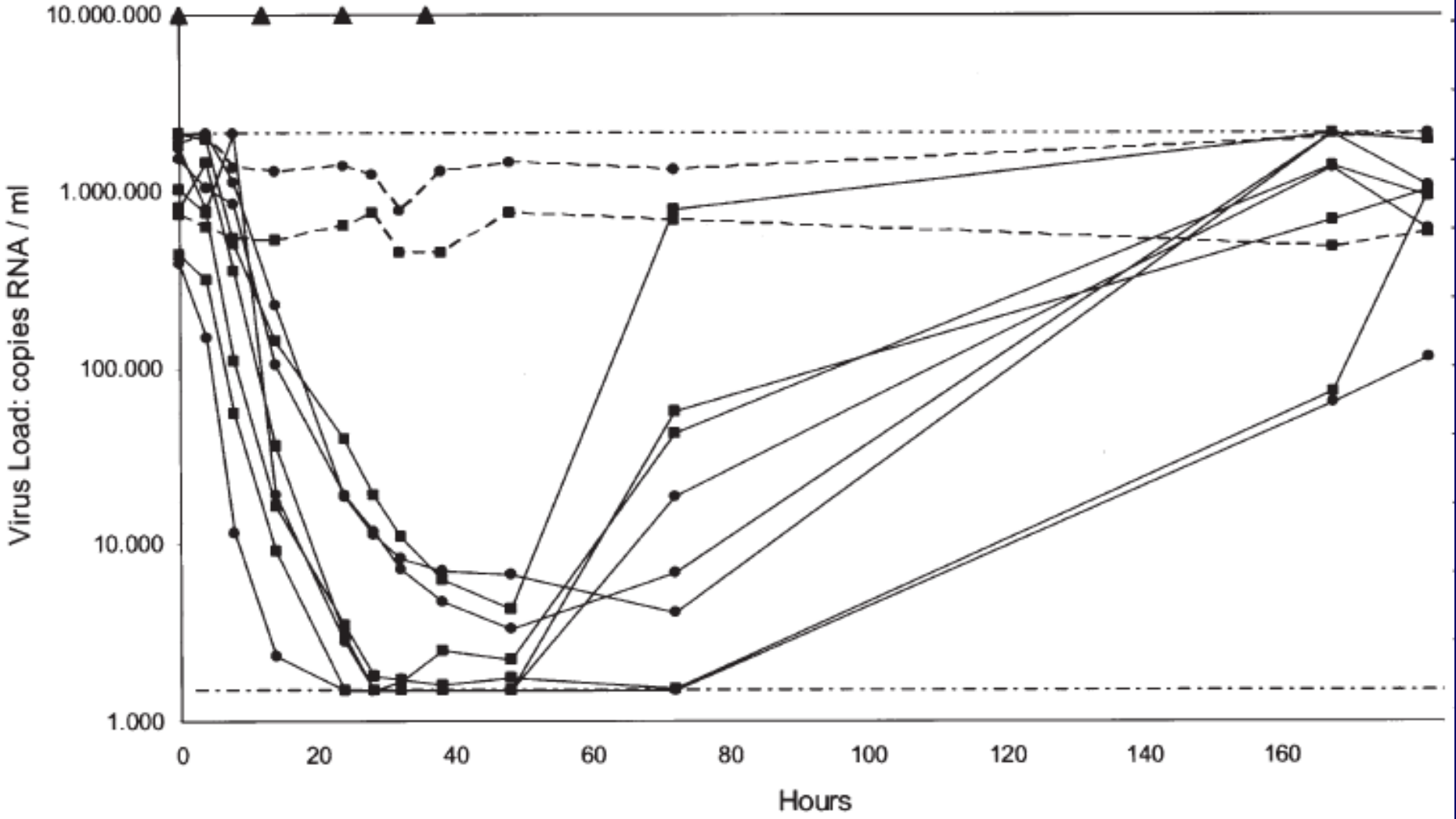
Viral Kinetics of HCV Genotype 1

	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

δ 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, and pretreatment steady state $\beta T_0 = c\delta/p$

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

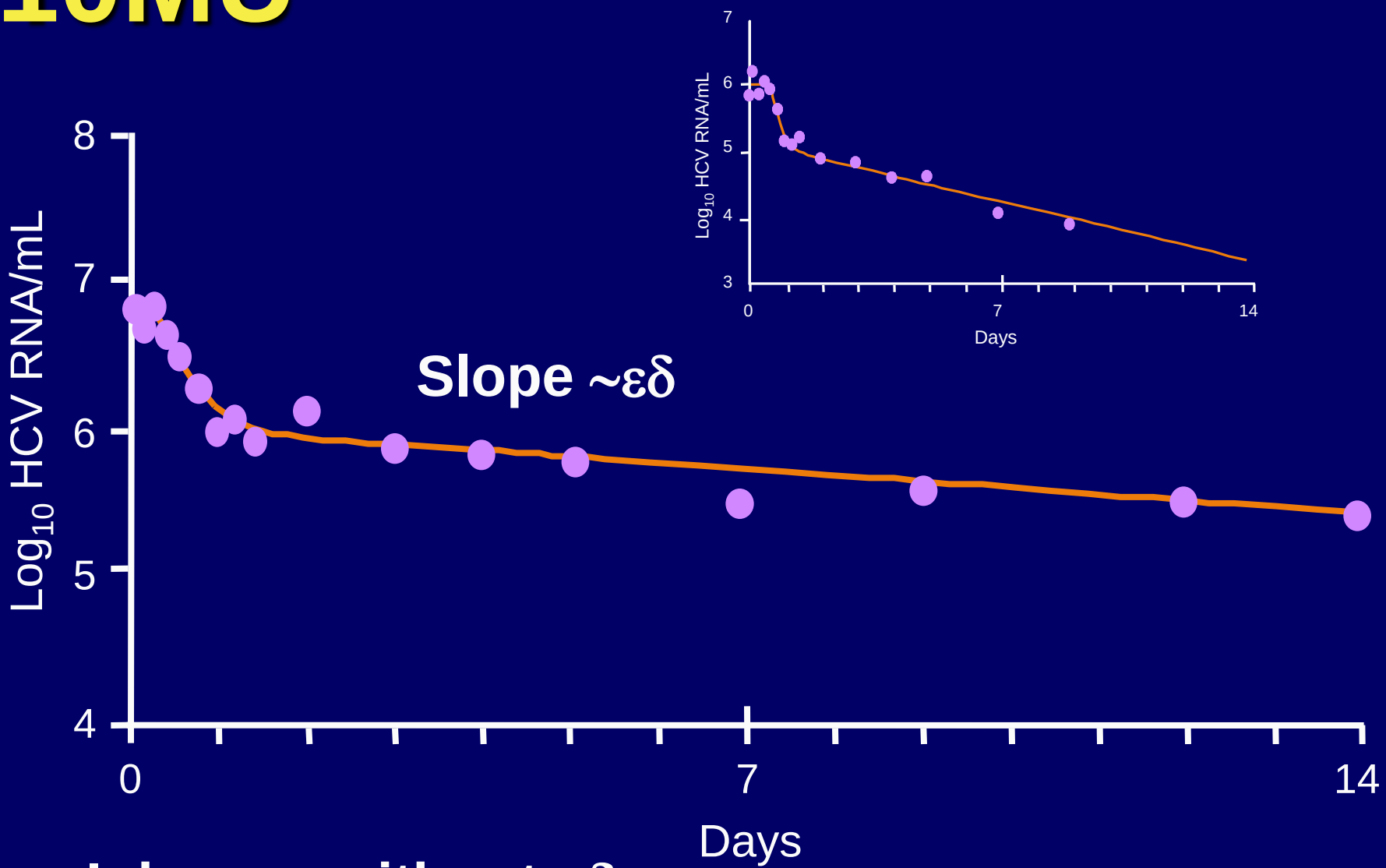
where

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

t_0 = delay between treatment commencement and onset of effect

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

10MU



I decays with rate δ

15MU

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

Viral kinetic theory was able to predict cure rate (SVR)

- Snoeck et al. Clin Pharm Therap 87:706 (2010) > 2000 pts; predicted cure rates after 1 year of treatment - PPV=99.3%, NPV=97.1%
- Prediction based on fitting viral kinetic model to early VL decline data and then predicting viral load and infected cell levels after one year of treatment.

.

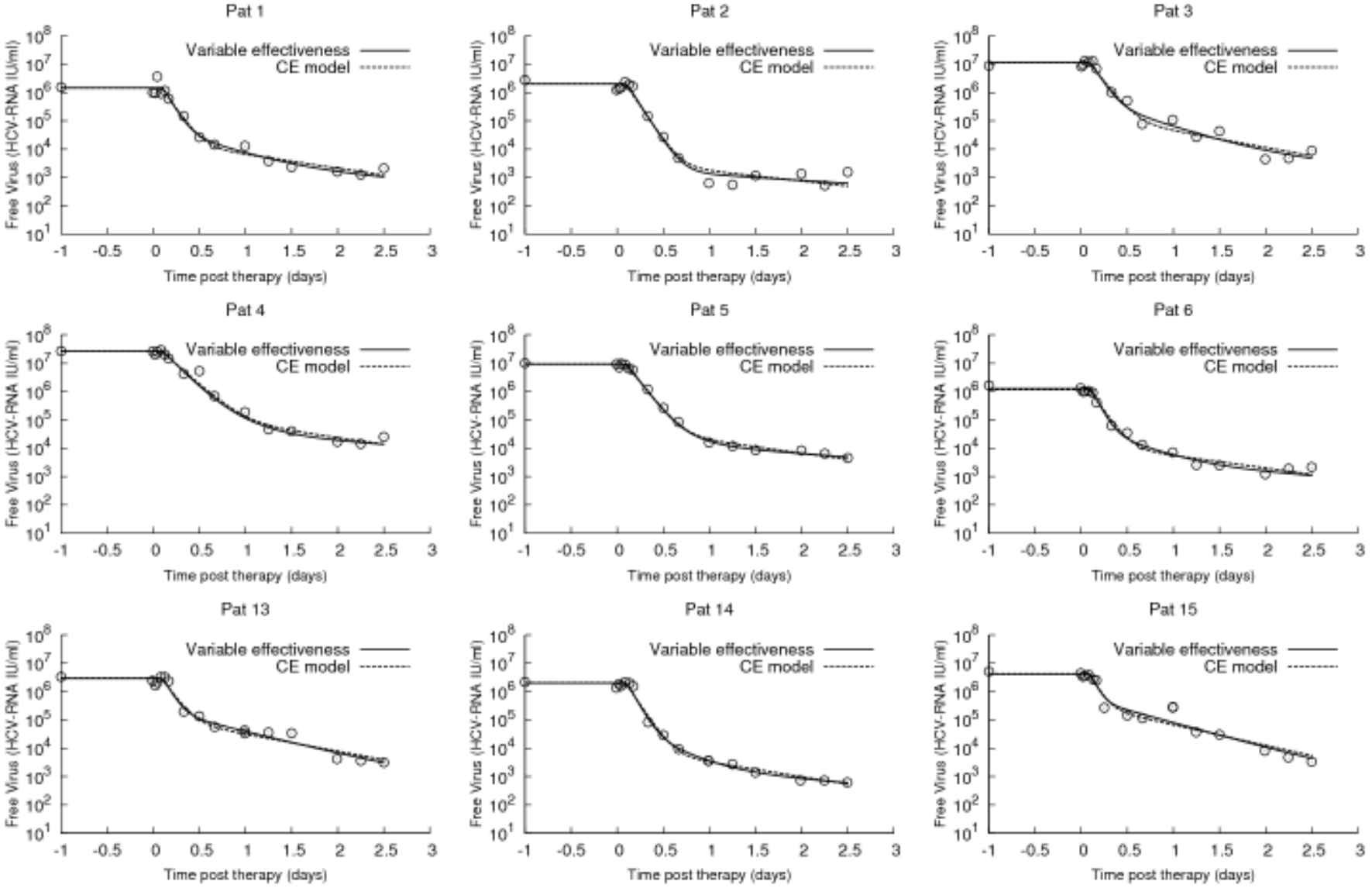
Everything looked neat and theory seemed to fit all available data

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

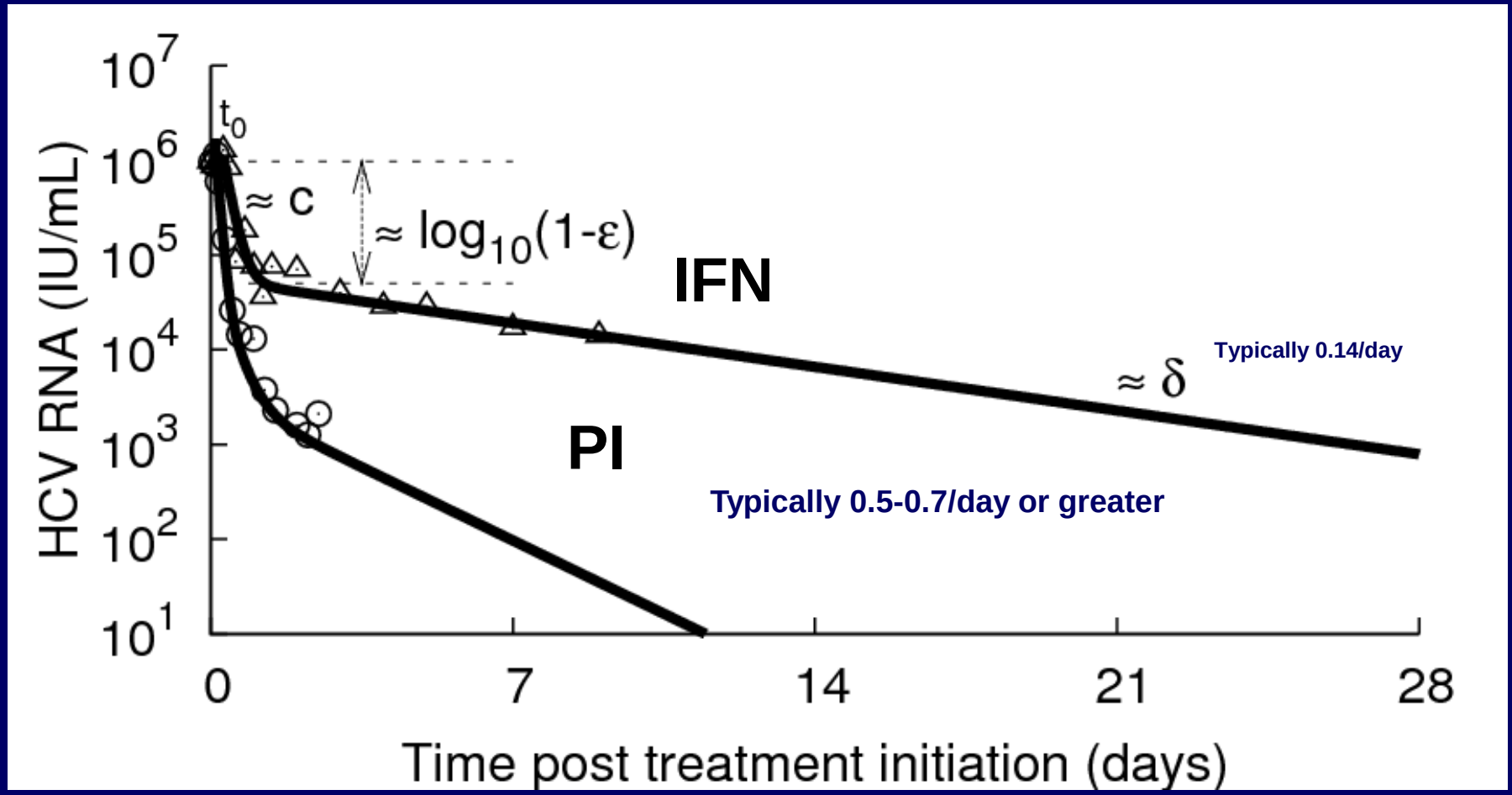
New Therapies

- Use direct acting antivirals (DAAs) – protease inhibitors, polymerase inhibitors, NS5A inhibitors,…
- Very potent compared to IFN
- Fewer side effects

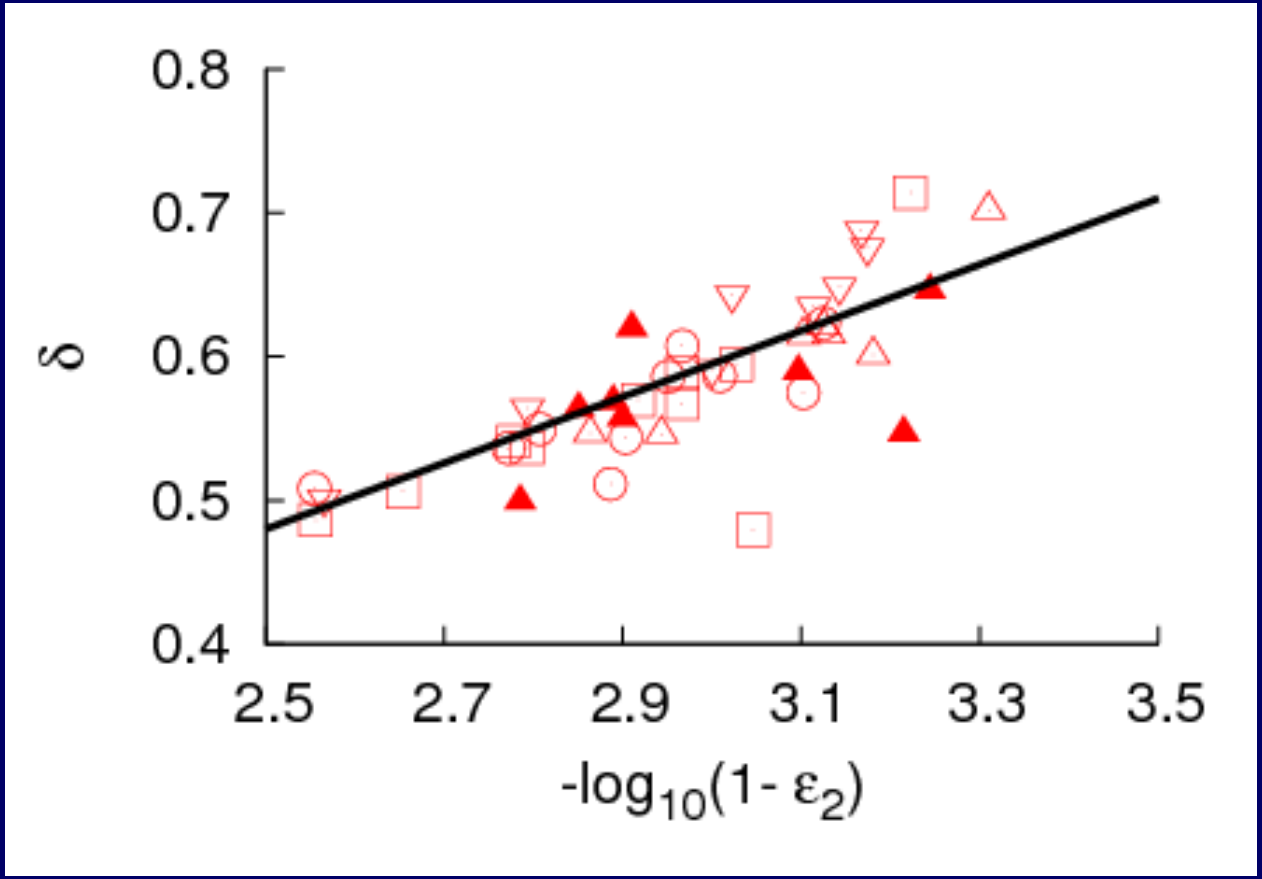
Model fits to data (n=44) from telaprevir monotherapy trial



IFN vs HCV protease inhibitor (telaprevir)

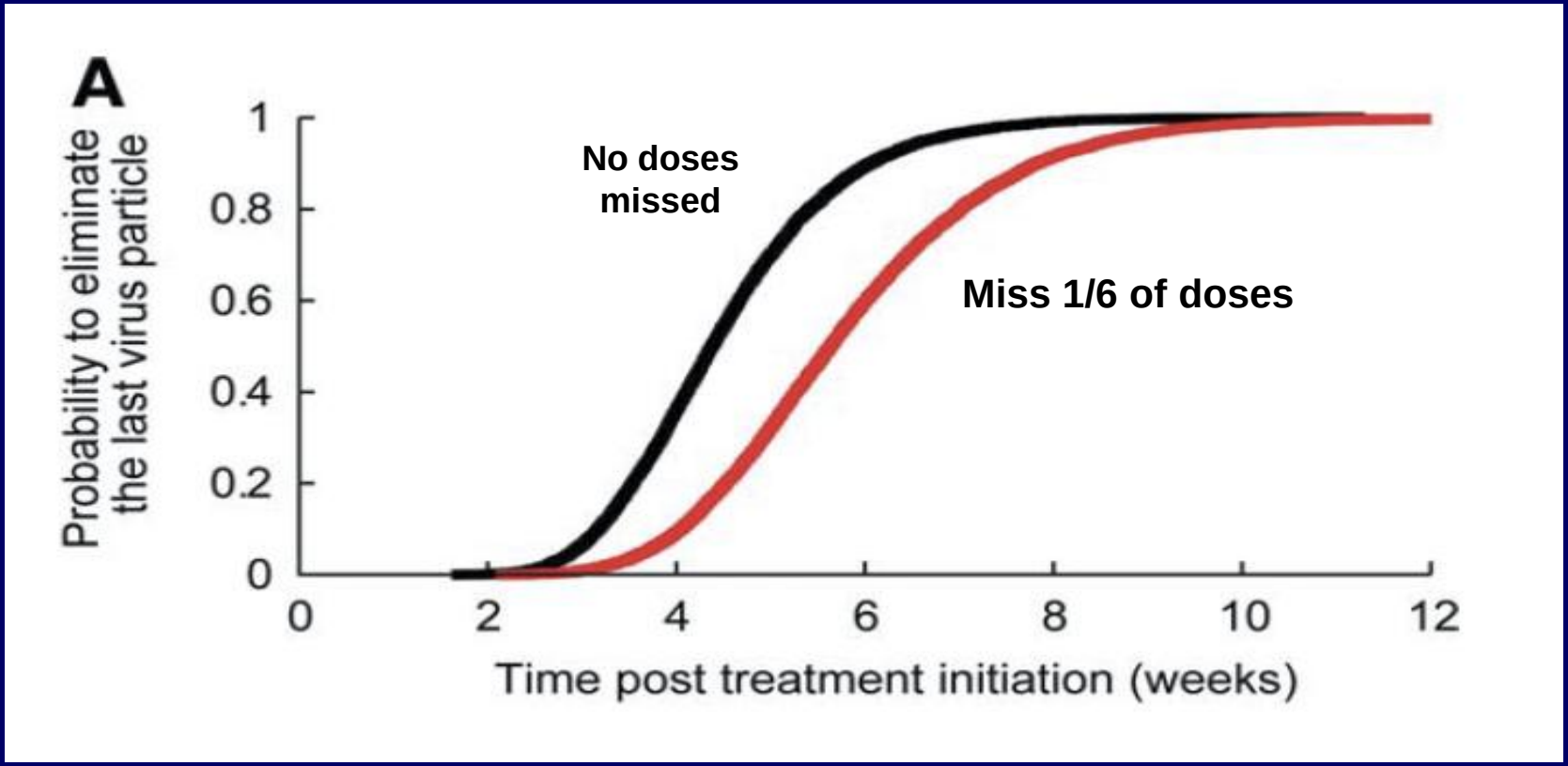


δ correlated with ε



r=0.79, p< 0.001

Time to reach < 1 virus



Should be able to eliminate virus in 95% of people in 7 weeks if no doses missed and in 9 weeks if 1/6 doses missed.

Calculations assume no drug resistance!!!

These predictions lead to short clinical trials

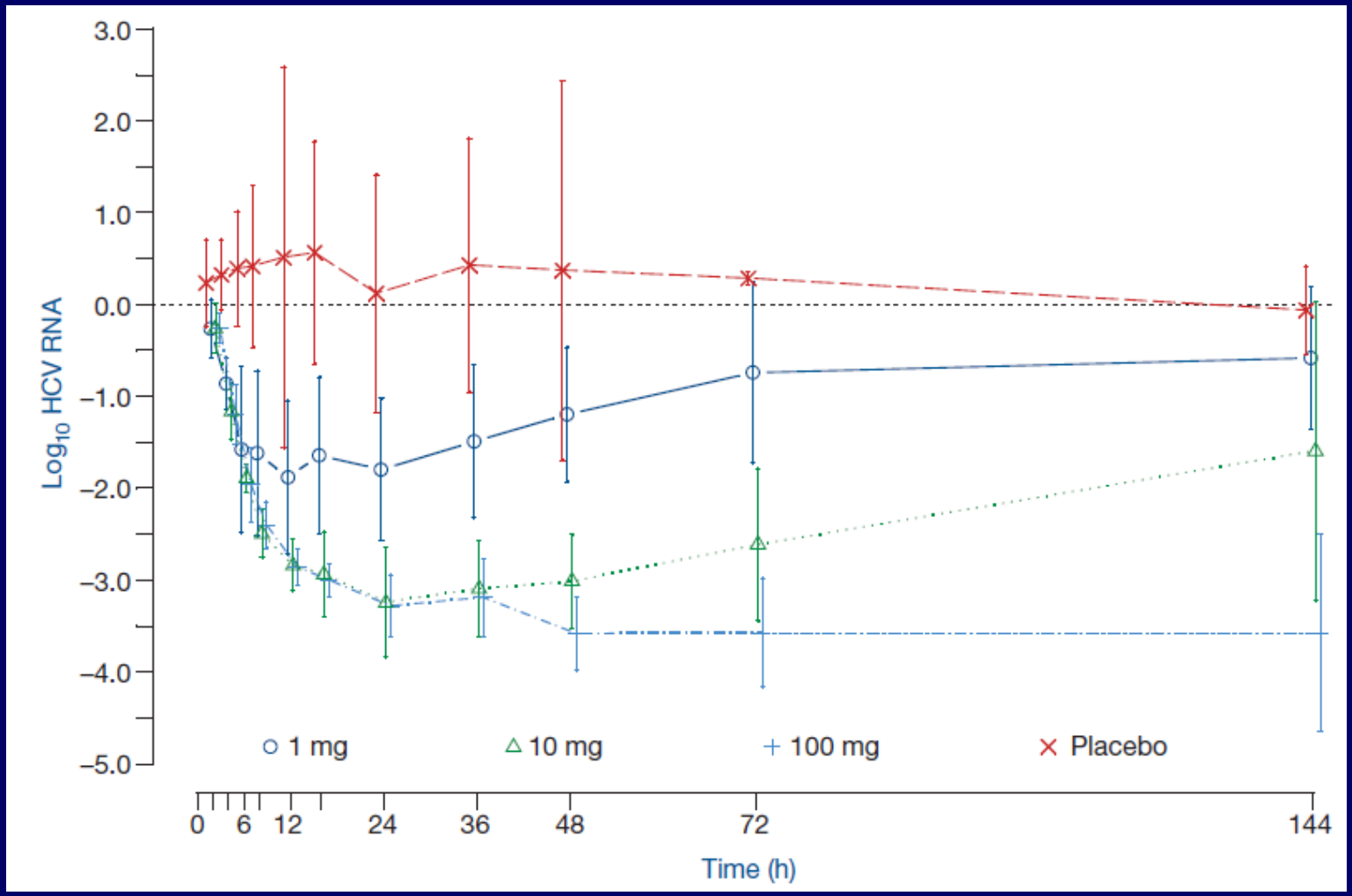
- SYNERGY trial had two arms each with 6 weeks of 3 drug combination therapy
- Cure obtained in 38 out of 40 treated pts. (Kohli et al., Lancet in press)

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

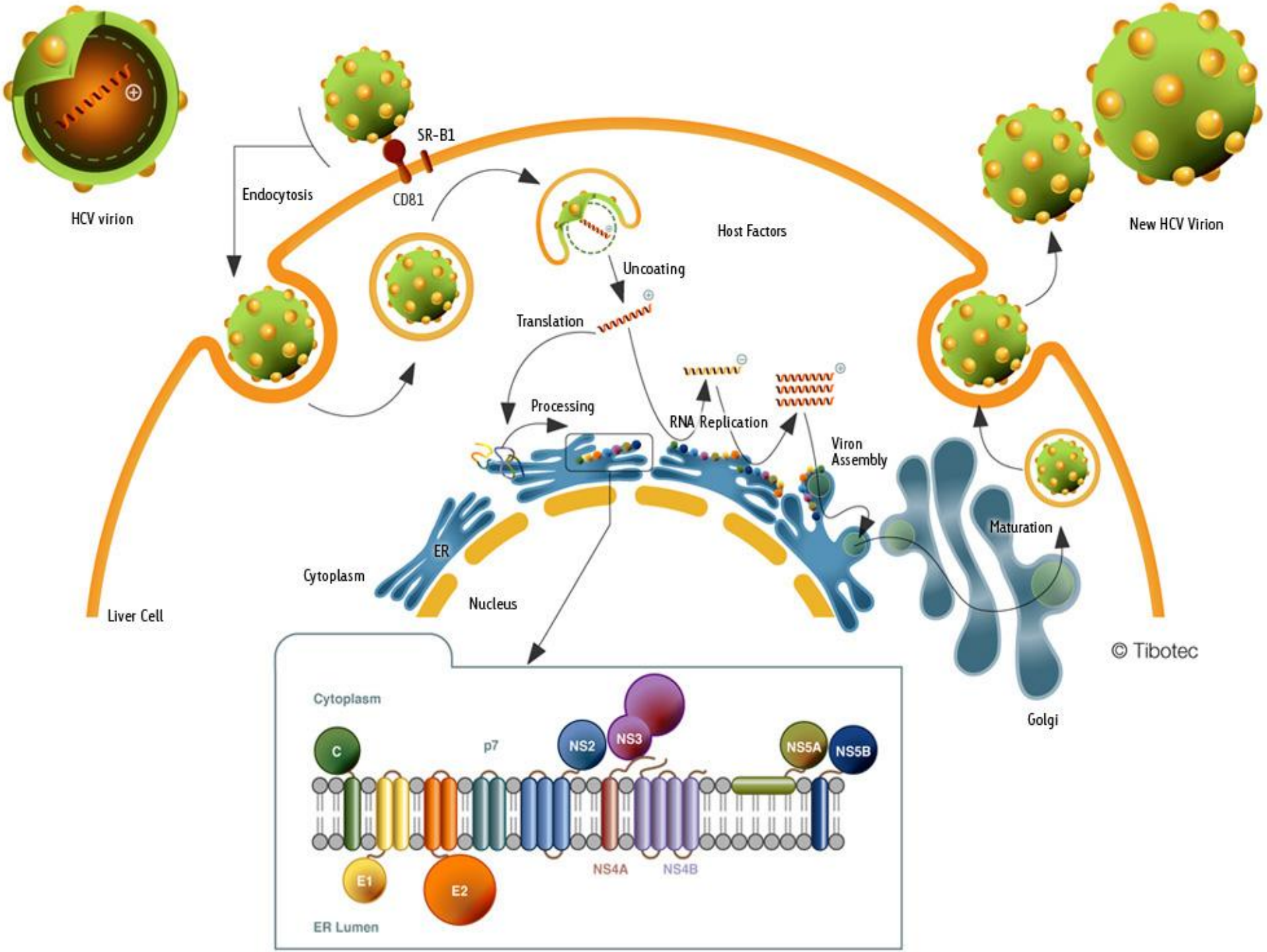
- IFN-therapy $c \sim 6\text{-}9\ d^{-1}$
- Telaprevir $c \sim 12\ d^{-1}$
- BMS-790052 $c > 20\ d^{-1}$ (NS5A inhibitor)

Models can not account for this.

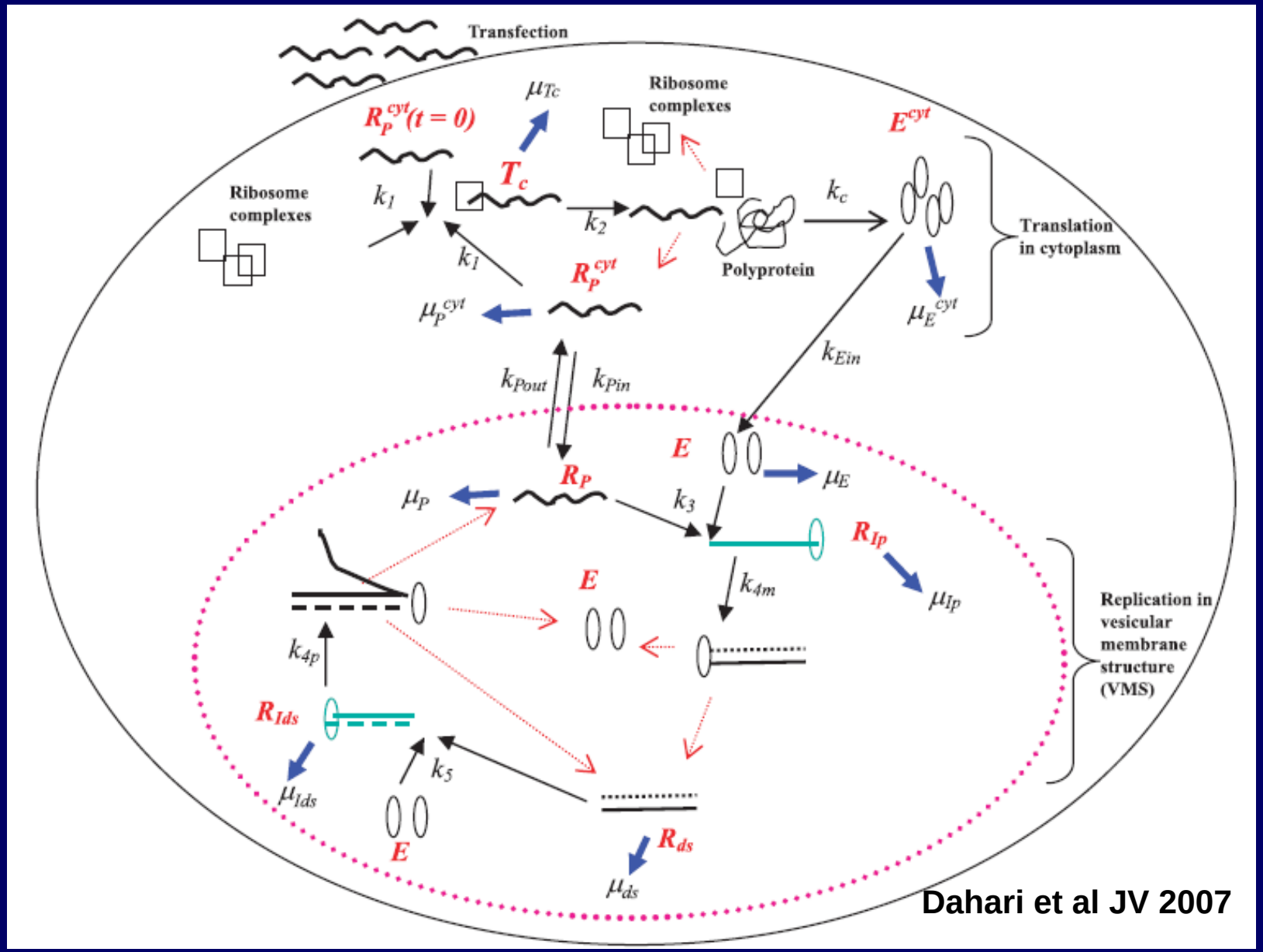
NS5A inhibitor; c > 20 d^{-1}



Gao et al, Nature May 2010

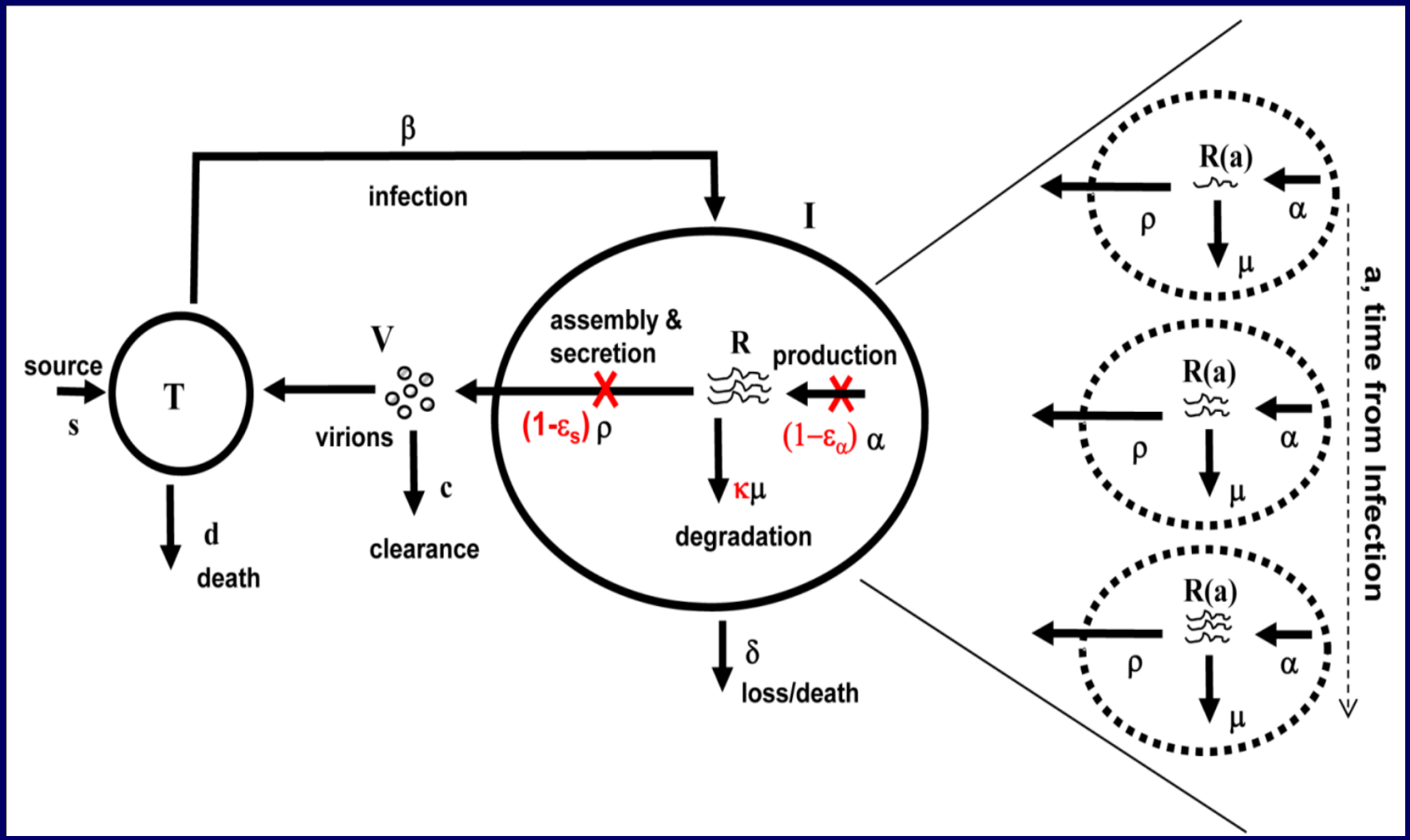

HCV virion
SR-B1
CD81
Endocytosis
Host Factors
Uncoating
Translation
Processing
RNA Replication
Viron Assembly
Maturation
New HCV Virion
Golgi
ER
Cytoplasm
Nucleus
Liver Cell
© Tibotec
Cytoplasm
C
p7
NS2
NS3
NS5A
NS5B
NS4A
NS4B
E1
E2
ER Lumen

Model of HCV RNA replication



Dahari et al JV 2007

Age-structured Multiscale Model



Guedj et al. *PNAS* 110: 3991 (2013)

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

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

$$\frac{dR}{da} = \alpha - (\rho + \mu)R$$ viral RNA

$$R(0) = 1$$

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

Effects of Treatment

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

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

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

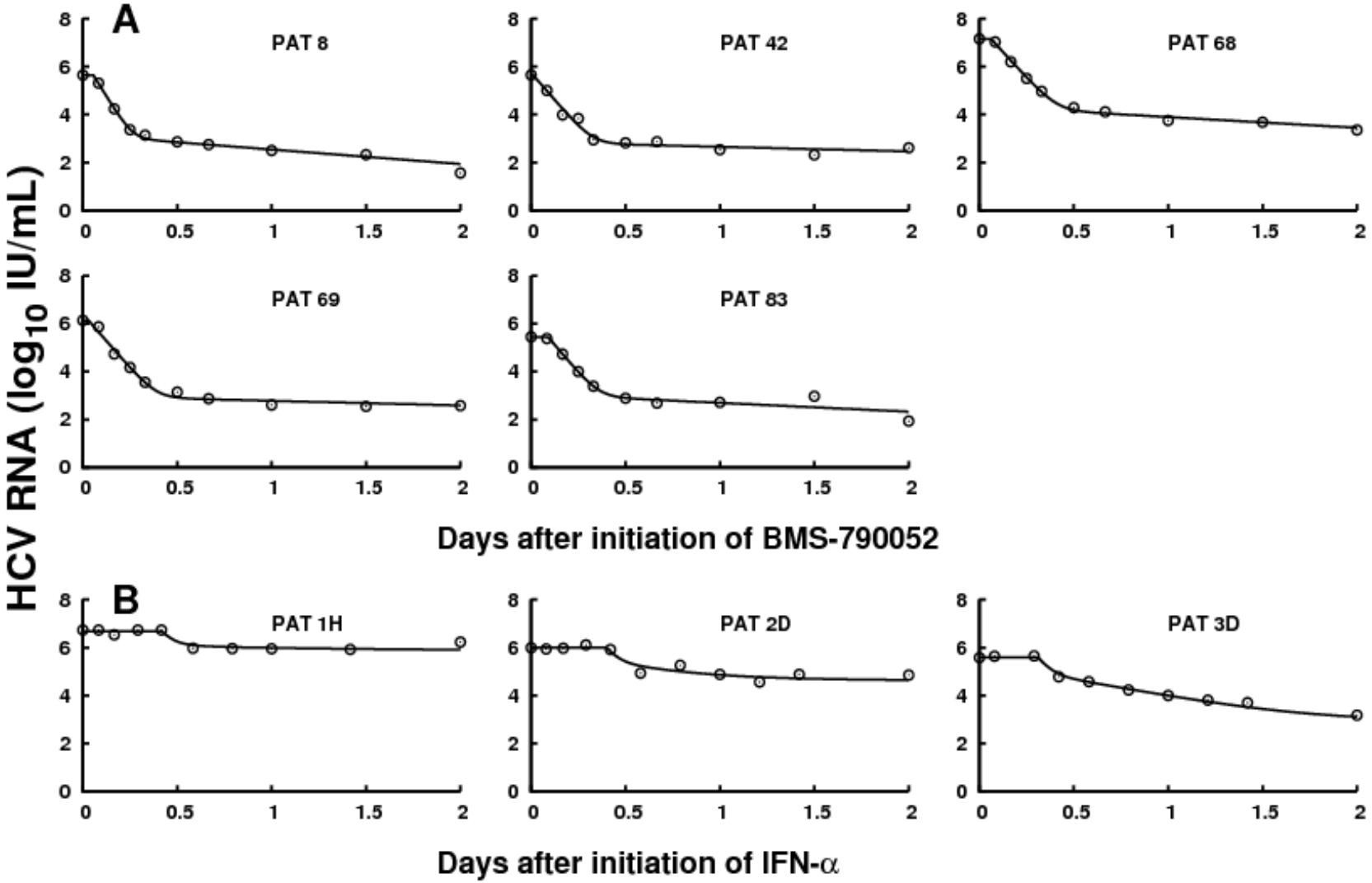
$$\frac{\partial R}{\partial t} + \frac{\partial R}{\partial a}\frac{da}{dt} = (1-\varepsilon_a)\alpha - ((1-\varepsilon_s)\rho + \kappa\mu)R$$

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

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

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

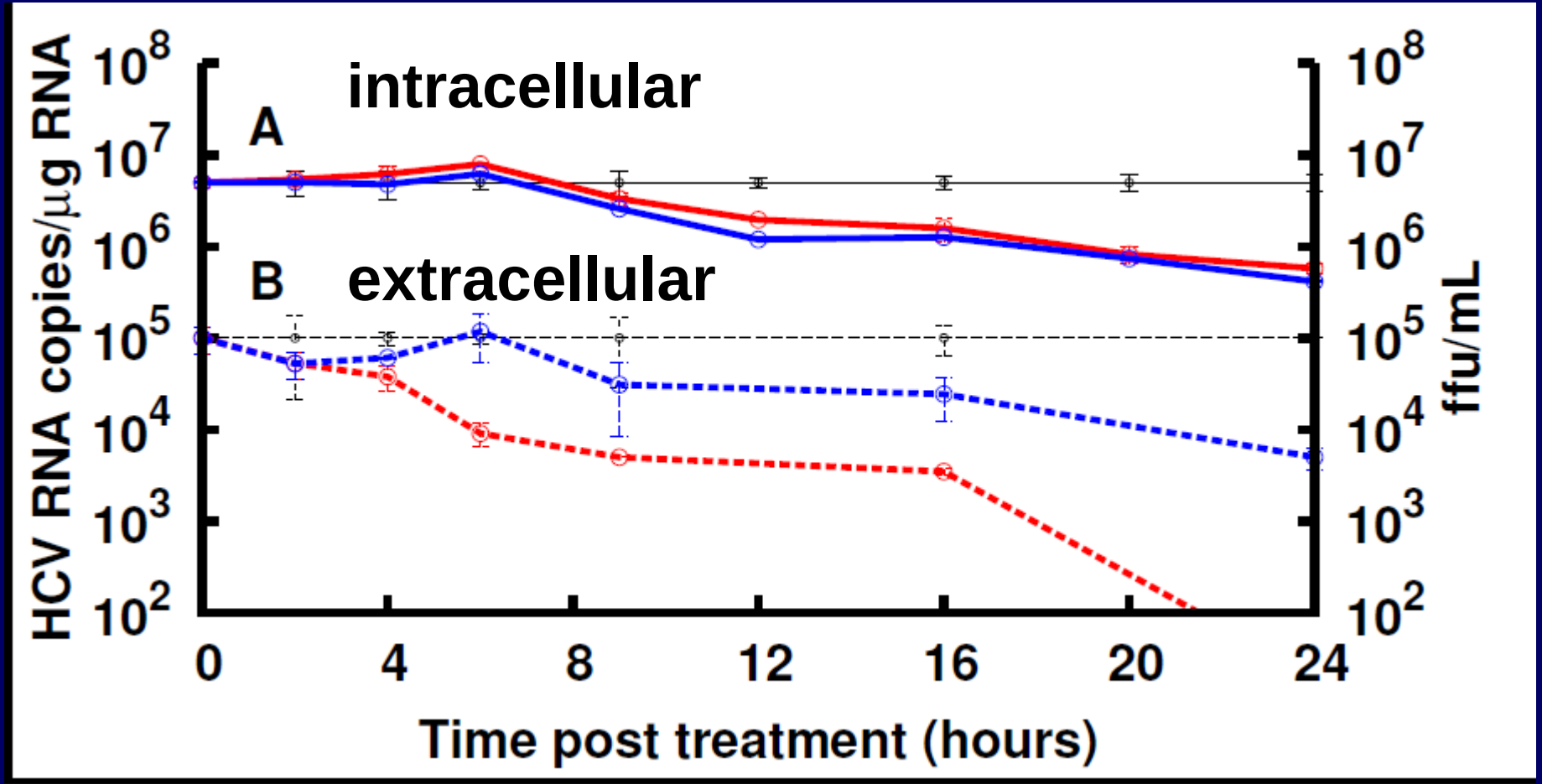
Fits to patient data



ε_α (BMS) = .993, ε_s (BMS)=.998, ε_α (15 MU IFN) = .98, ε_s (IFN) = .41, $\kappa\mu$ =1.46/day

In vitro confirmation that BMS-790052 inhibits secretion

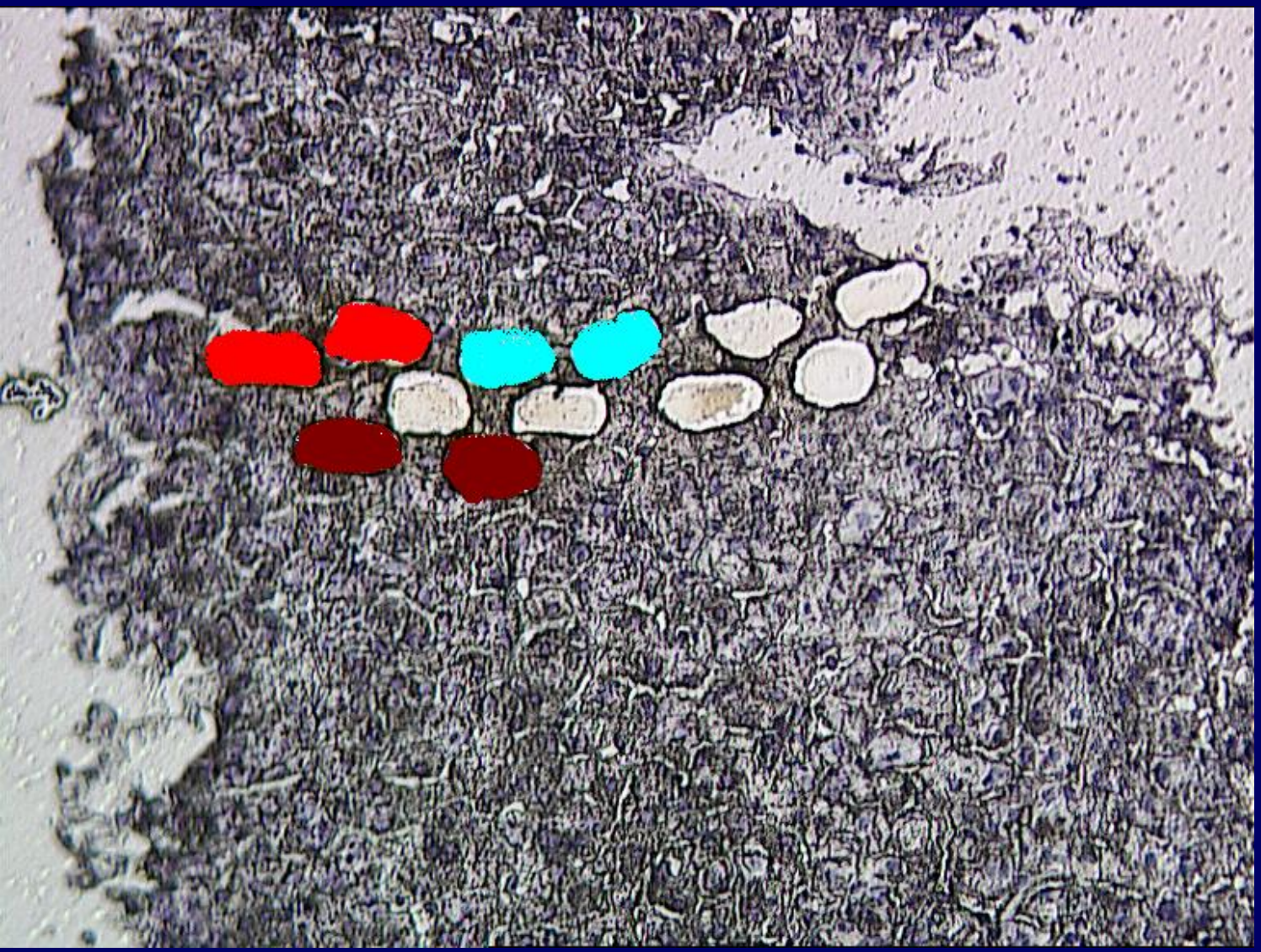
Red = BMS Blue = Polymerase inhibitor, NM107



Guedj et al. *PNAS* 110: 3991 (2013)

Spatial Models

- Liver is a solid tissue
- Virus can spread both through blood and cell-to-cell
- Models have ignored cell-to-cell spread
- In vitro, cells in suspension – can block 97% of infection with a monoclonal Ab;
- If cells allowed to form a monolayer, same Ab now blocks ~50% of infection.
- Developing lattice model where infected cells can infect neighbors and long range infection via free virus.



< 10 IU/ext

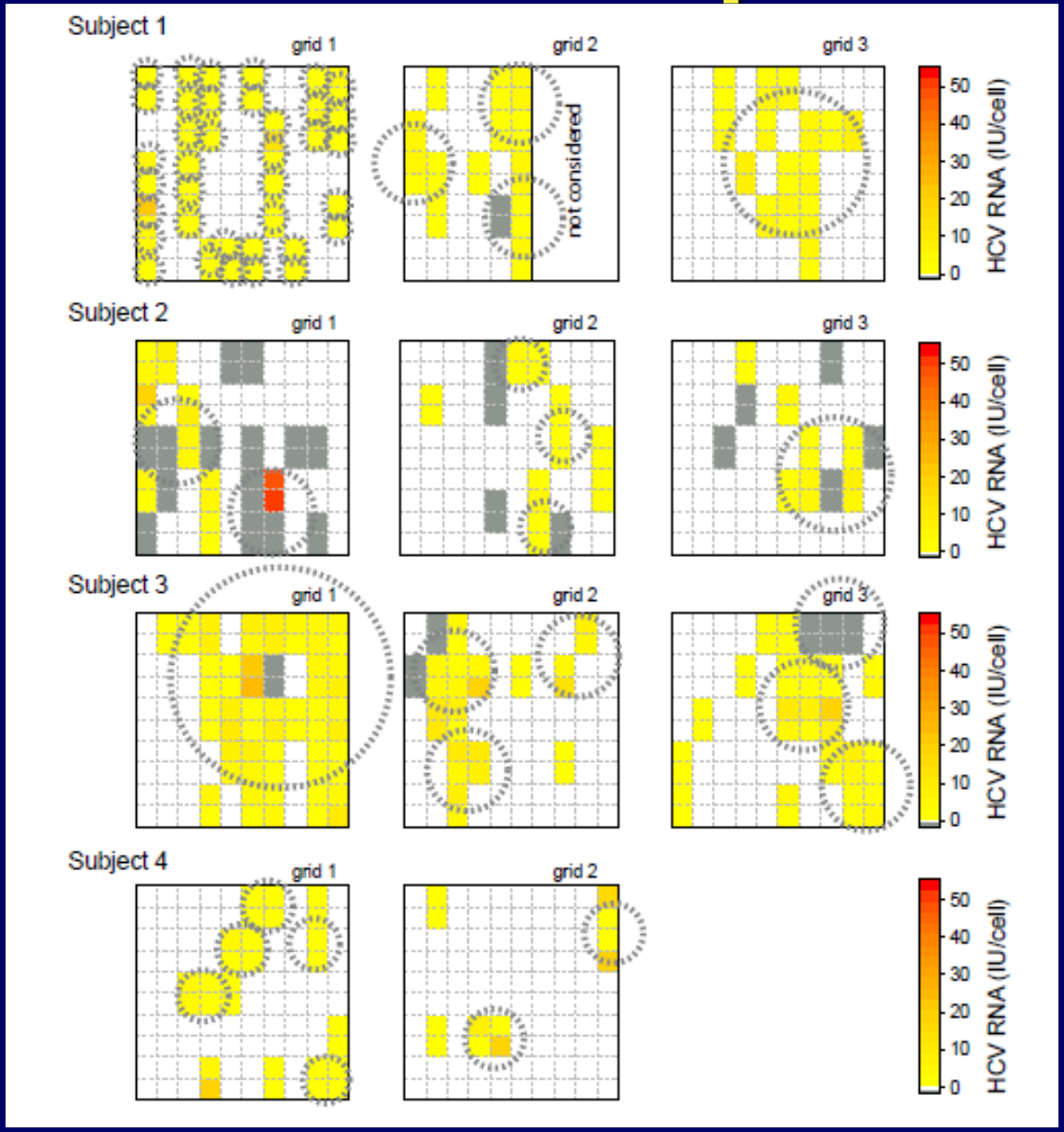
10-20 IU/ext

21-40 IU/ext

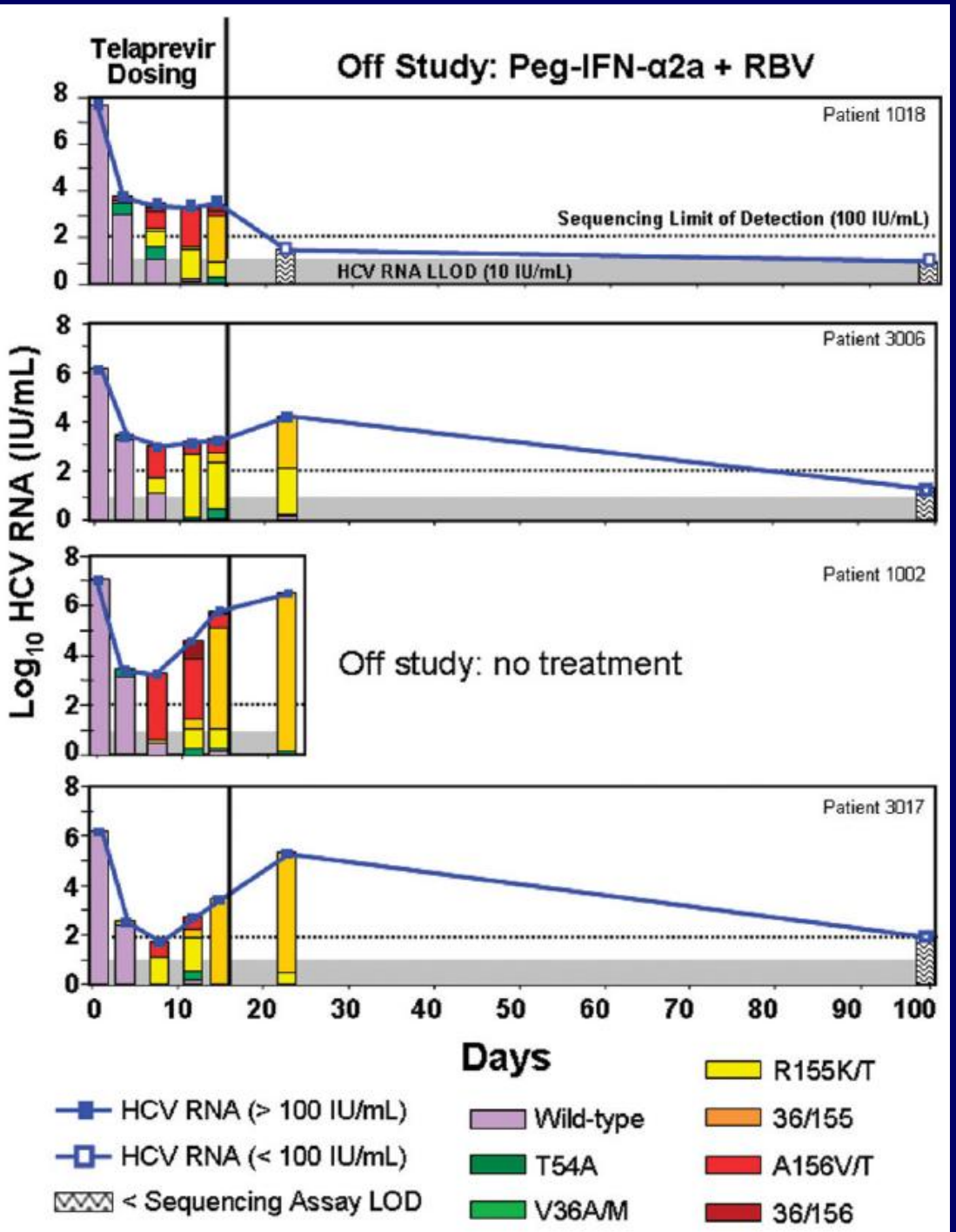
41-80 IU/ext

>80 IU/ext

Infection is spatial



New antivirals lead to drug resistance



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

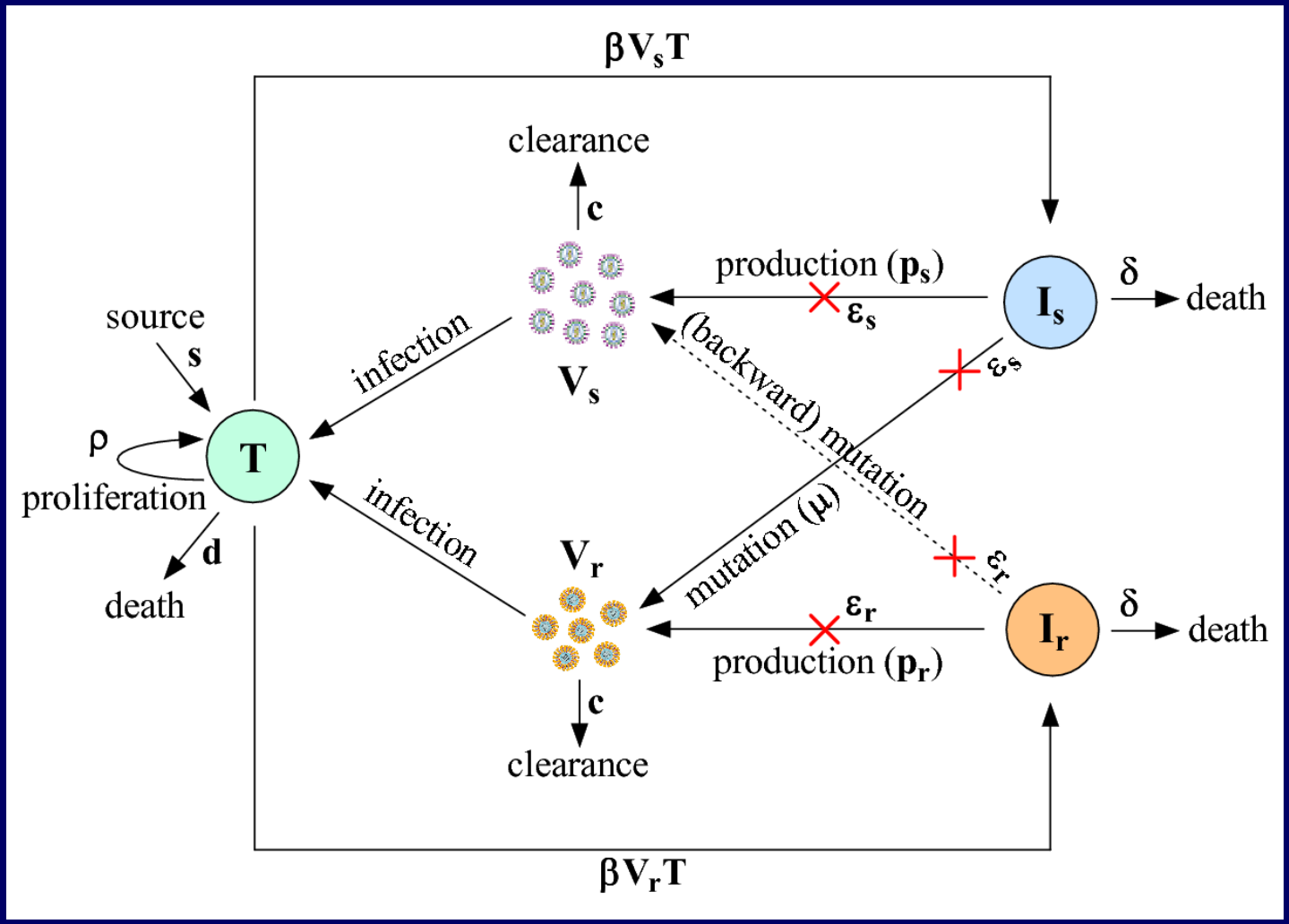
By day 14, close to 100% is resistant

Kieffer et al. Hepatol 2007 genotype 1a pts

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

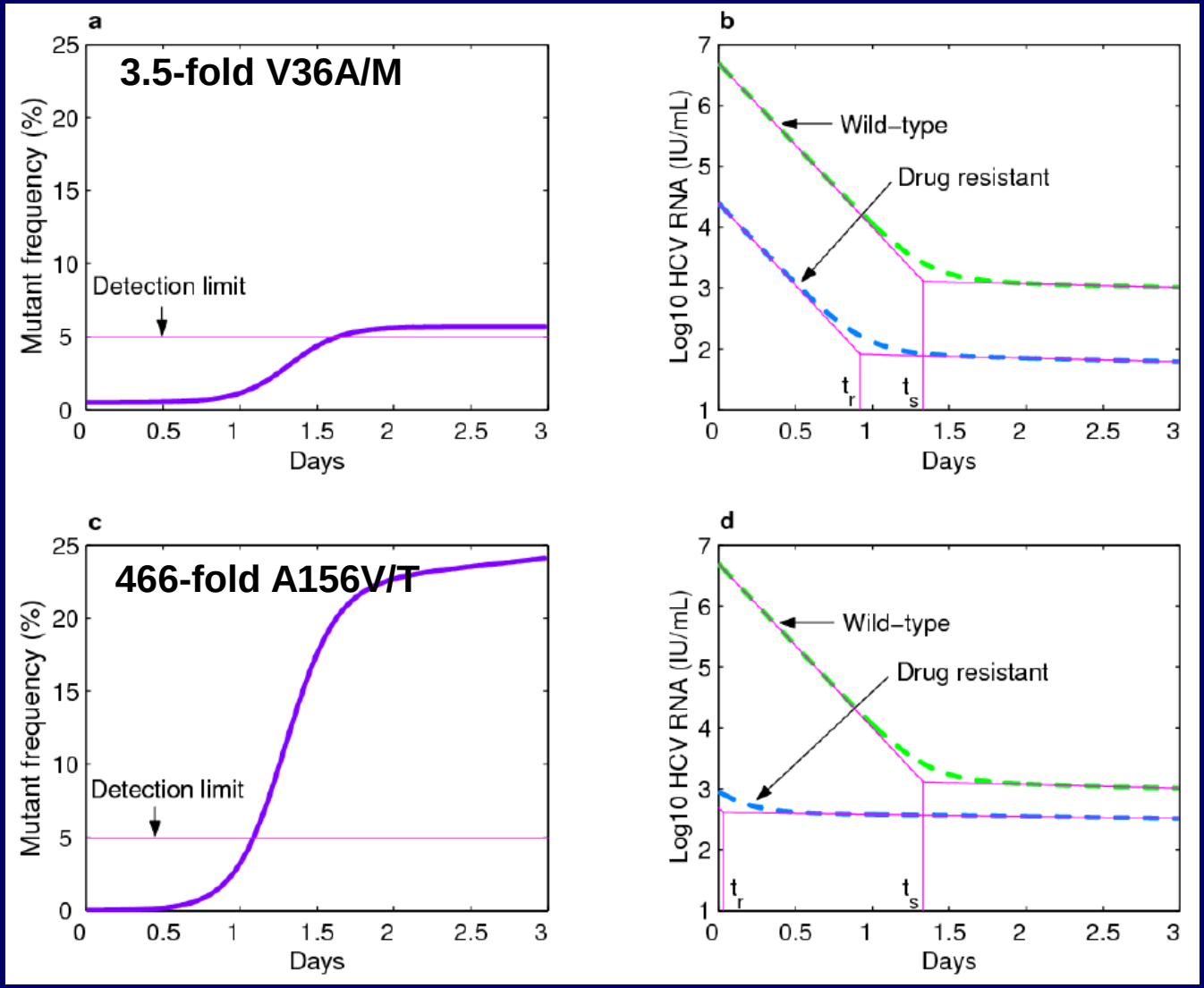
Base changes	Expected number (10^{12}/day) HCV RNA	# possible variants	% produced/ day
1 (9%)	9×10^{10}	3×10^{4}	100%
2 (0.45%)	4.5×10^{9}	4.5×10^{8}	100%
3 (0.015%)	1.5×10^{10}	4.5×10^{12}	.003%

Two-strain model

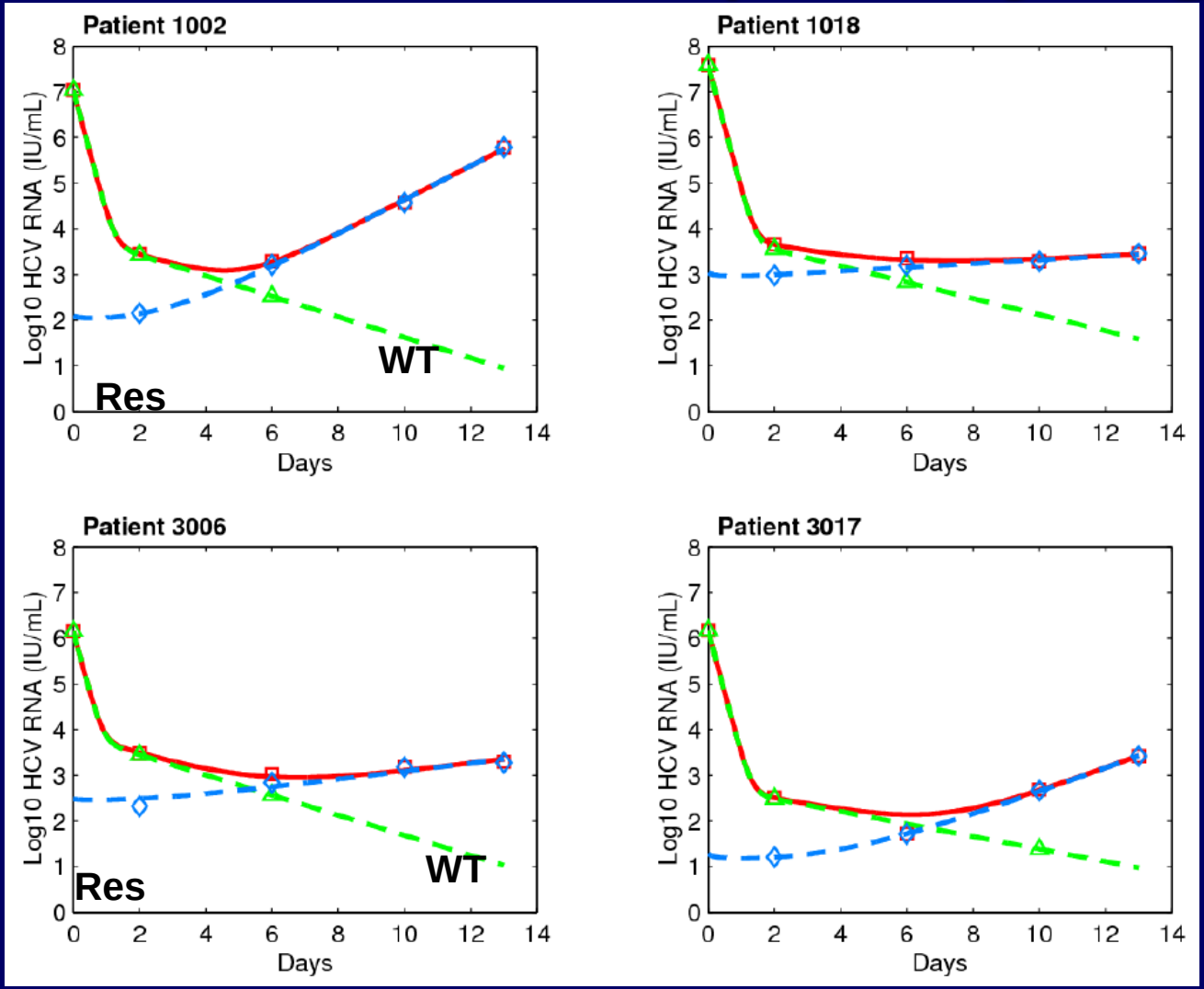


V_s =drug sensitive, V_r= drug resistant

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



Viral rebound (T varying)



Rong Perelson et al., Science Trans Med 2010

Parameter estimates for viral rebound

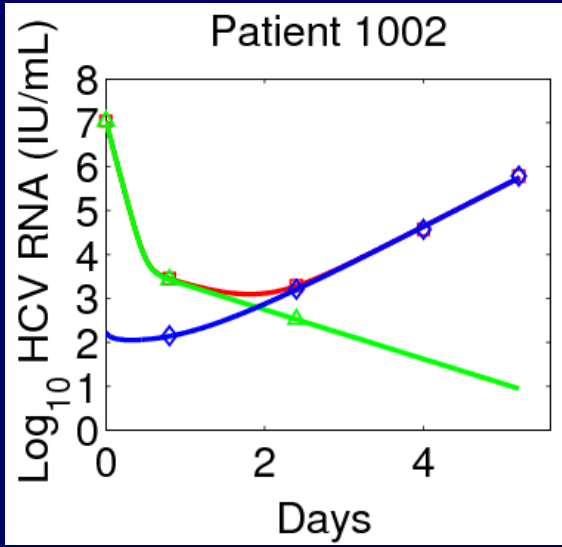
Patient	ρ_T (day^{-1})	δ (day^{-1})	μ (10^{-6})	ε_s	ε_r	β (10^{-8} mL day^{-1} $virion^{-1}$)	p_s (virions $cell^{-1}$ day^{-1})	r	Infected cells at baseline (%)	Increase in total hepatocytes (fold)
1002	1.91	0.52	2.68	0.99943	0.001	4.30	44.36	0.80	16	1.32
1018	2.38	0.41	13.91	0.99982	0.036	0.58	131.08	0.70	16	1.11
3006	2.50	0.50	5.98	0.99548	0.003	11.21	6.60	0.97	11	1.07
3017	1.21	0.32	1.99	0.99965	0.002	19.41	6.17	0.84	16	1.32
Average ±SD	2.00 ±0.59	0.44 ±0.09	6.14 ±5.46	0.99860 ±0.00210	0.011 ±0.017	8.88 ±8.29	47.05 ±58.81	0.83 ±0.11	15 ±2.5	1.21 ±0.13

Model requires rapid hepatocyte proliferation and death (~0.4/day) and about 20% increase in total liver cells

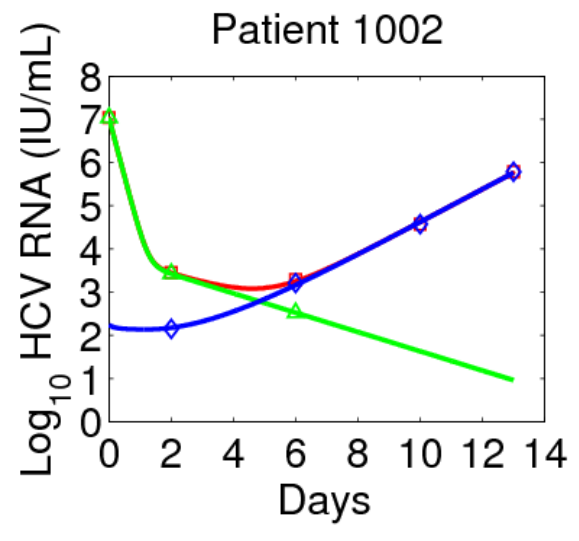
To get growth of resistant virus need “replication space:

- Could be loss of infected cells and replacement by new target cells generated by proliferation
- Could be due to cure of infected cells
- 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

Loss of Antiviral State



Ave lifetime of antiviral state 3 d



One day delay, then decay of antiviral state with rate .42/d, i.e. 2.4 day lifetime

Conclusions- Resistance

- Models of within host HCV infection can accurately represent measured changes in viral load during therapy and be used to estimate key parameters
- Due to the rapid turnover of virus, all single and double mutants are expected to be produced each day so pre-existing resistant variants are to be expected and combination therapies are needed.
- When protease inhibitor monotherapy is used resistant variants can be a sizeable fraction of the viral population after a few days of therapy and can completely dominate the population after 1-2 weeks.

Conclusions

- The fraction of resistant virus initially increases rapidly due the loss of the wildtype, which uncovers pre-existing resistant variants.
- The subsequent rapid expansion of the resistant variants requires replication space, the nature of which is under investigation but appears to involve generation of new target cells by proliferation, cure of infected cells and possibly by release from an antiviral state.
- Superinfection and take over of already infected cells also seems to play a role in the rapid turnover of resistant populations.
- Resistant forms evolve rapidly, presumably gaining fitness so that they can persist even when therapy is stopped.

Future

- The future of HCV treatment is IFN-free therapy.
- However, drug resistance is a problem and combination therapy will be needed.
- Choosing the best combinations to explore is difficult. For HCV there are ~50 drugs in development, i.e. ~ 20,000 three drug combinations. Theory can help identify the mode or modes of action of drugs in vivo.
- Predicting how combinations will work in vivo is still a challenge as is understanding the nature of replication space for resistant variants.

Collaborators

- Avidan Neumann, Bar-Ilan
- Harel Dahari, Loyola Univ, Chicago
- George Shaw, Univ Penn
- Ruy Ribeiro, LANL
- Libin Rong, Oakland Univ
- Jeremie Guedj, INSERM, Paris
- Ruian Ke, LANL