How we cured HCV and a bit of HCV evolution and drug resistance

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Chronic viral infections

- HIV
- HBV – hepatitis B
- HCV – hepatitis C
- HPV – Human papillomavirus
- EBV – Epstein-Barr virus
- CMV - cytomegalovirus

Hepatitis C Virus Infection

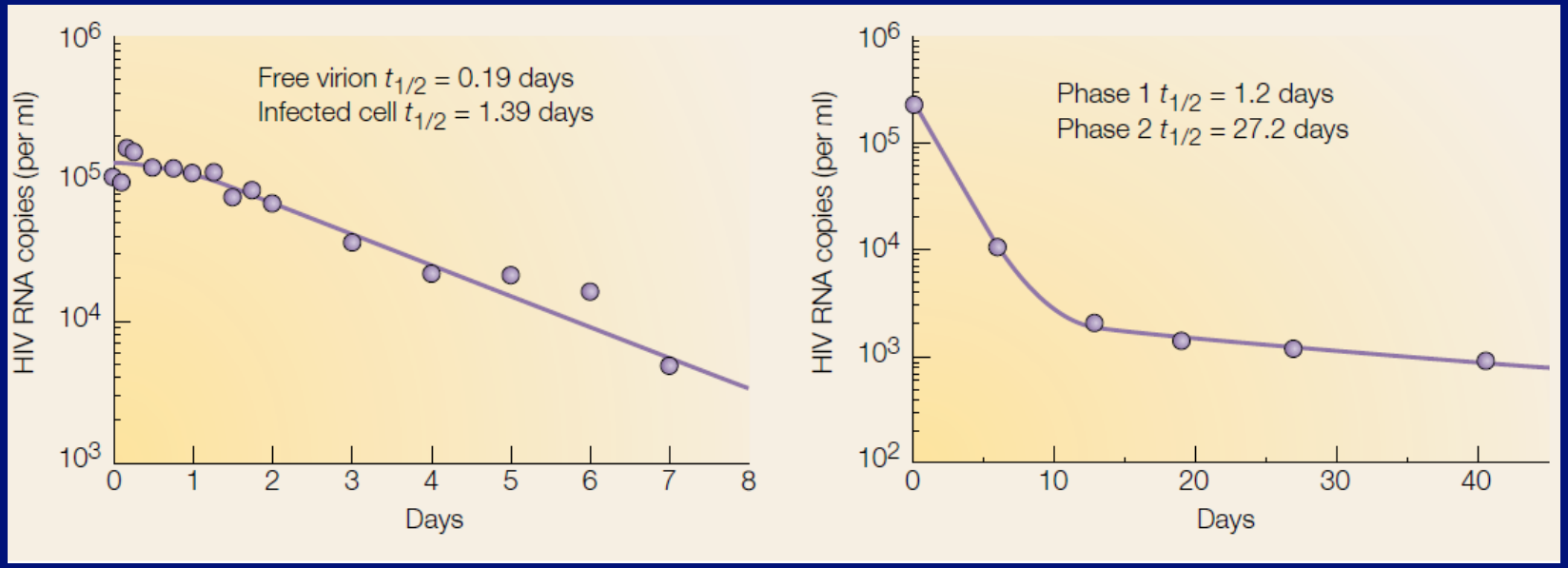
- HCV is a positive strand RNA virus that infects the liver; 9.6 kb; error prone RdRp
- It can lead to cirrhosis and liver cancer with a varying time course, from a few years (fulminant hepatitis) to > 30 years
- ~ 4 million infected in the US, 170 million world-wide
- First chronic viral infection that can be cured with drug therapy.
- No vaccine available yet.

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 a number of small molecule inhibitors of the HCV polymerase, protease and NS5A have been approved and when used in combination can cure 95-97% of people.

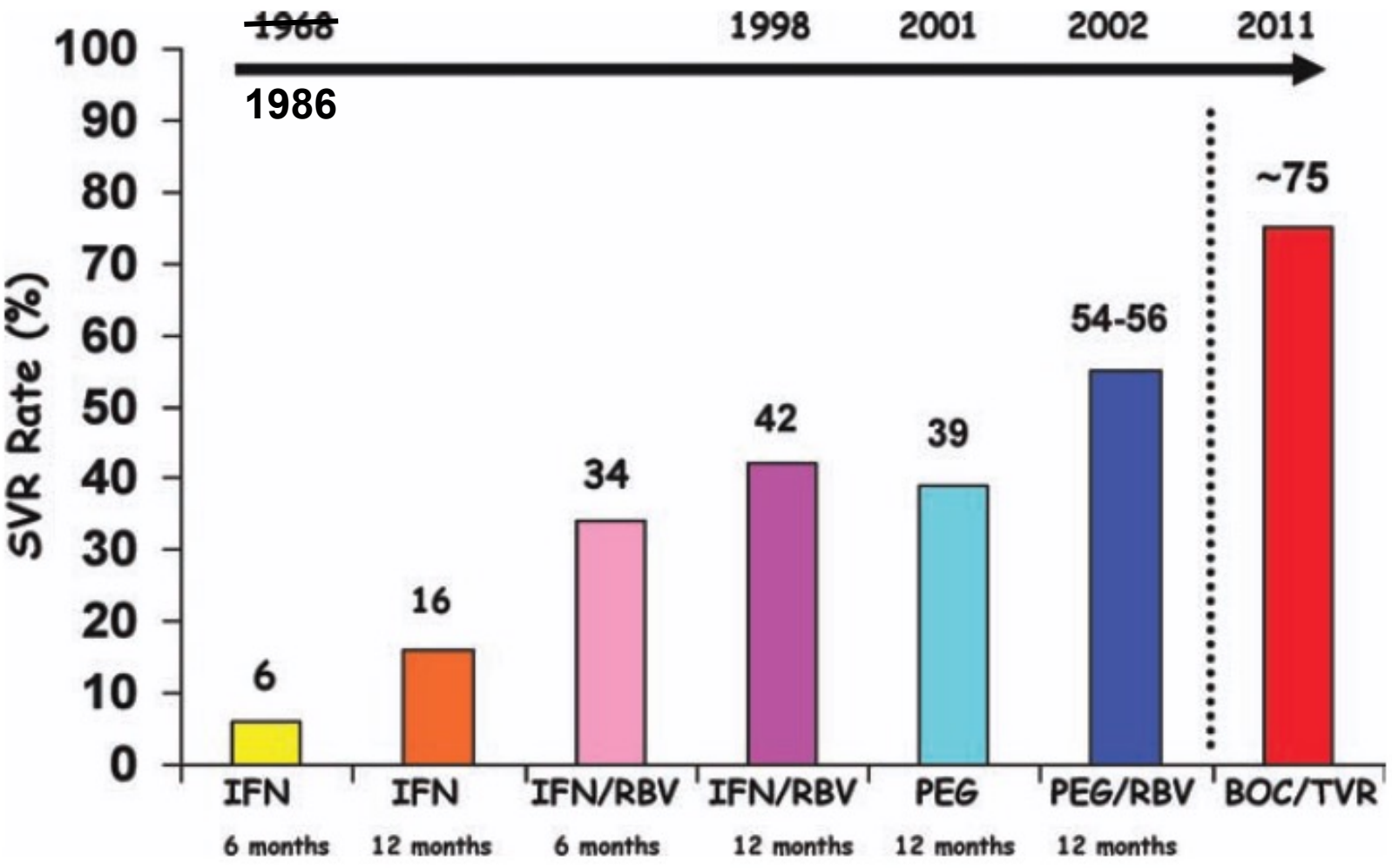
HIV Dynamics



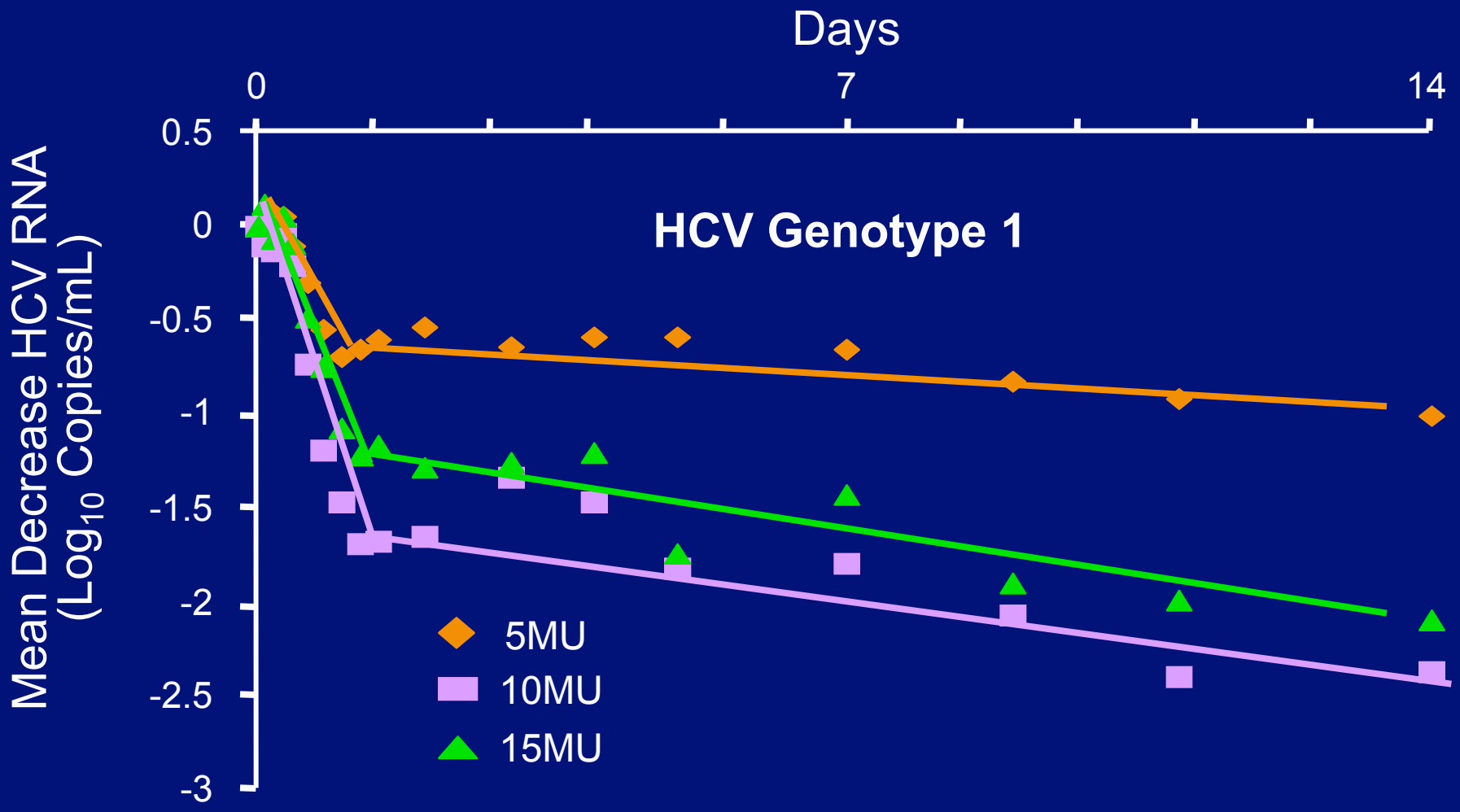
Ho et al. Nature 1995
Perelson et al. Science 1996
Perelson et al. Nature 1997

Perelson Nelson SIAM Rev 1999
Perelson Nature Rev Immunol 2002

History of interferon use

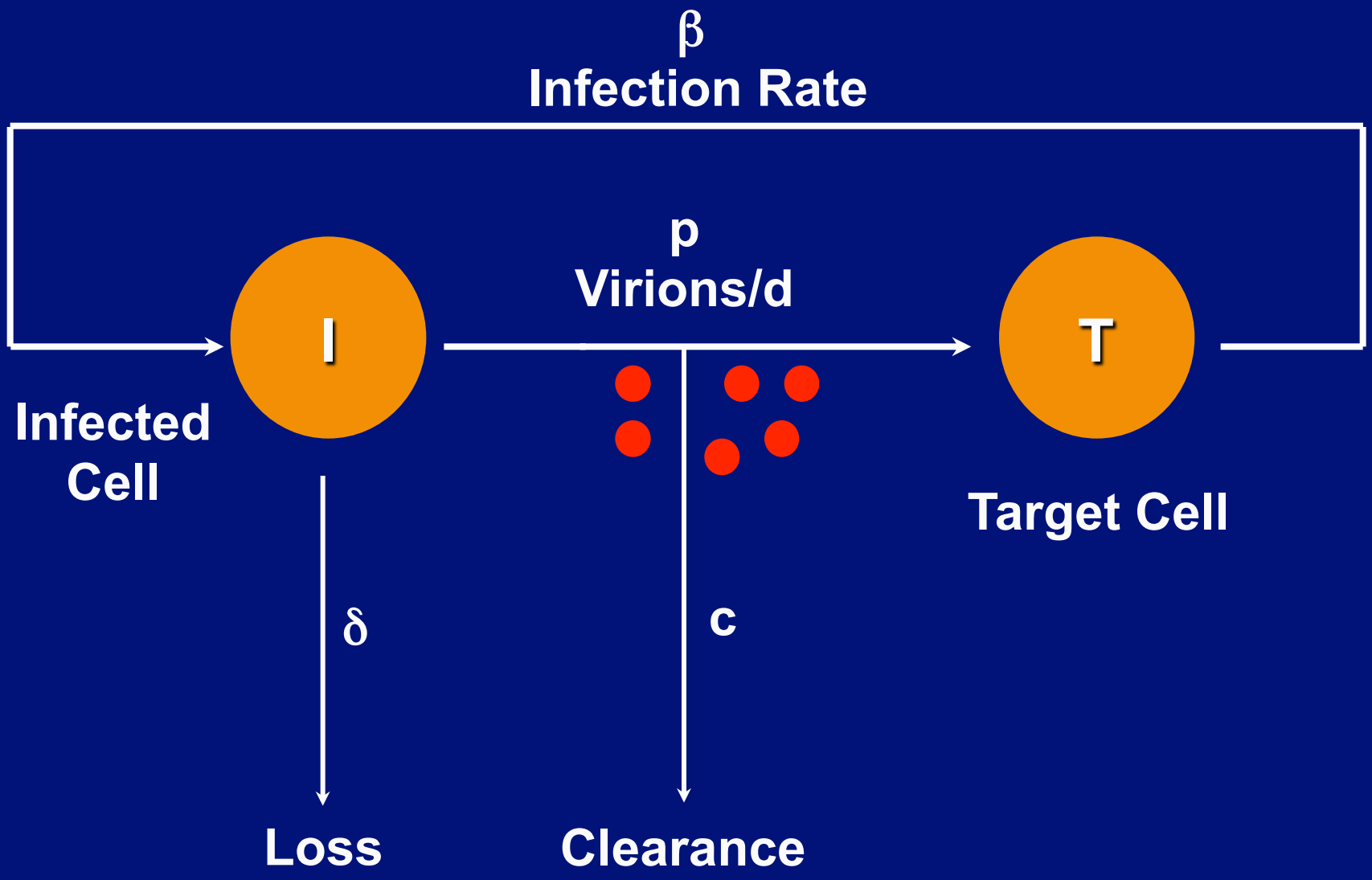


Mean Decrease in HCV RNA Levels Over First 14 Days of QD IFN-α Treatment

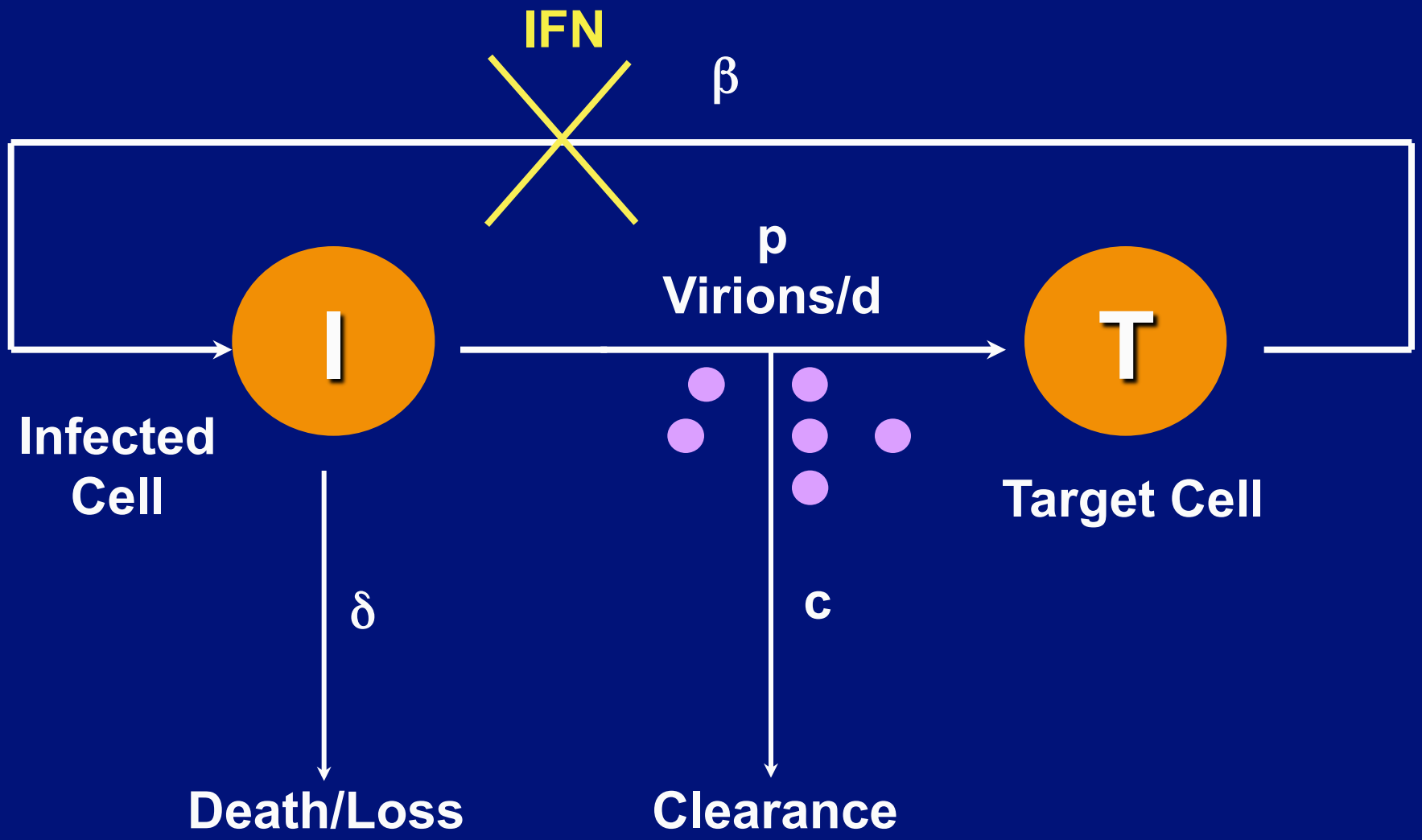


Lam N. DDW. 1998 (abstract L0346).

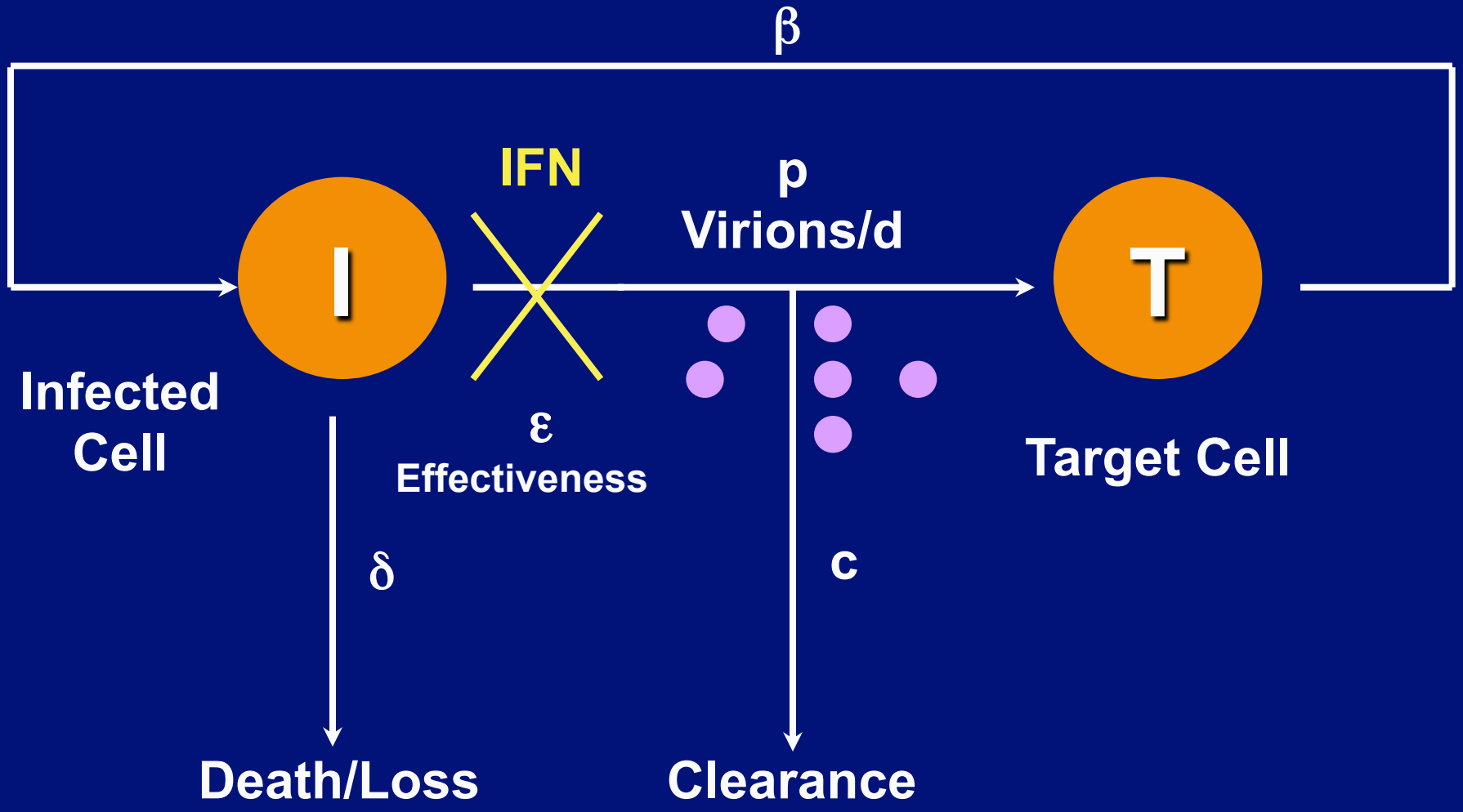
Model of HCV Infection



What If IFN Blocks 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, assume steady state so that $pI_0 = cV_0$. Also, assume at short times, $I = \text{constant} = I_0$, so that

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

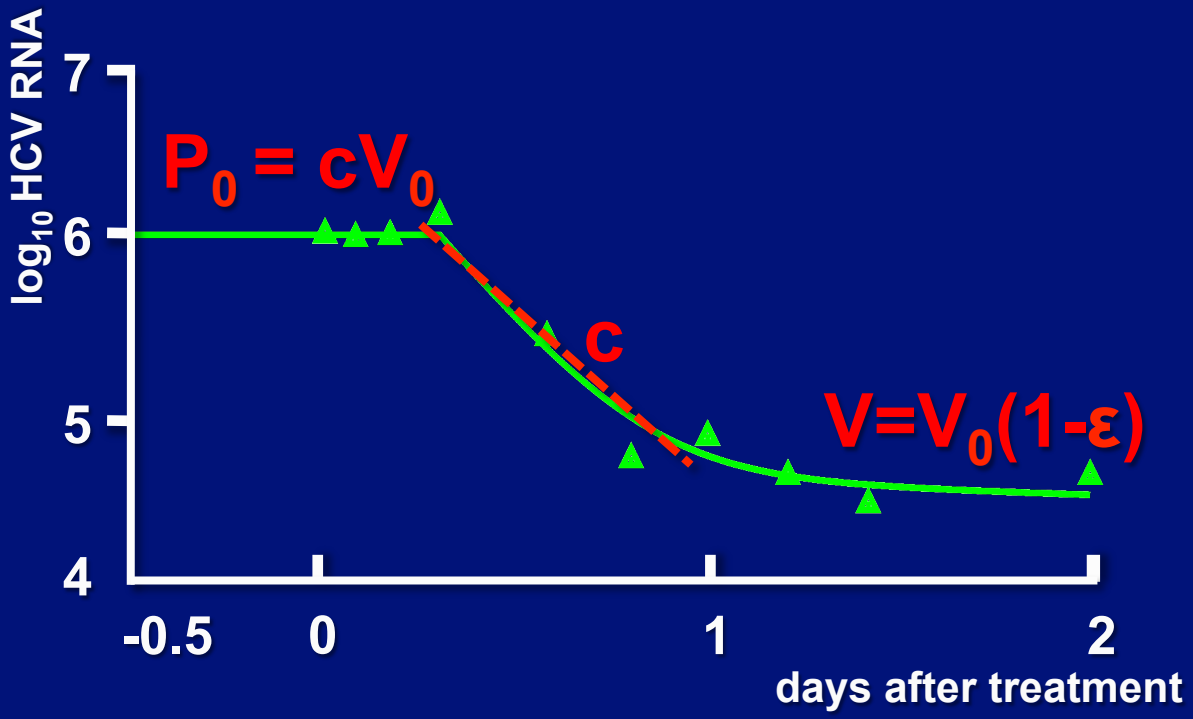
- Model predicts that after therapy is initiated, the viral load will initially change according to:

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

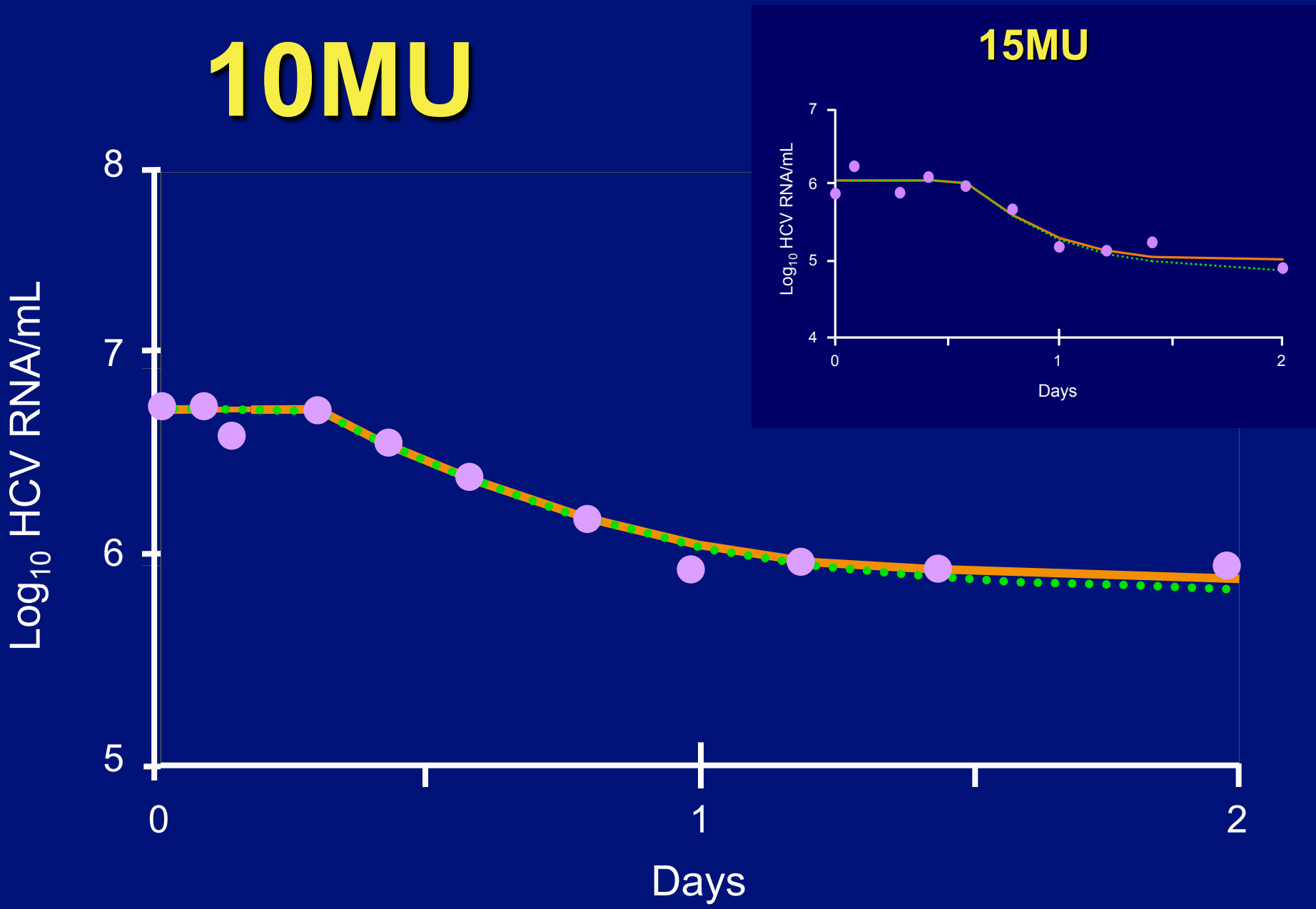
- This equation can be fit to data and **c** and ε estimated.

- This suggests drug effectiveness can be determined within the first few days of treatment!

First phase decline



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



Orange line, assumes I=constant

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)

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 $\beta T_0 = c\delta/p$

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

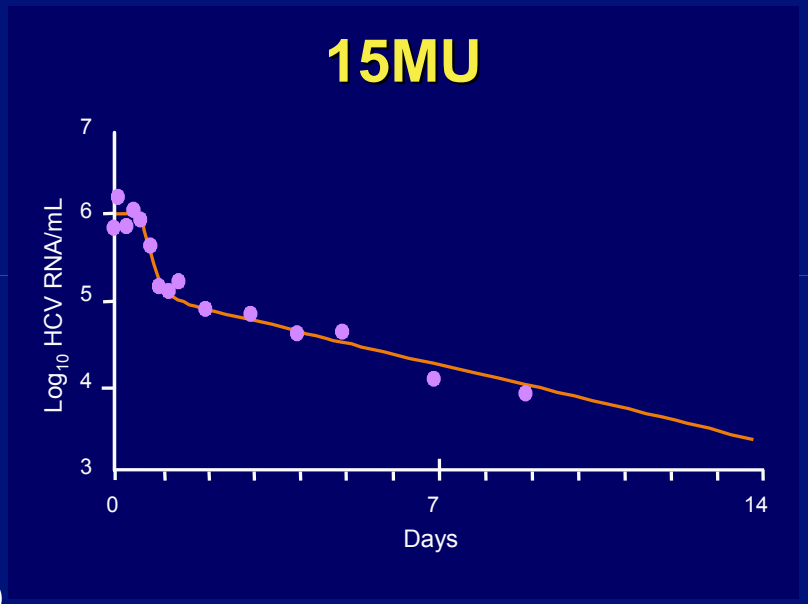
where

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

t_0 = delay between treatment commencement and onset of effect

- When c>>δ, $\lambda_1 \approx c$ and $\lambda_2 \approx \varepsilon\delta$ (death rate of infected cells)

10MU



Slope ~δ

Log10 HCV RNA/mL

8
7
6
5
4

0
7
14

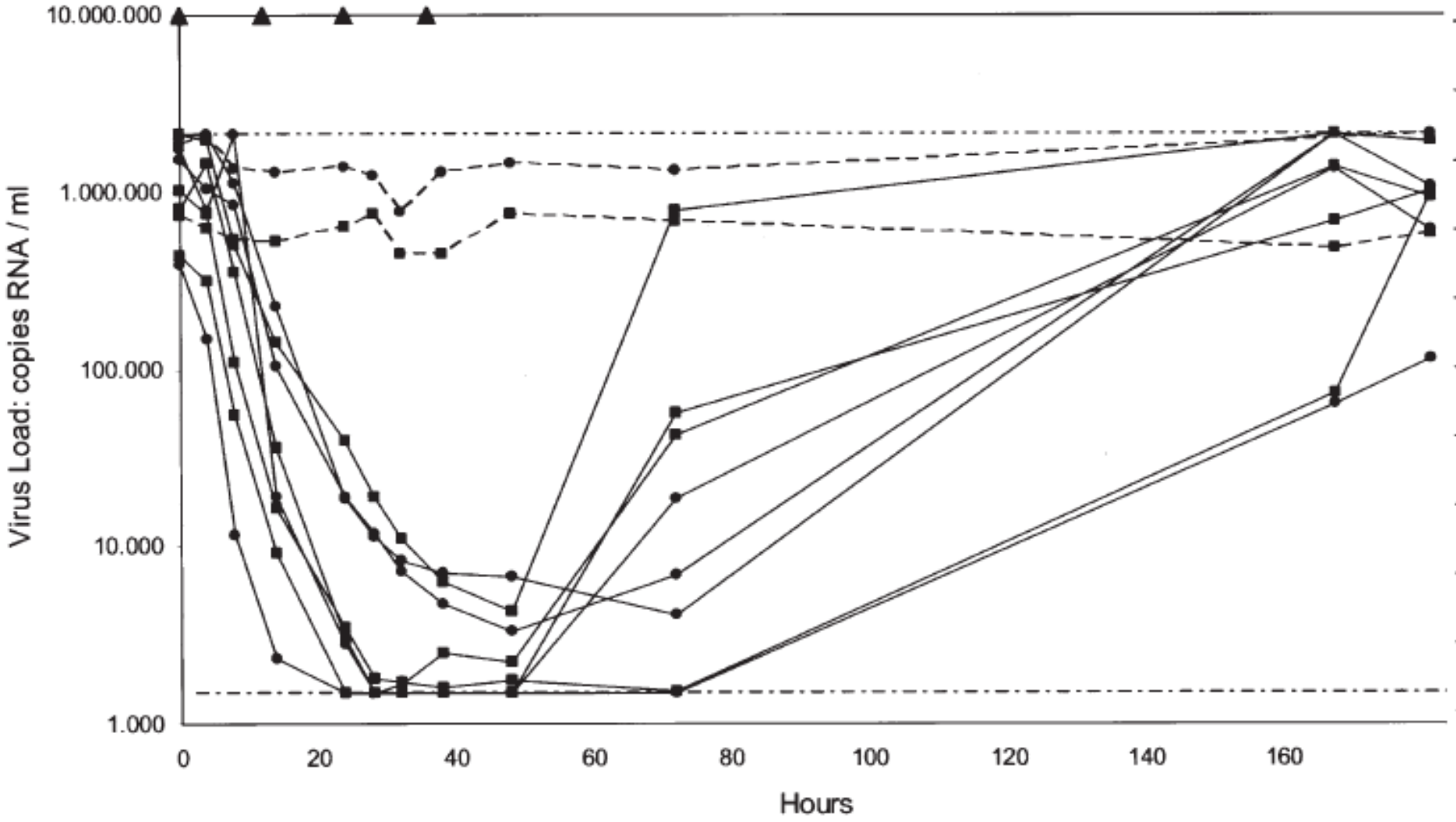
Days

I decays with rate δ

Neumann et al. Science 1998

The first 2 day clinical trial (BILN 2061)

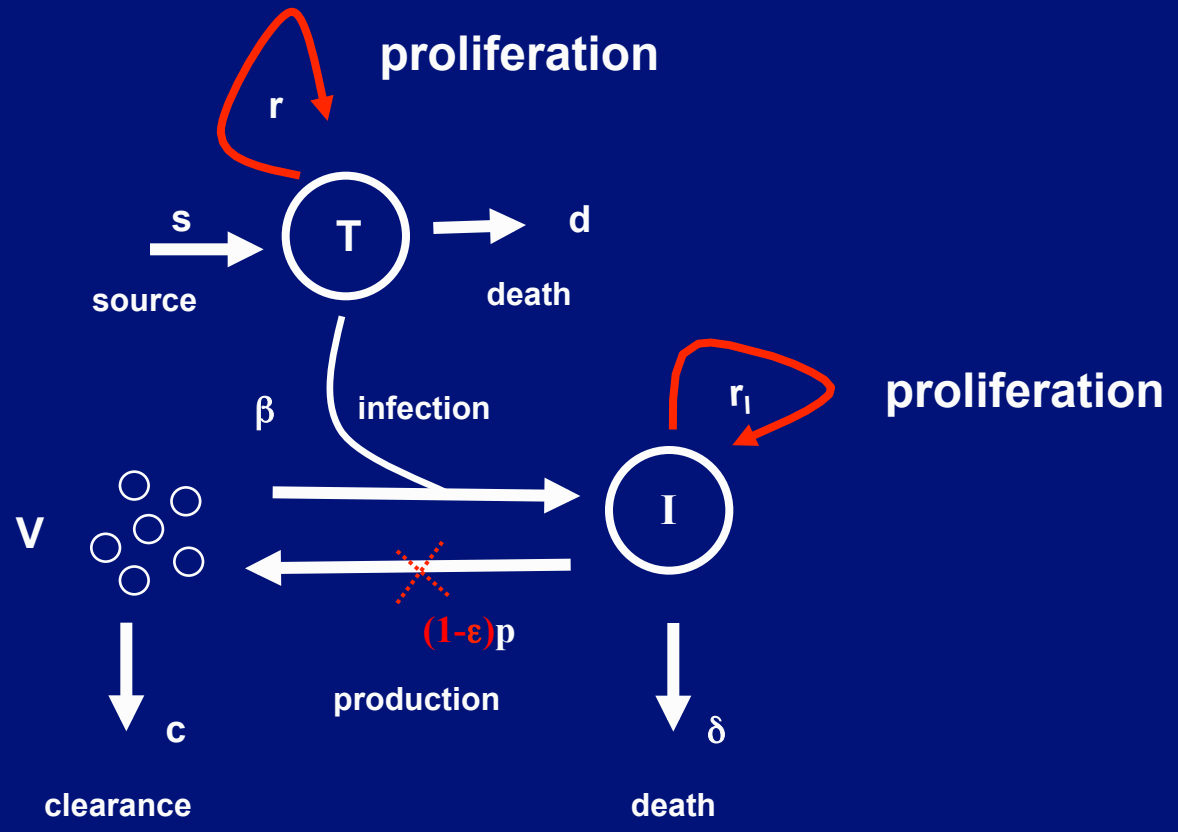
Hinrichsen et al. Gastro. 127: 1347 (2004)



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

Extended Model: Proliferation



Dahari et al., Hepatology 2007; JTB 2007

Model with proliferation

$$\frac{\mathrm{d}T}{\mathrm{d}t} = s + rT\left(1 - \frac{T+I}{T_{max}}\right) - \mathrm{d}T - (1-\eta)\beta VT,$$

$$\frac{\mathrm{d}I}{\mathrm{d}t} = (1-\eta)\beta VT + rI\left(1 - \frac{T+I}{T_{max}}\right) - \delta I,$$

$$\frac{\mathrm{d}V}{\mathrm{d}t} = (1-\varepsilon_p)pI - cV,$$

Model has 2 steady states

- An uninfected steady state, with V=I=0
- An infected steady state:

$$\overline{V} = \frac{(1-\varepsilon_p)p\overline{I}}{c}, \quad \overline{I} = \overline{T}(A-1) + T_{max} - B,$$

$$\overline{T} = \frac{1}{2}\left[-D + \sqrt{D^2 + \frac{4sT_{max}}{rA^2}}\right],$$

where

$$A = \frac{(1-\eta)(1-\varepsilon_p)p\beta T_{max}}{cr}, \quad B = \frac{\delta T_{max}}{r},$$

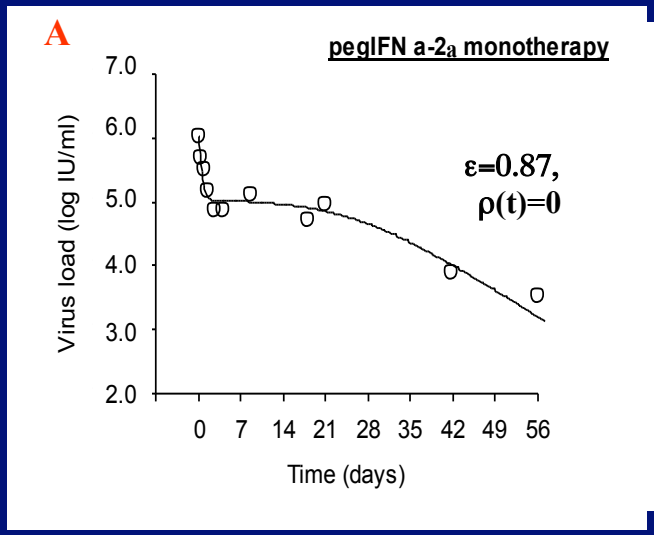
$$D = \frac{1}{A}\left(T_{max} + \frac{dB}{\delta A} - B\left(\frac{1}{A} + 1\right)\right).$$

Transcritical bifurcation

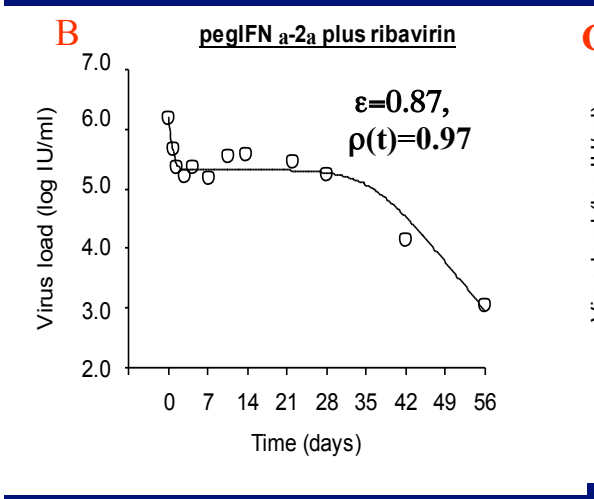
Critical Drug Efficacy

- Drug effectiveness ε, where $1-\varepsilon = (1-\varepsilon_p)(1-\eta)$ is in steady state expressions.
- There exists a drug effectiveness, called the critical effectiveness, ε_c, at which the infected steady state amount of virus goes to zero.
- Thus, with $\varepsilon > \varepsilon_c$ model predicts elimination of virus.

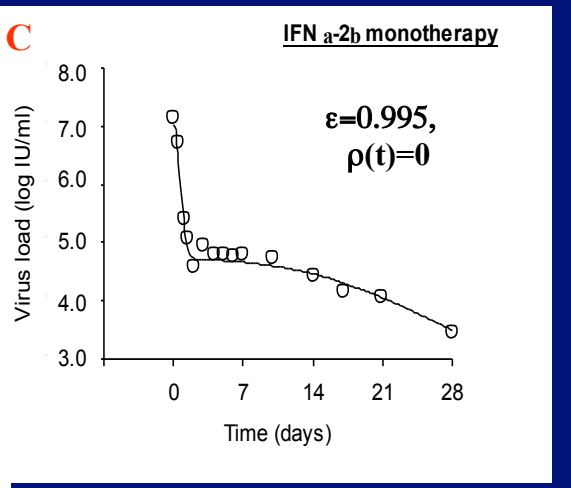
Extended model: Fits to data



Herrmann et al.
(Hepatology 2003)



Herrmann et al.
(Hepatology 2003)



Bekkering et al.
(BMC Gastro 2001)

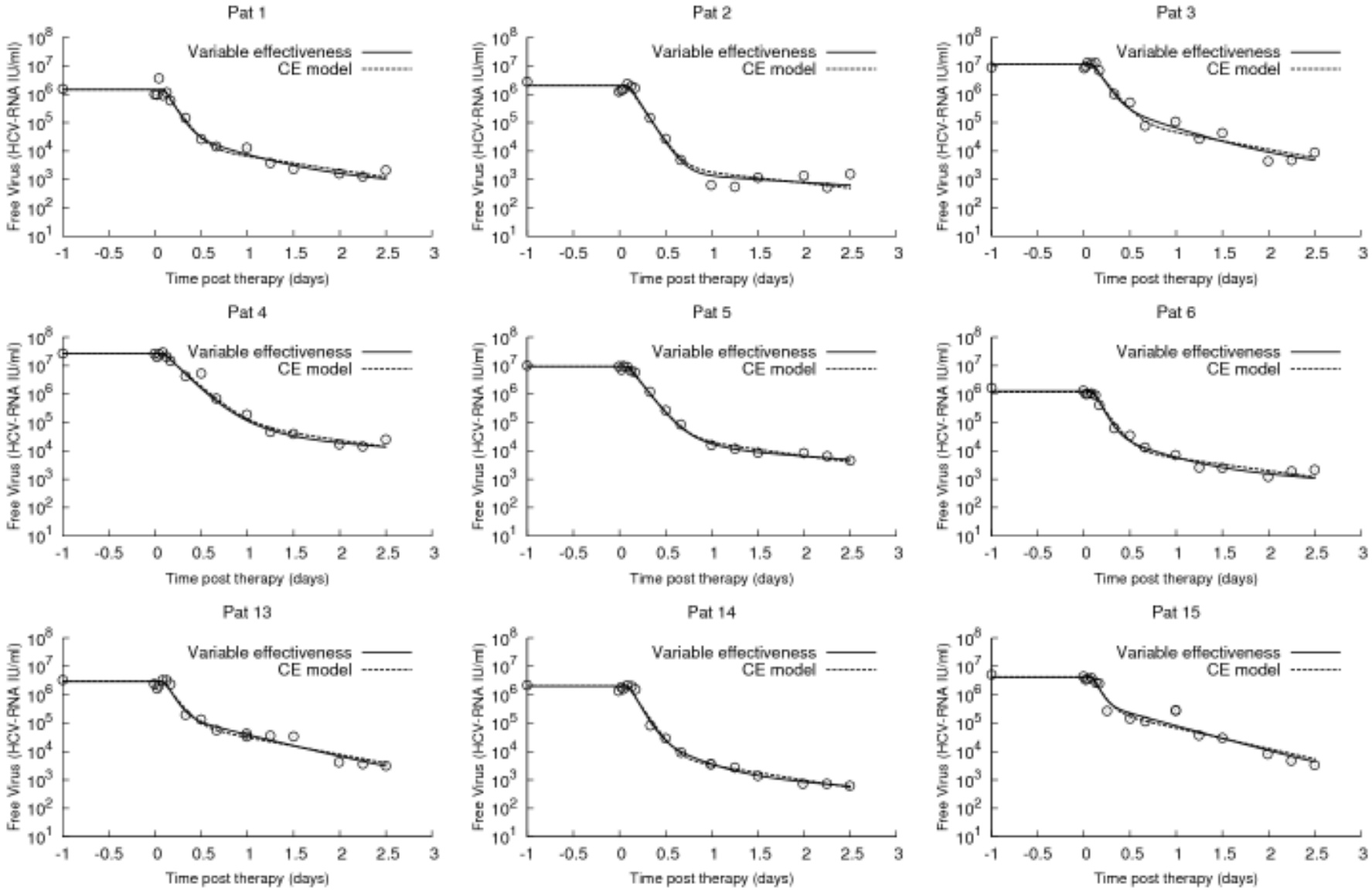
Everything looked neat and viral kinetic theory seemed to fit all available data

- Snoeck et al. Clin Pharm Therap 87:706 (2010) > 2000 pts; predicted SVR - PPV=99.3%, NPV=97.1%
- However, unlike HIV there were no cell culture systems and confirming predicted parameter estimates was difficult.
- Discrepancies with theory started arising when used for new therapies.

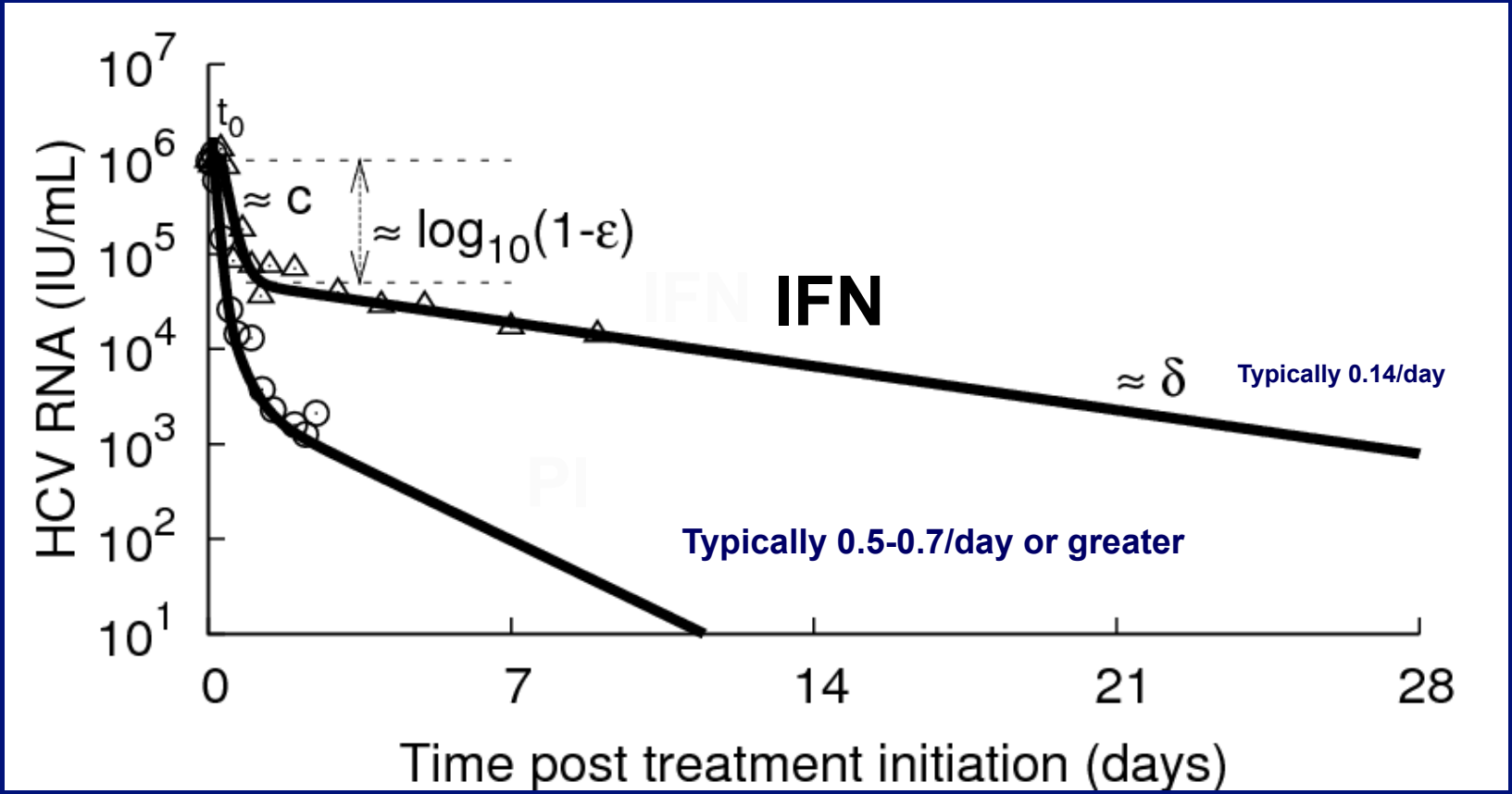
New Therapies

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

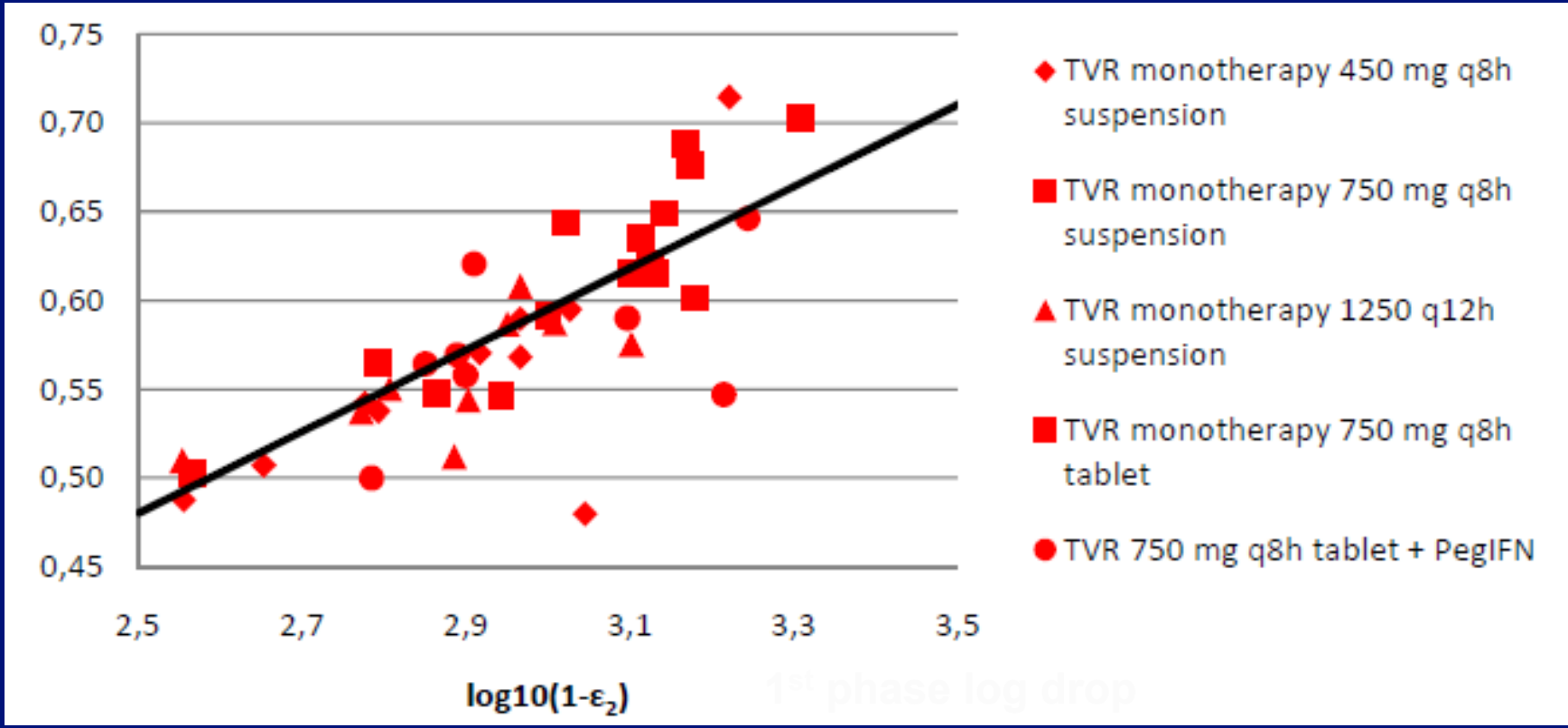
Model fits to data (n=44) from telaprevir monotherapy trial



IFN vs HCV protease inhibitor = PI (telaprevir)



Second phase slope, δ, higher than seen with IFN and correlates with antiviral efficacy, ε



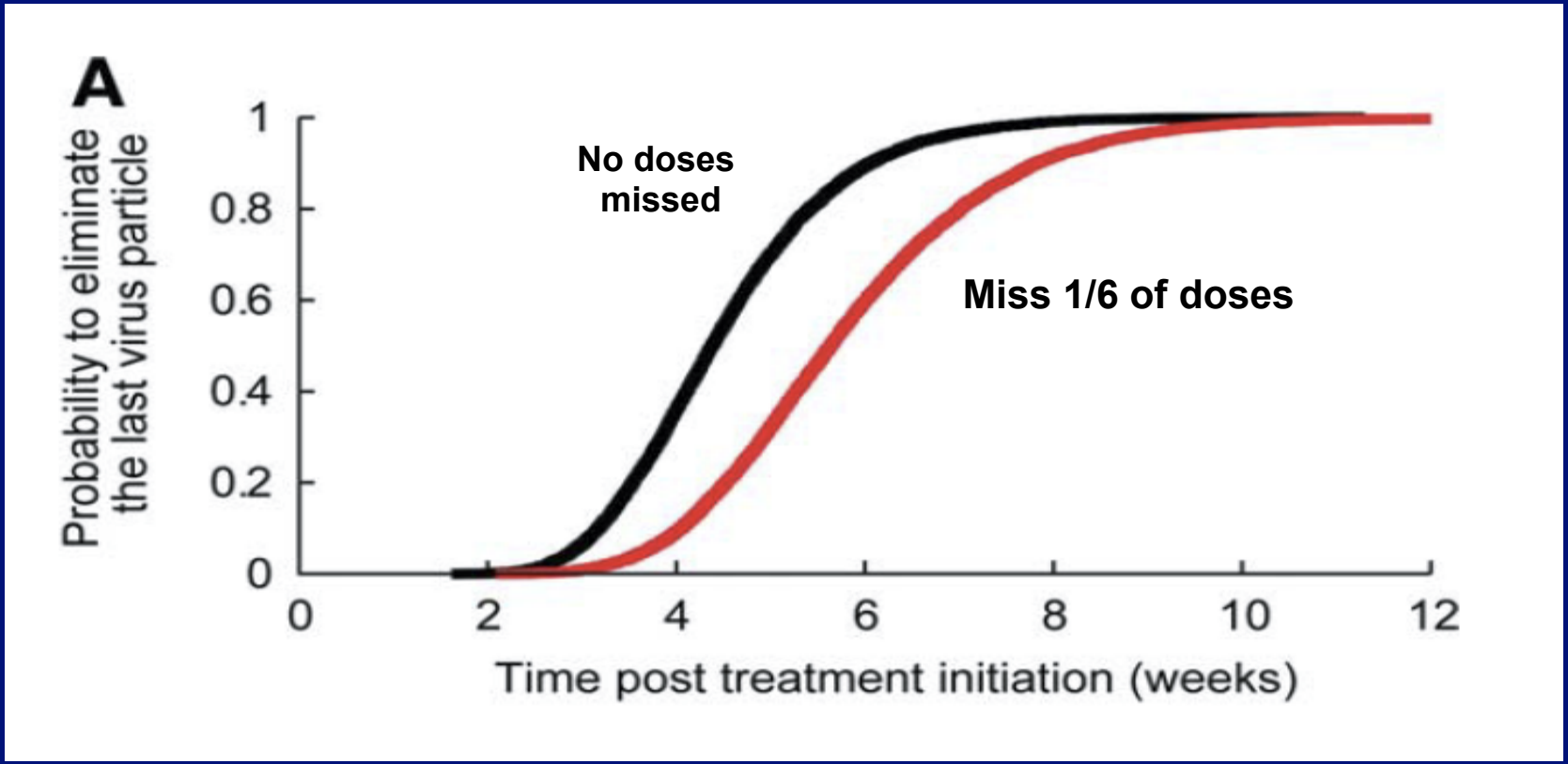
r=0.79, p< 0.001 Guedj et al Hepatol 2010

Current theory

- δ is rate of infected cell death and should be independent of drug effectiveness, ε.
- However, if δ increases with ε it has great practical implications for shortening treatment.
- Believe mechanism is that with high effectiveness cells can be “cured” of infection – viral RNA lost – so infected cells lost by cure and death.

Time to reach < 1 virus

(Current therapy 24 – 48 weeks)



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!!!

Current theory

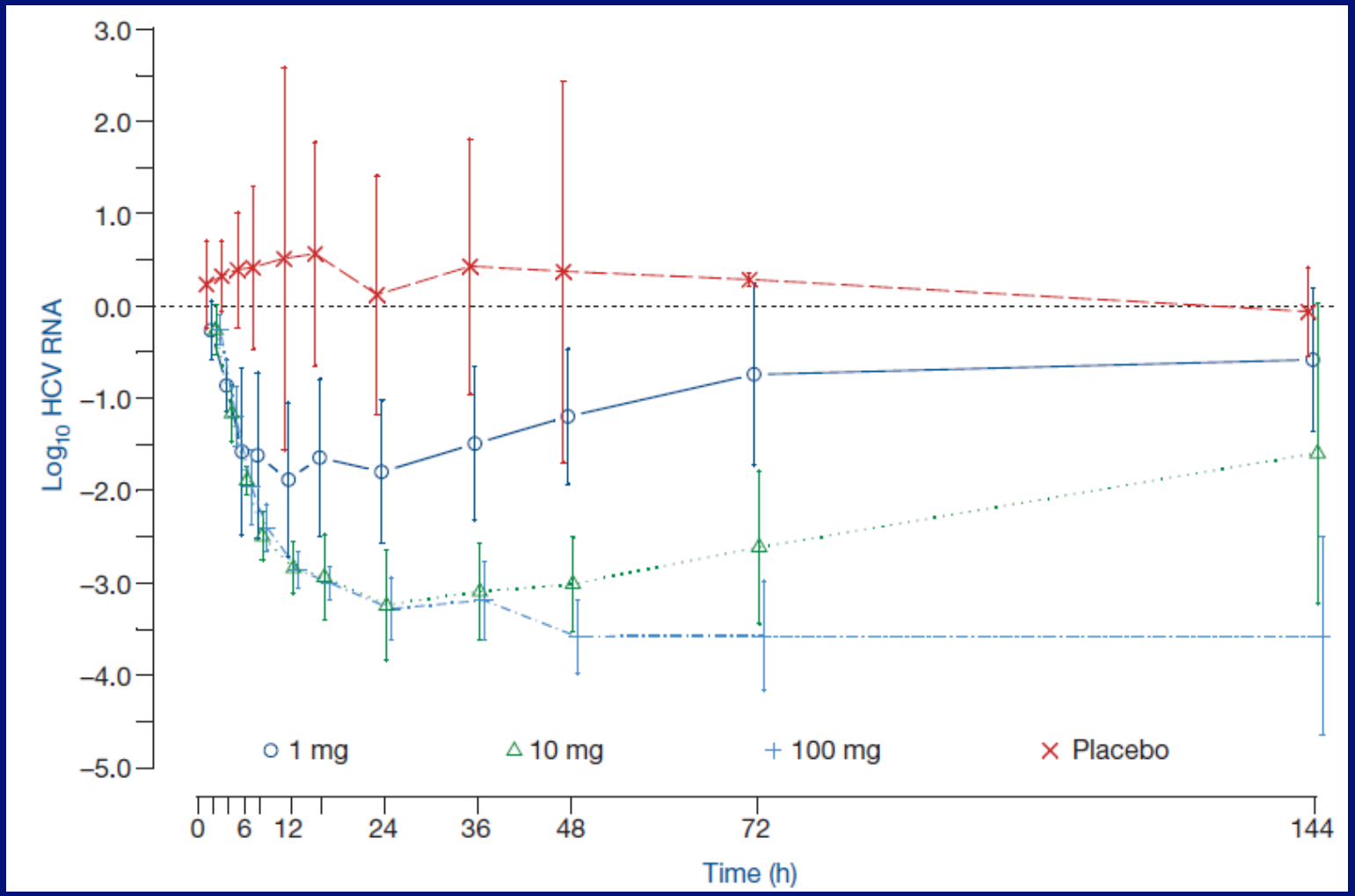
- In current theory, c, the slope of first phase decay, is the rate of viral clearance by processes such as phagocytosis, and hence should be independent of the antiviral drug used.

Estimated rate of virus clearance, c, may change with the drug being used

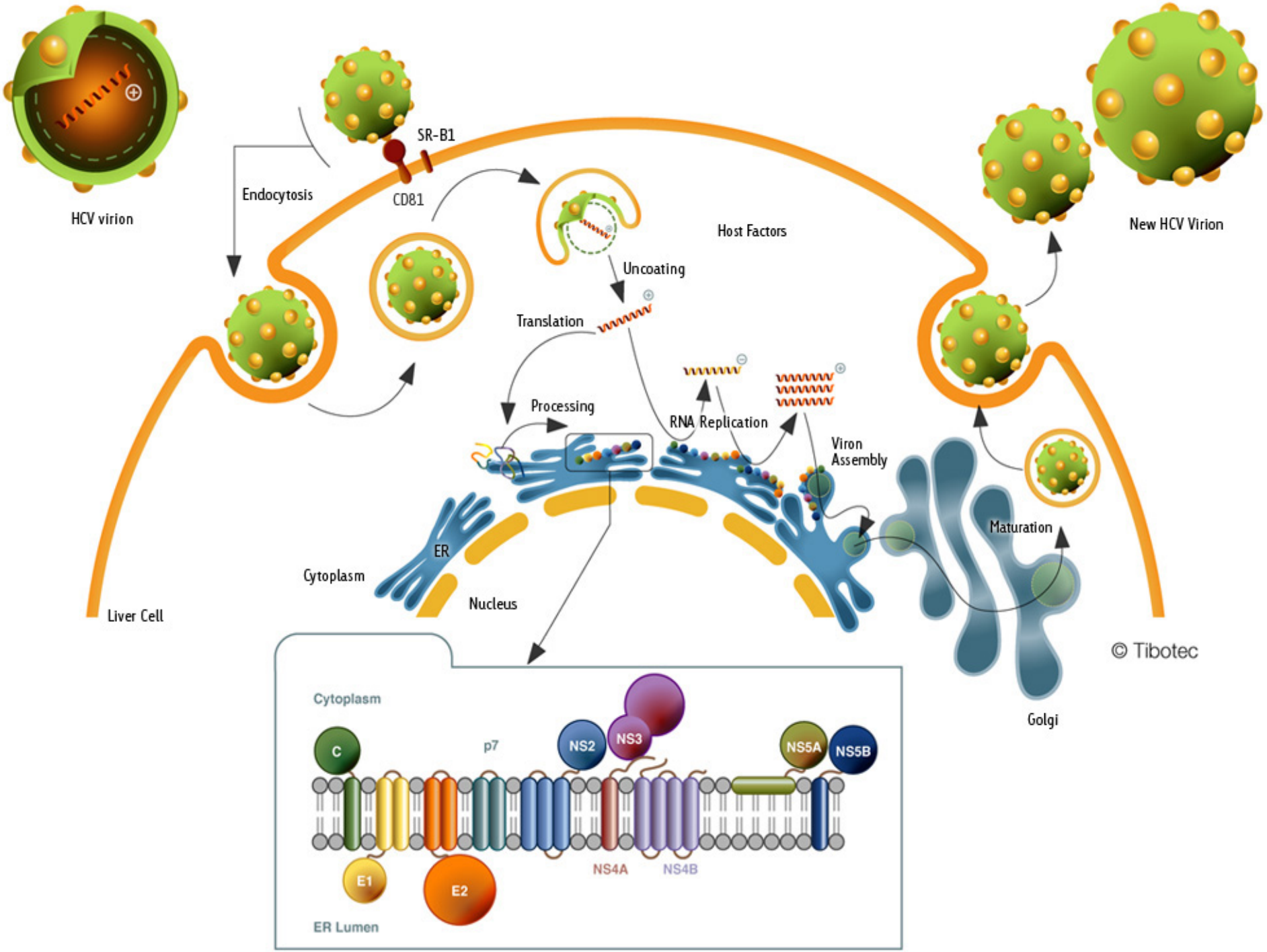
- IFN-therapy $c \sim 6\text{-}9\ d^{-1}$
- Telaprevir $c \sim 12\ d^{-1}$
- BMS-790052 (daclatasvir) $c \sim 23\ d^{-1}$ (NS5A inhibitor)

Standard models can not account for this.

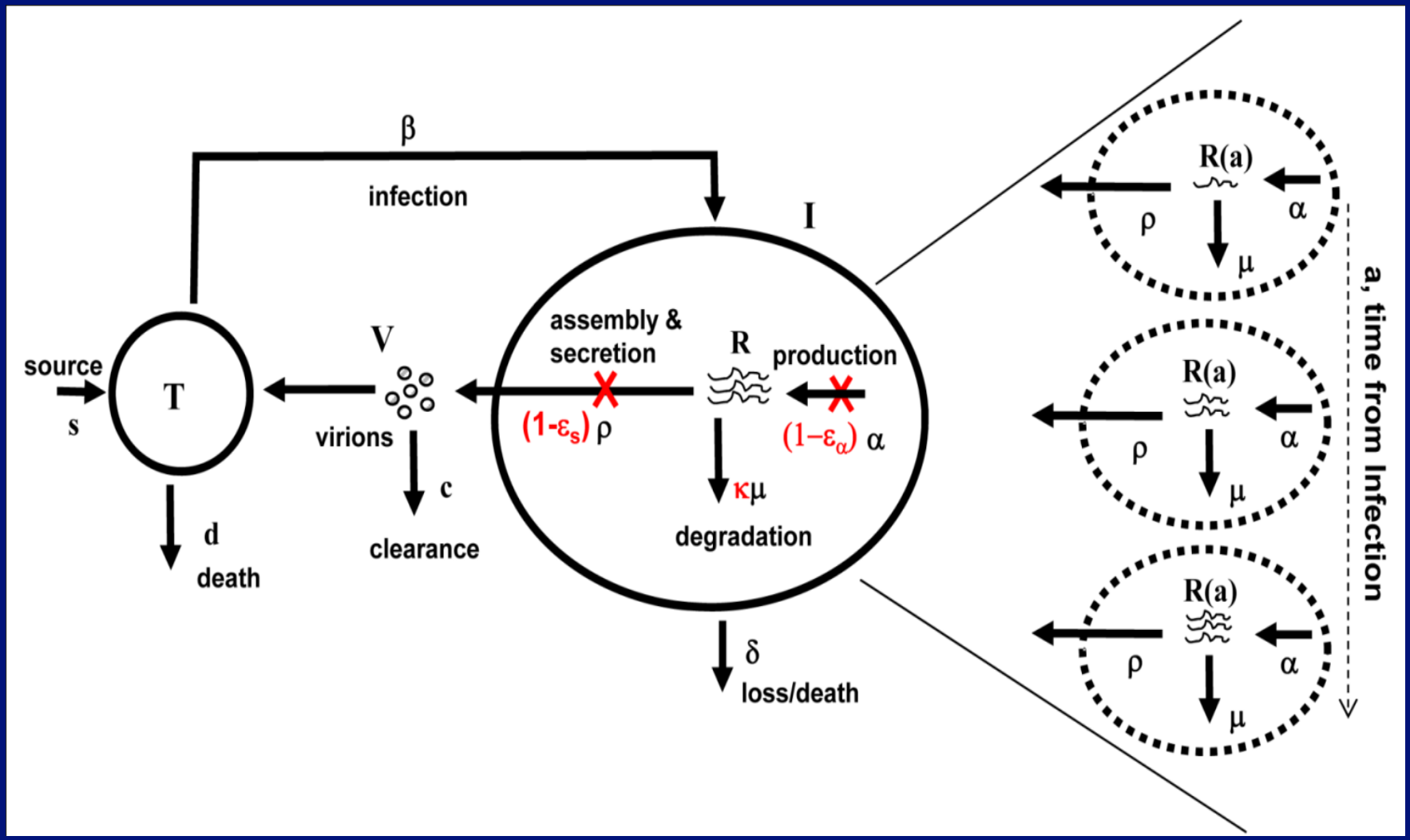
NS5A inhibitor; c > 20 d^{-1}



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
E1
E2
NS4A
NS4B
ER Lumen

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

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

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

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

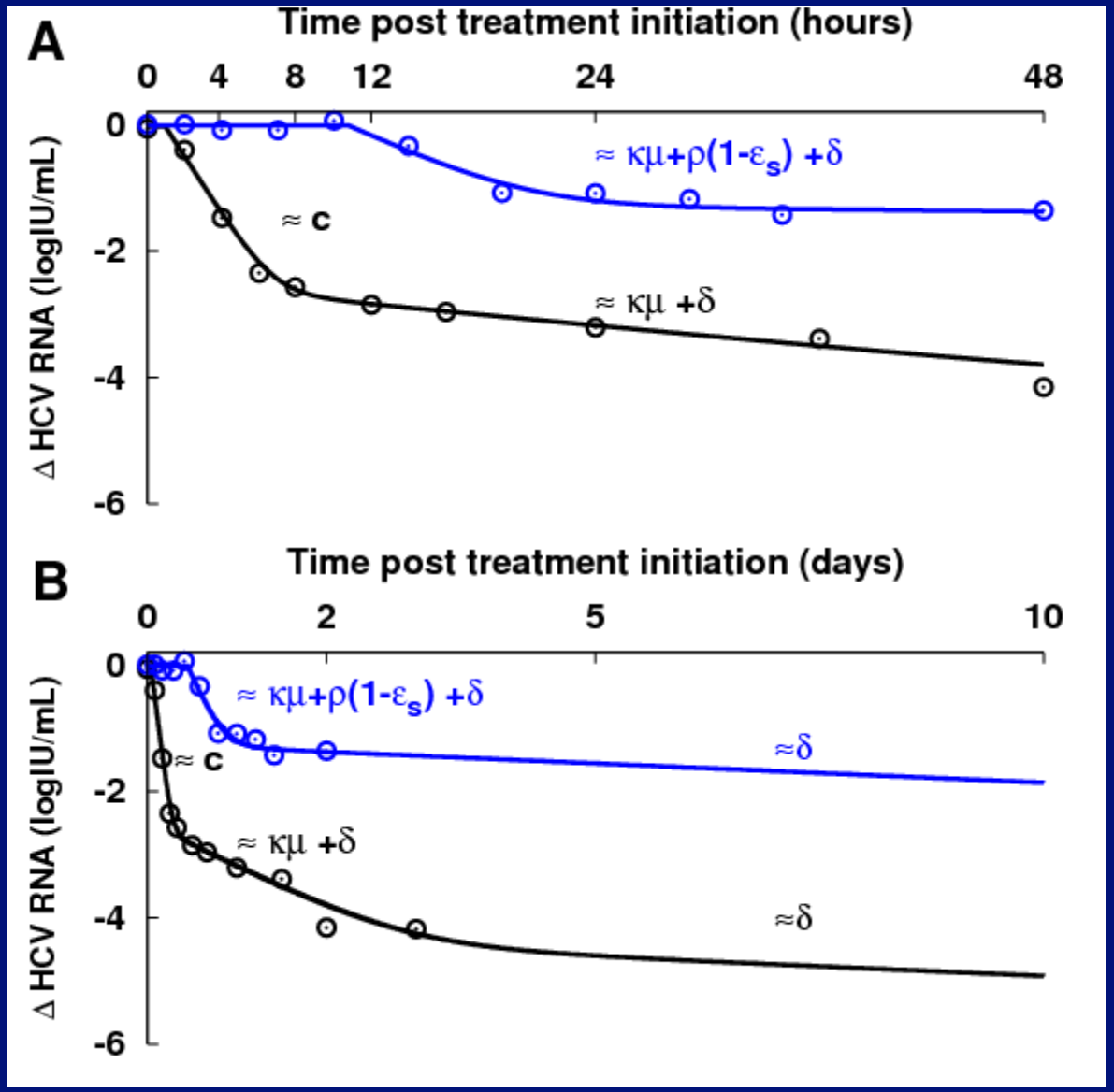
$$\frac{\partial R}{\partial t} + \frac{\partial R}{\partial a}\frac{da}{dt} = (1-\varepsilon_\alpha)\alpha - ((1-\varepsilon_s)\rho + \kappa\mu)R$$

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

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

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

Model fits data and explains why different drugs gave different values for c



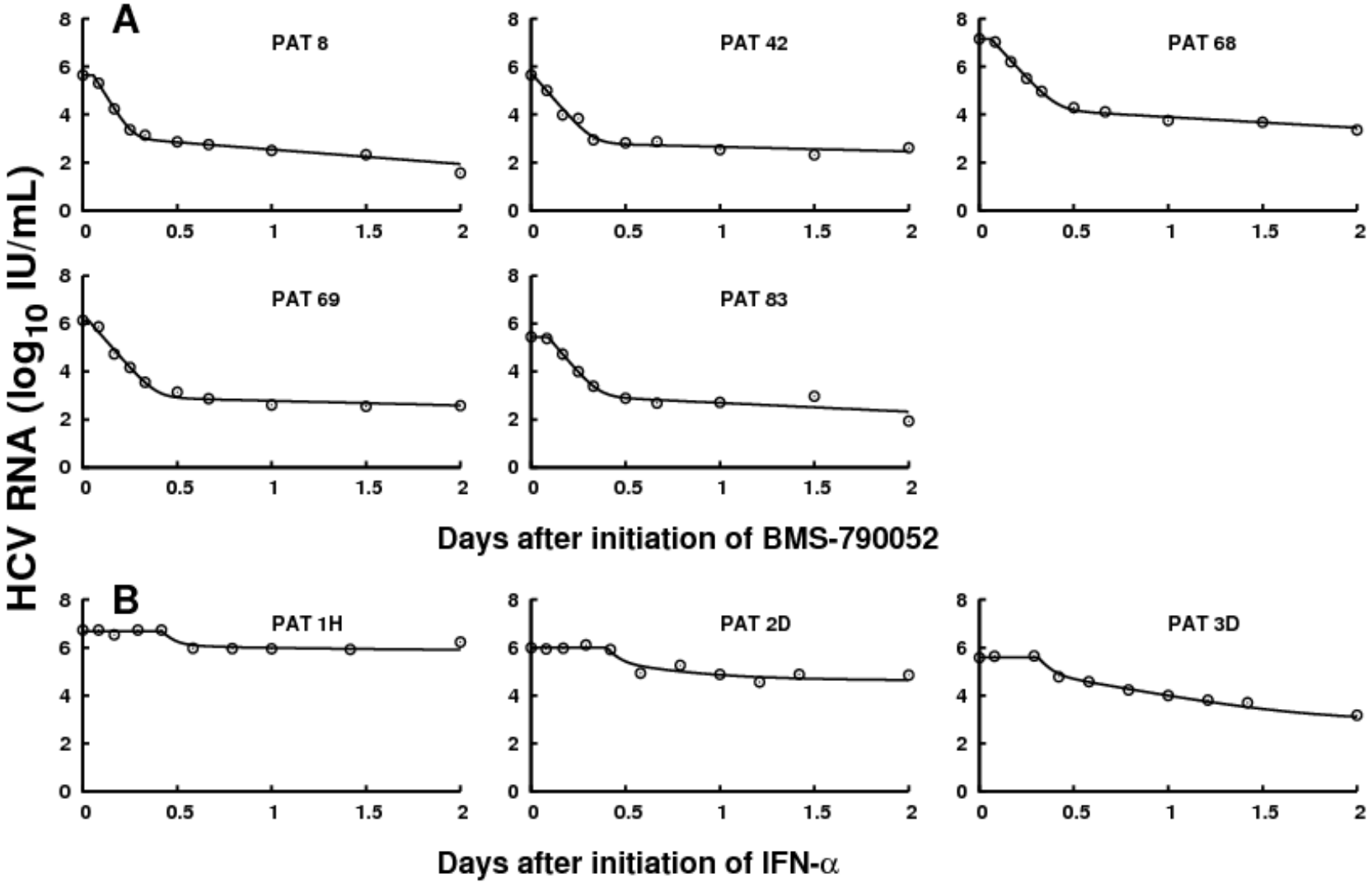
IFN

BMS-790052

For IFN first phase ~ Ae^{-ct} not visible A<1, c =23 d^{-1}

For BMS, A much larger and decay **is visible**

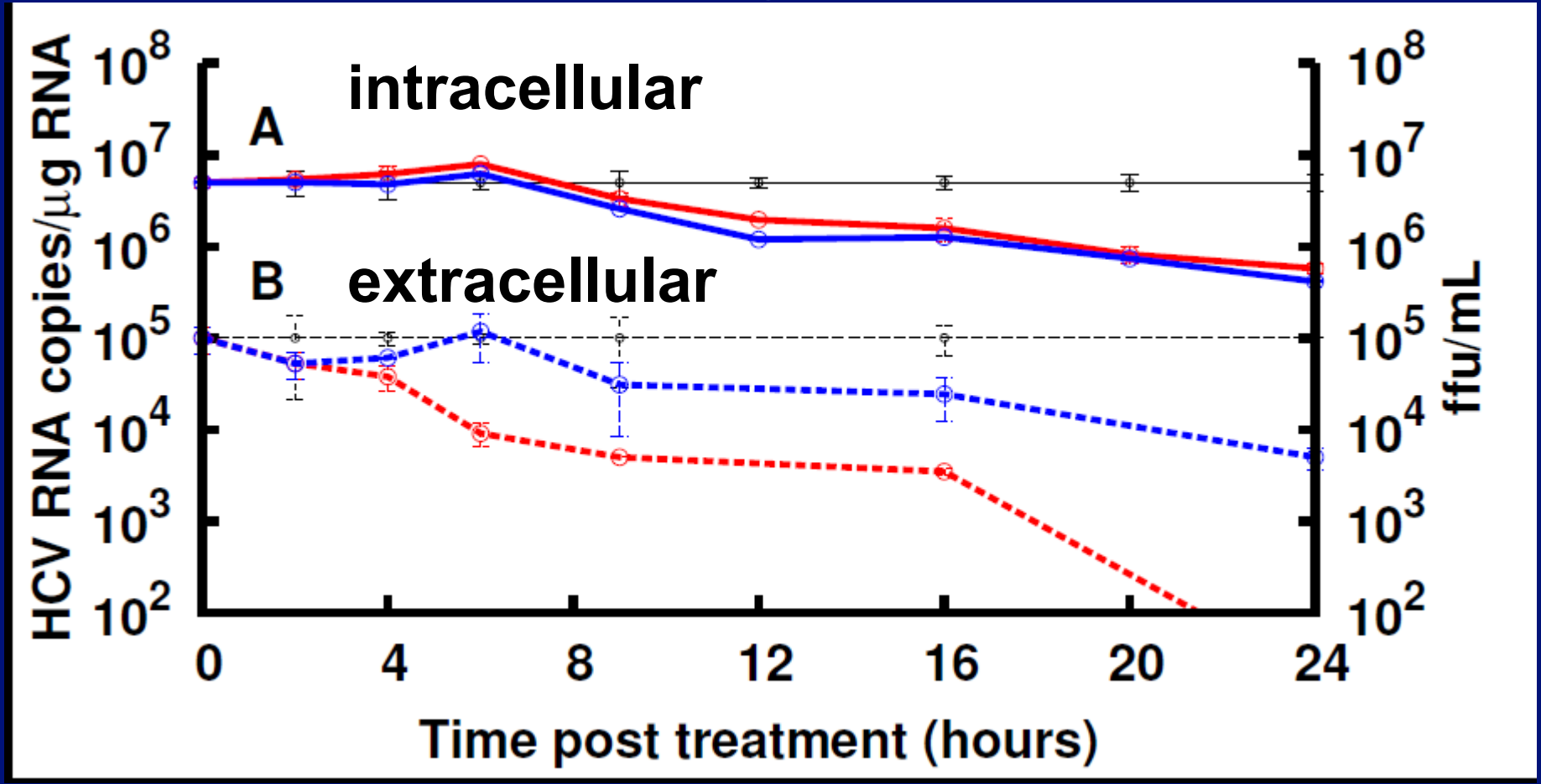
Fits to patient data



ε_α (BMS) = .993, ε_s (BMS)=.998, ε_α (15 MU IFN) = .98, ε_s (IFN) = .41

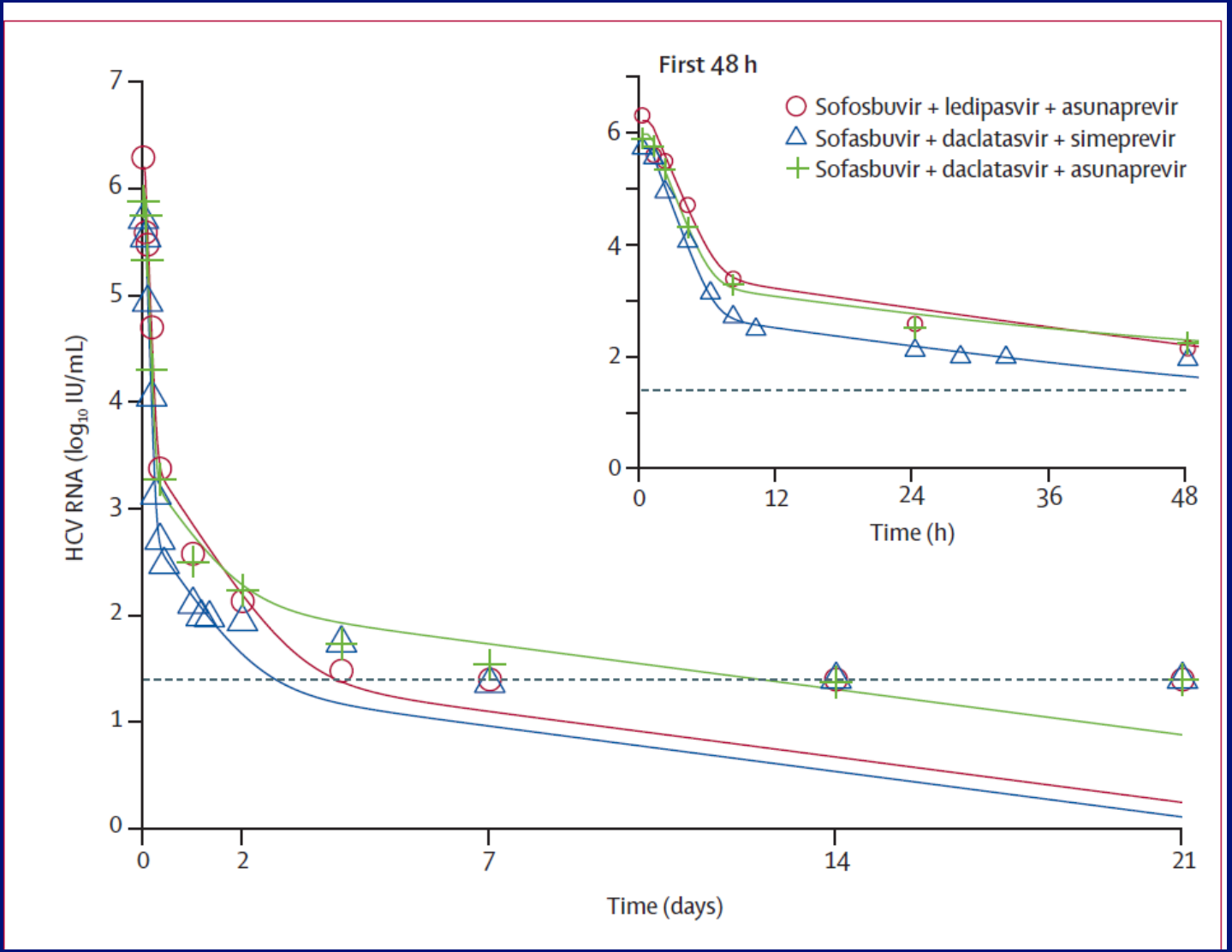
In vitro confirmation that daclatsvir inhibits secretion

Red = BMS Blue = Polymerase inhibitor, NM107



Guedj et al. PNAS (2013)

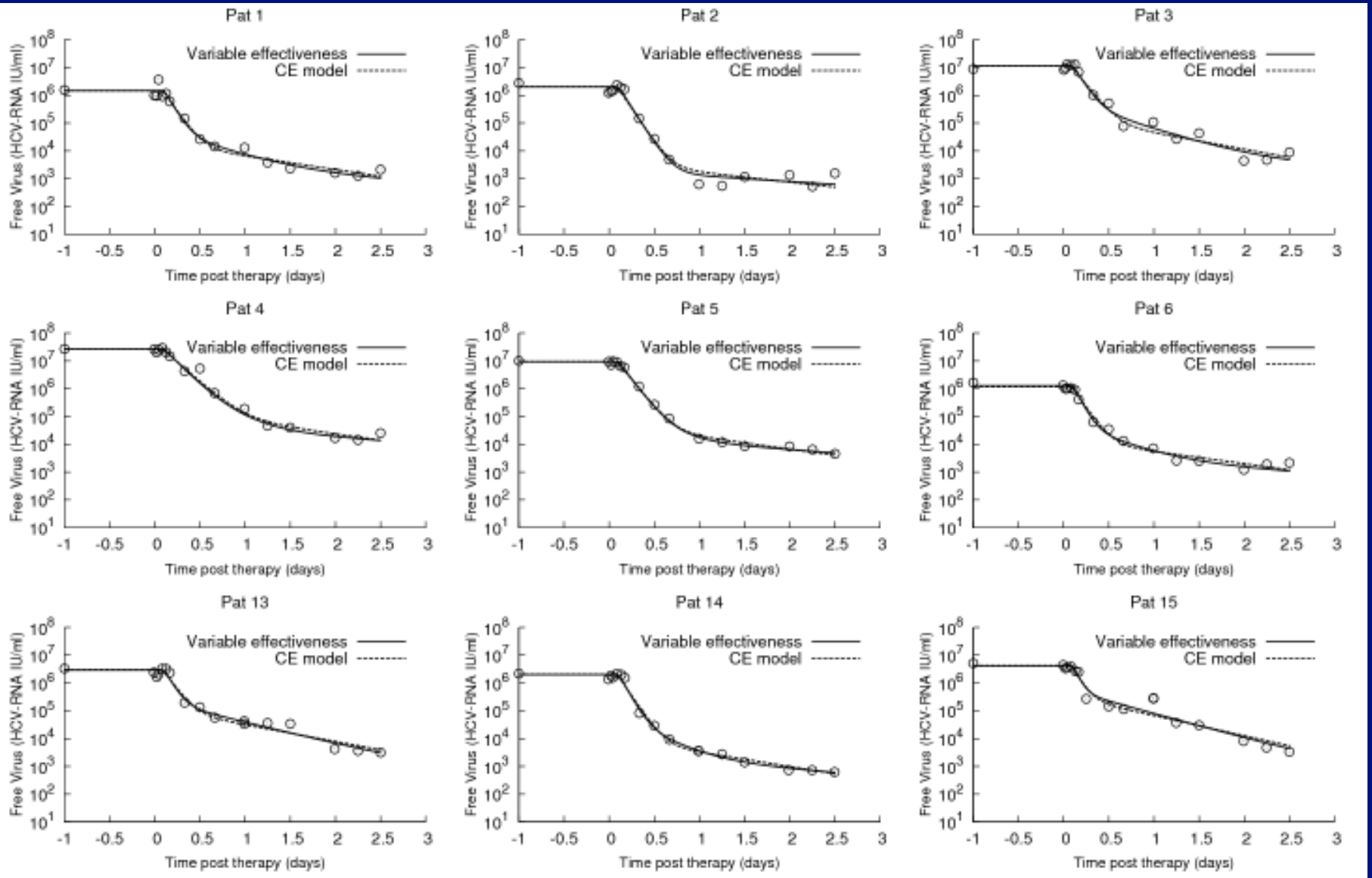
3 drug combo therapy

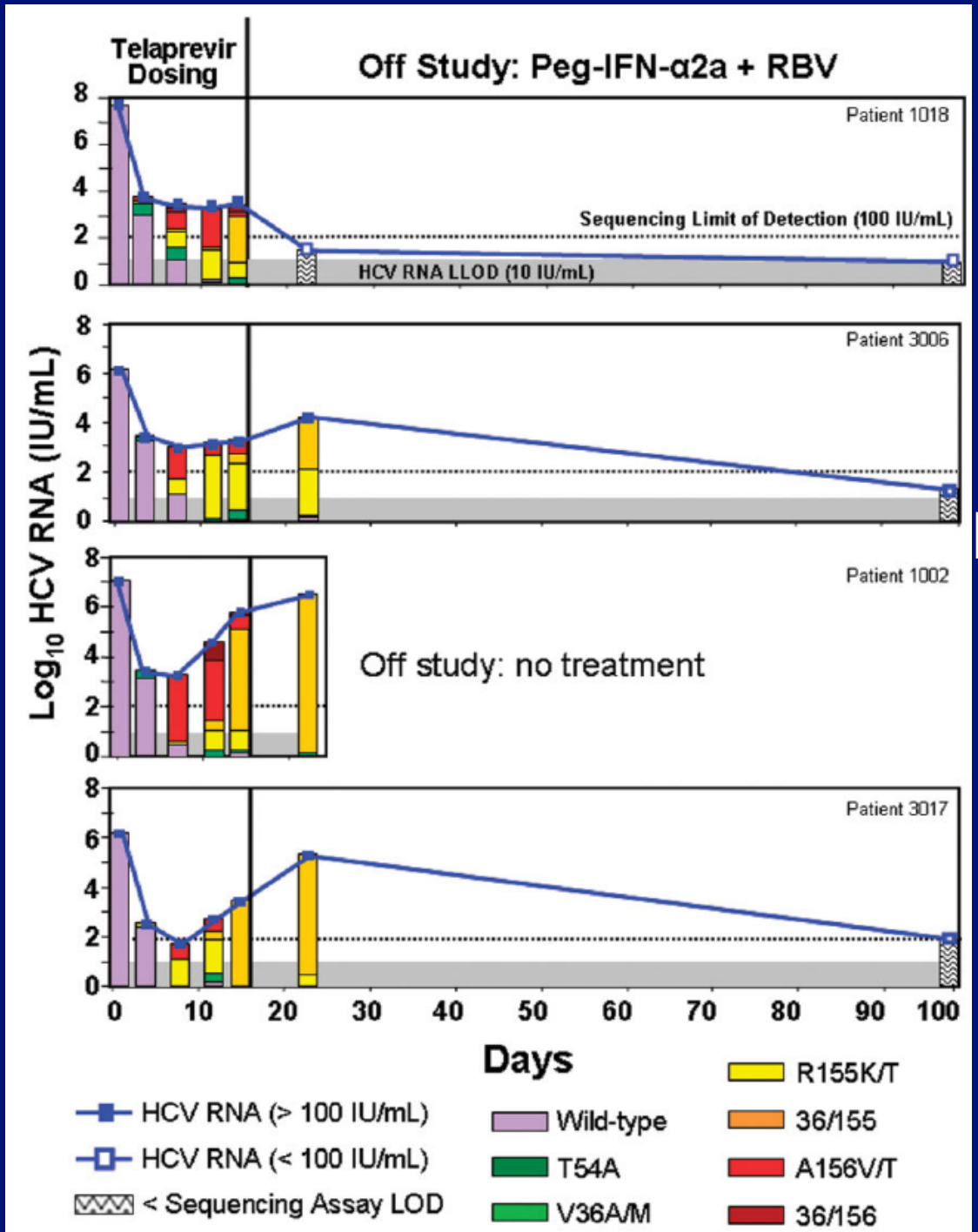


Lau et al. Lancet GH 1: 97-104 (2016)

New antivirals lead to drug resistance

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





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

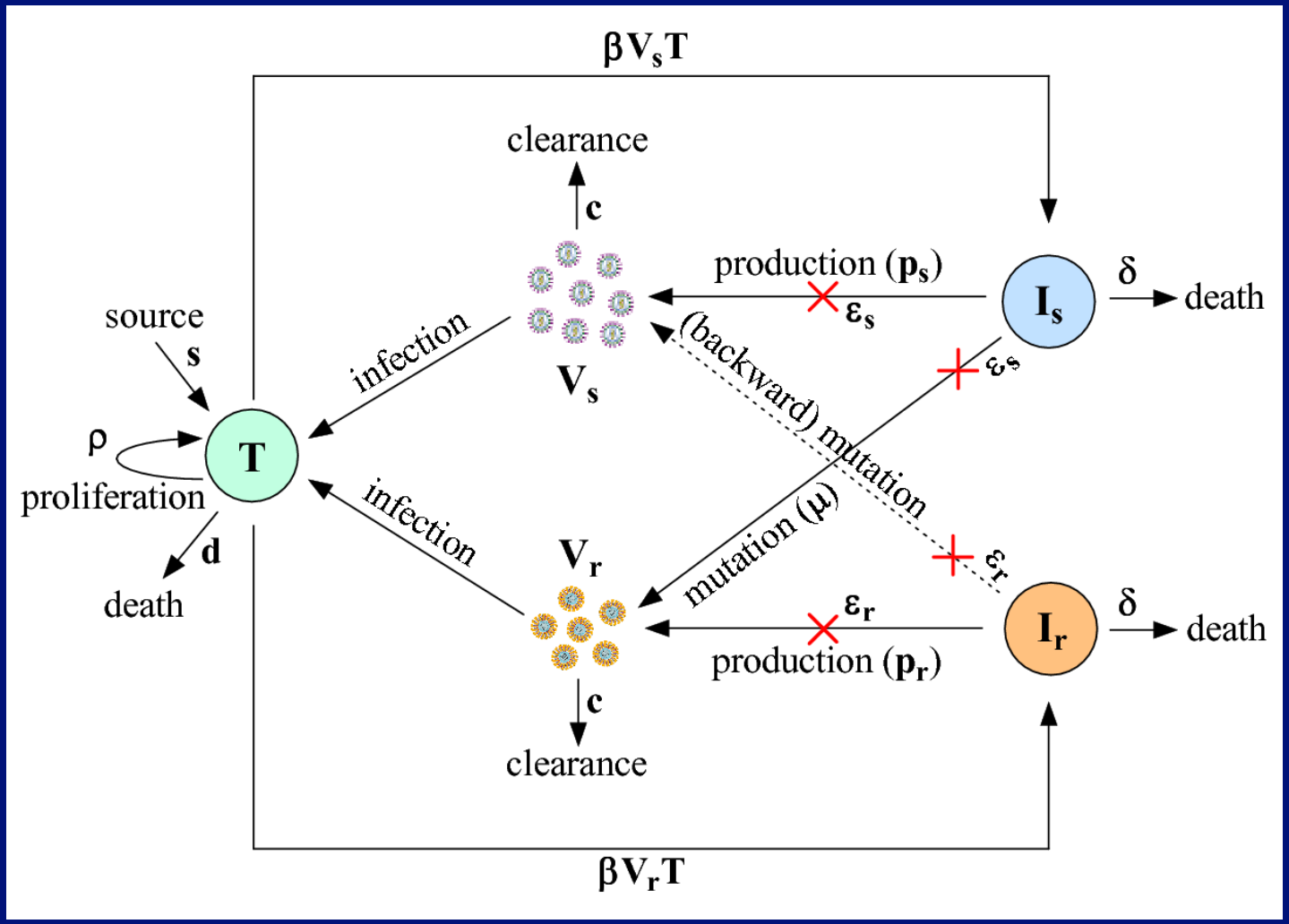
By day 14, close to 100% is resistant

Kieffer et al. Hepatol 2007 genotype 1a pts

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

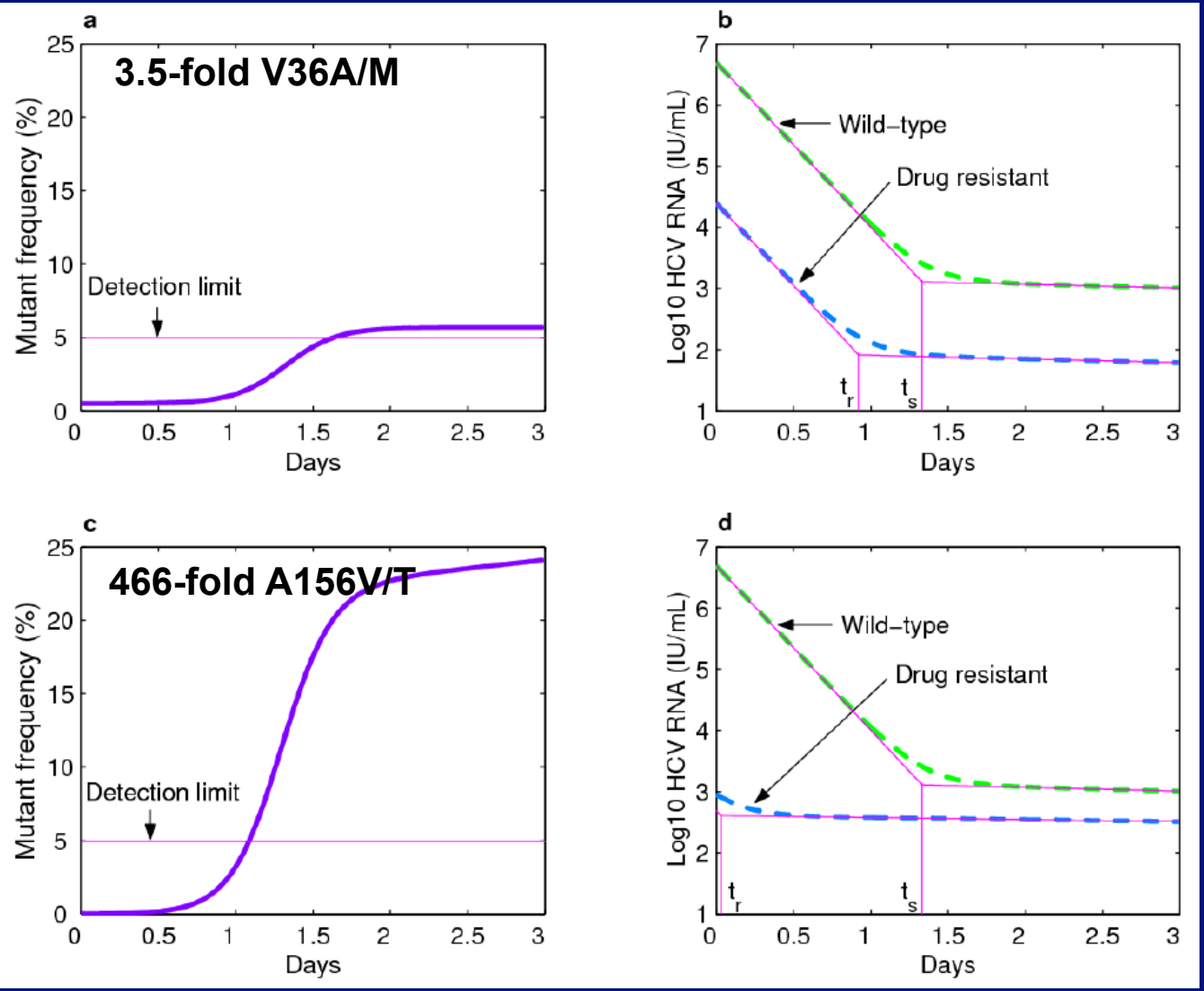
Base changes	Expected number (10^{12}/day) HCV RNA	# possible variants	% produced/ day
1 (9%)	9×10^{10}	3×10^{4}	100%
2 (0.45%)	4.5×10^{9}	4.5×10^{8}	100%
3 (0.015%)	1.5×10^{10}	4.5×10^{12}	.003%

Two-strain model

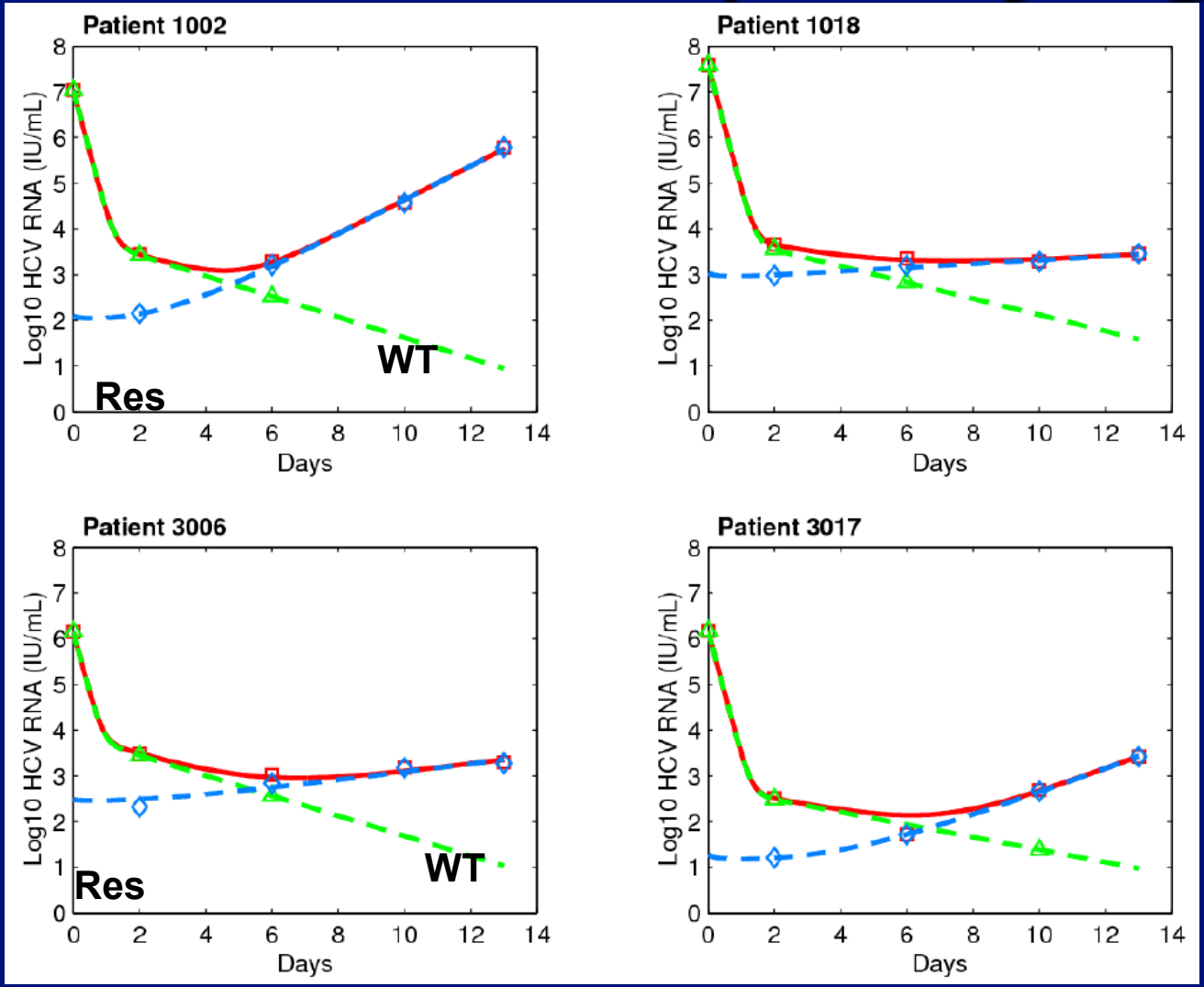


V_s =drug sensitive, V_r= drug resistant

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



Viral rebound (T varying)



Rong 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

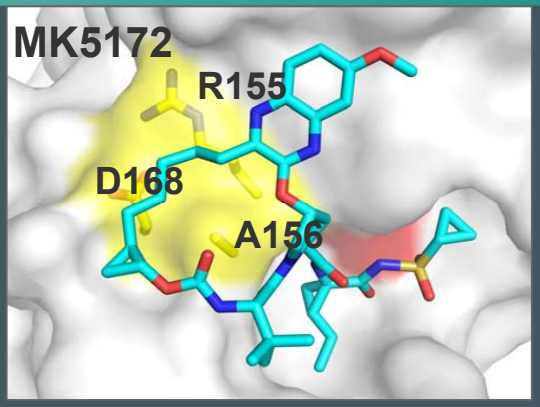
Drug efficacy of telaprevir against wt and resistant virus

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 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

Introduction



- MK-5172 is a once-daily macrocyclic next-generation HCV Protease Inhibitor
  - Expected to provide a high barrier to resistance development in treatment-naïve patients
  - Has potent activity against early generation HCV PI resistant variants
  - Pangenotypic (with EC_{50} values ranging from 1-200nM against all six HCV genotypes *in vitro*)

MERCK
Be well

MK-5172 Monotherapy Study Design

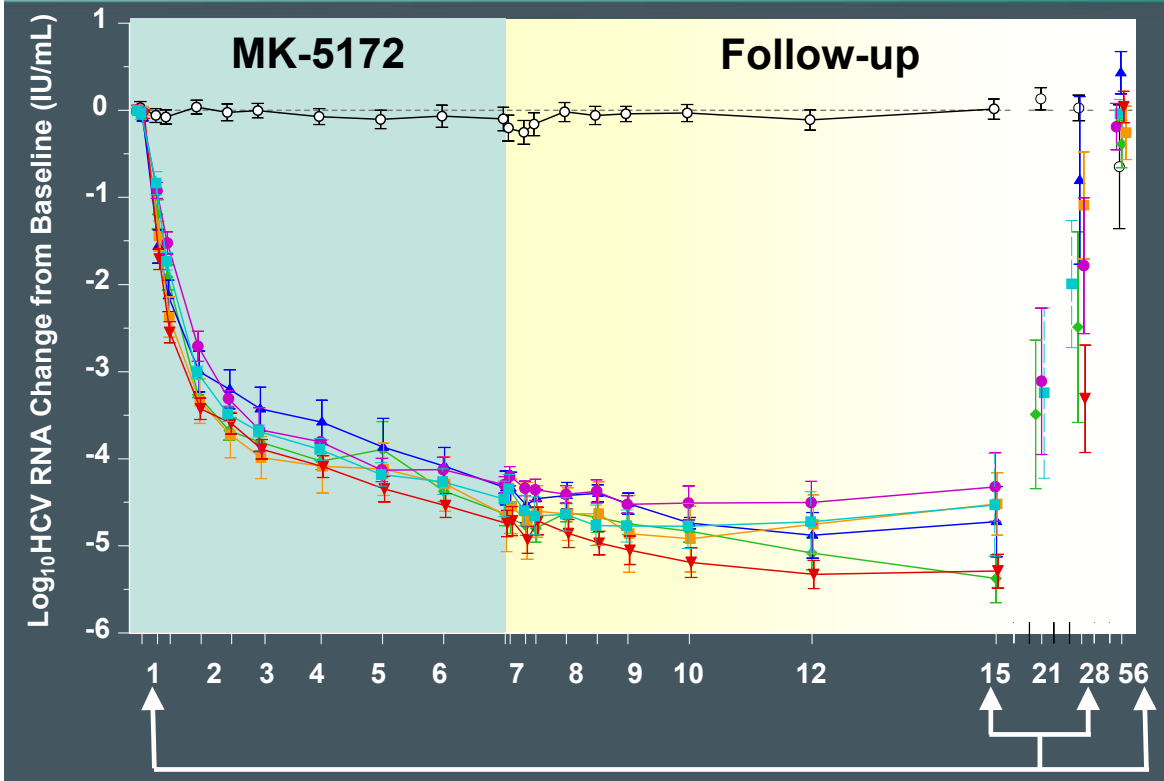
1-3 placebo patients in each arm

Samples Collected for Population Sequence Analysis

MERCK
Be well

Petry et al., American association for the study of Liver Diseases (AASLD, 2011), San Francisco, CA
Fraser et al., HEPDART (2011) Kauai, HI

MI-5172 Monotherapy Results by Dose in Genotype 1 Treatment-Naïve Patients



Dose	Pts (N)	Pts with VL <LOQ (n)
PBO	8	--
50	5	2
100	5	4
200	5	3
400	5	5
600	5	3
800	15	13
Total	40	30

Population Sequencing if VL >1,000 IU/ml

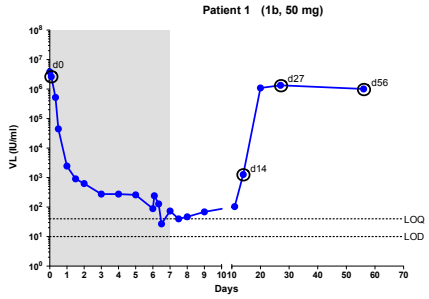
Placebo Pooled Across Panels. 1/2 LOD used to impute values that were BLOD, 1/2 LOQ used to impute values that were BLOQ; PBO = Placebo

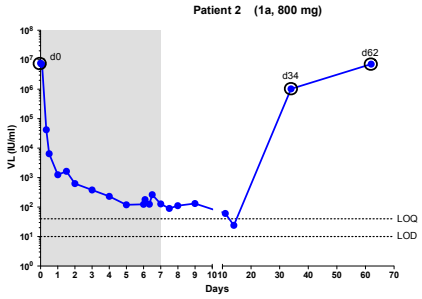
Petry et al., American association for the study of Liver Diseases (AASLD, 2011), San Francisco, CA
Fraser et al., HEPDART (2011) Kauai, HI

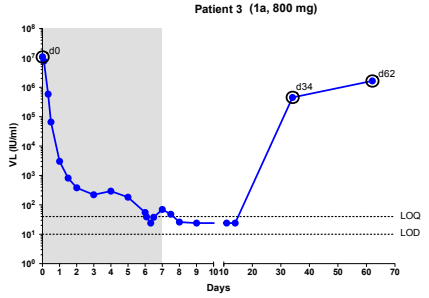
MERCK Be well

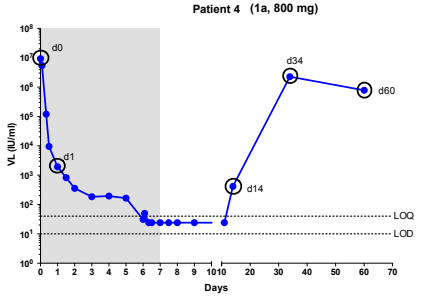
Treatment period

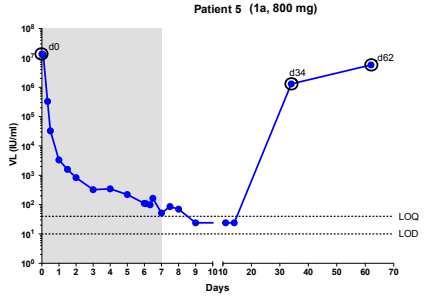
Time point sequenced

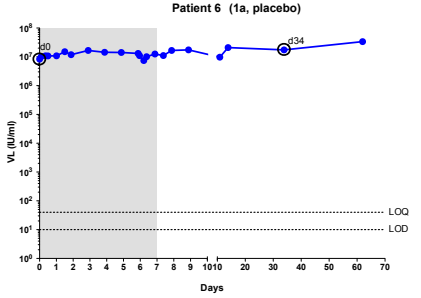


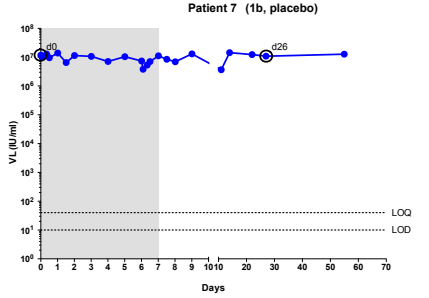


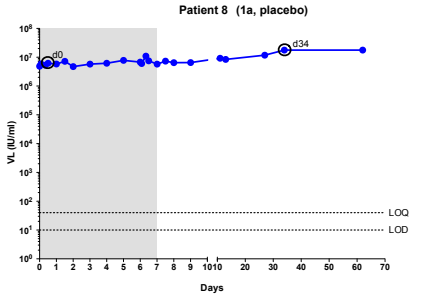






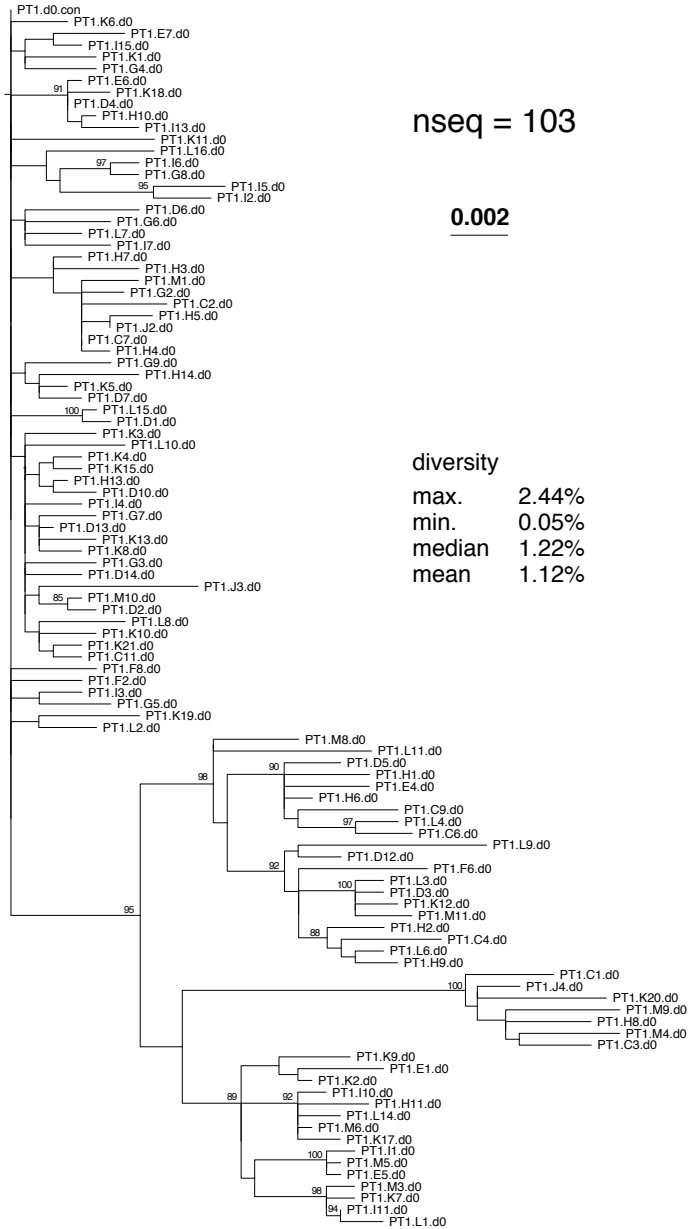






Patient 1 - day0 (VL: 2,641,151 IU/ml)

NO resistance-conferring mutations

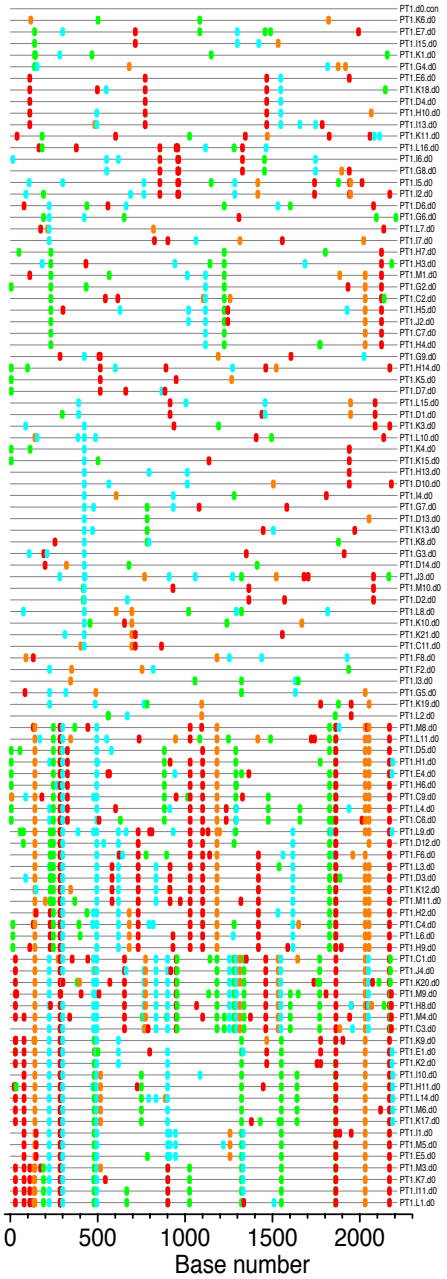


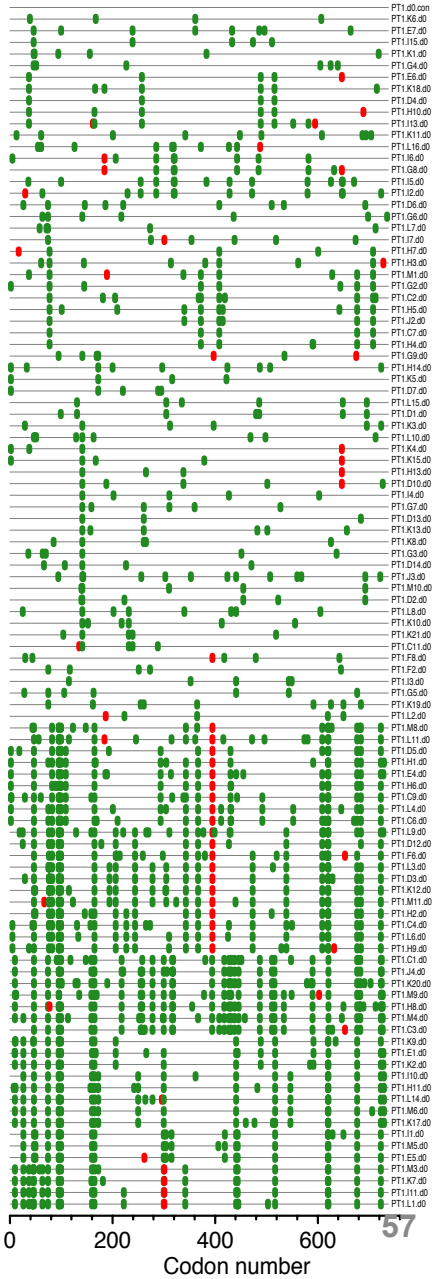
nseq = 103

0.002

diversity

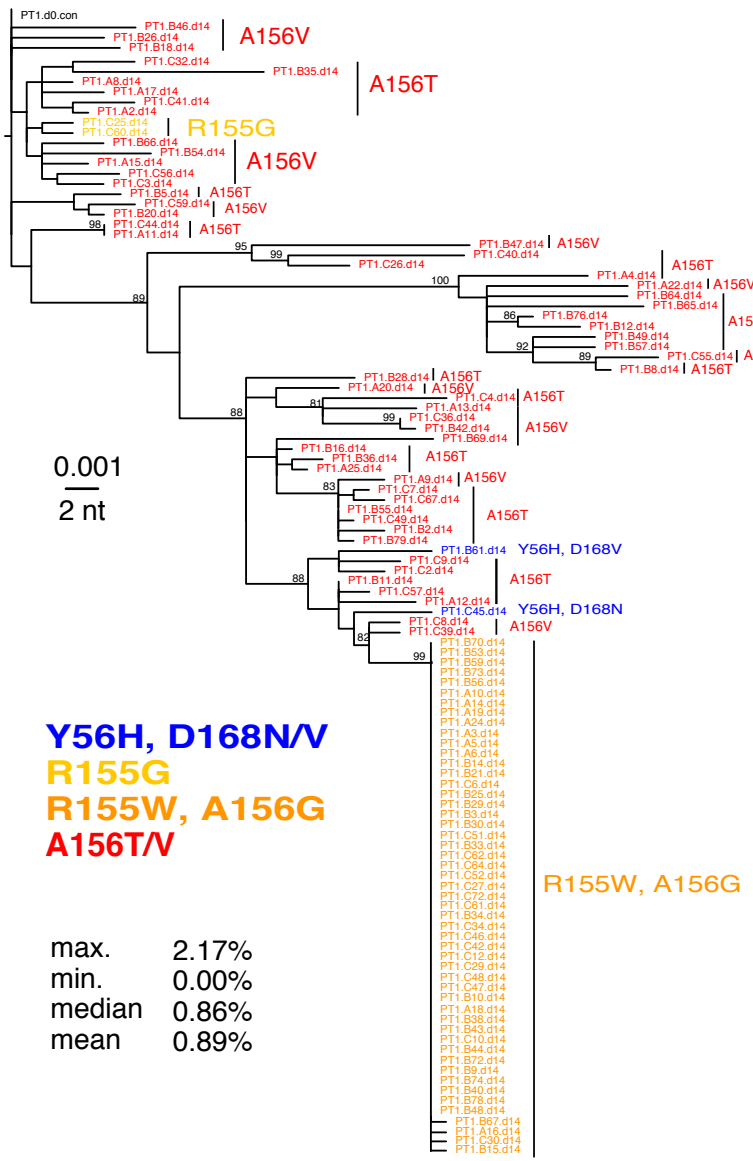
max.	2.44%
min.	0.05%
median	1.22%
mean	1.12%





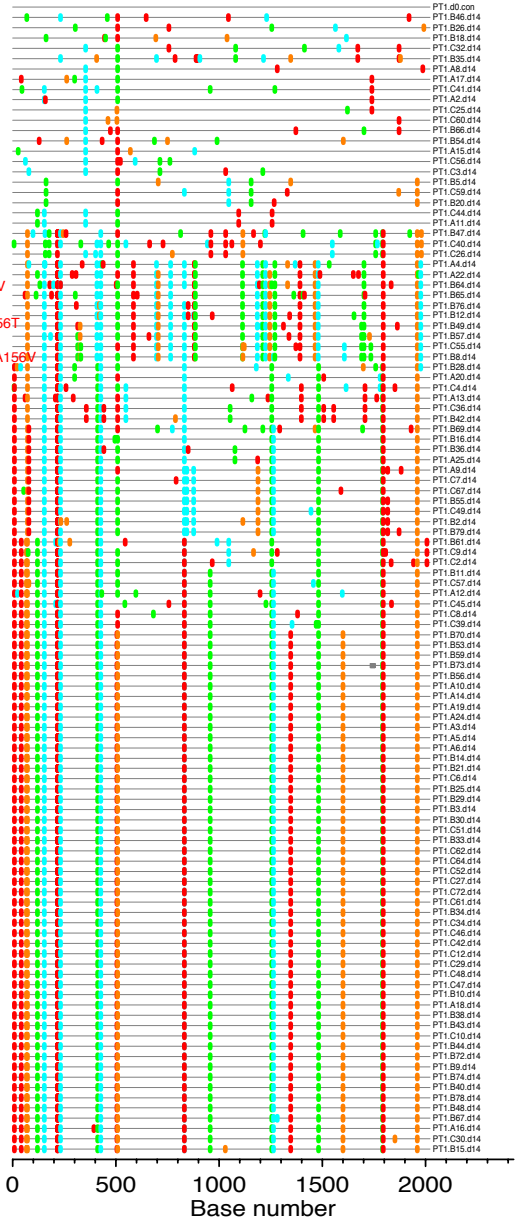
Patient 1 - day 14 (VL : 1270 IU/ml)

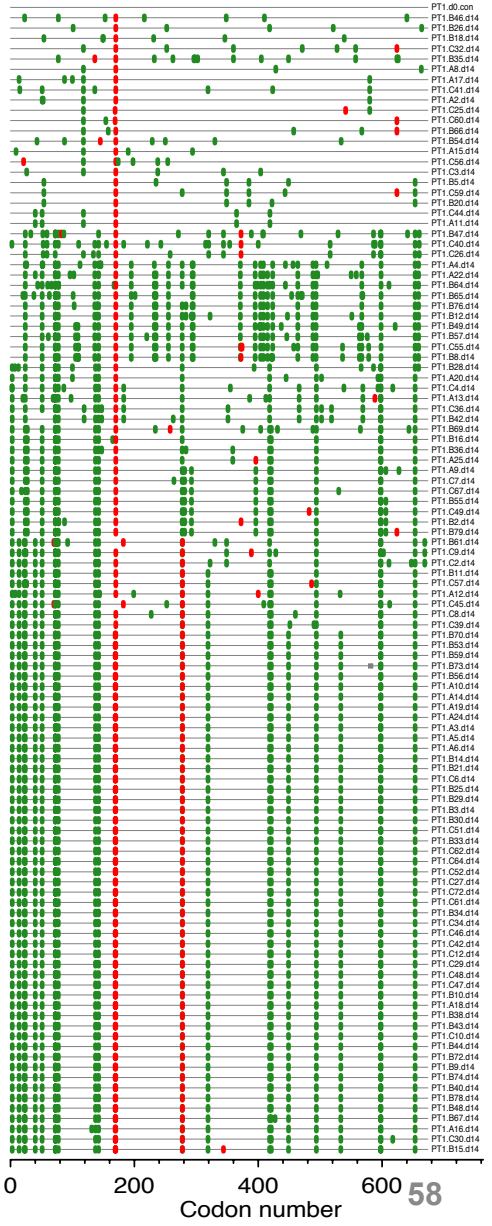
nseq = 111



Y56H, D168N/V
R155G
R155W, A156G
A156T/V

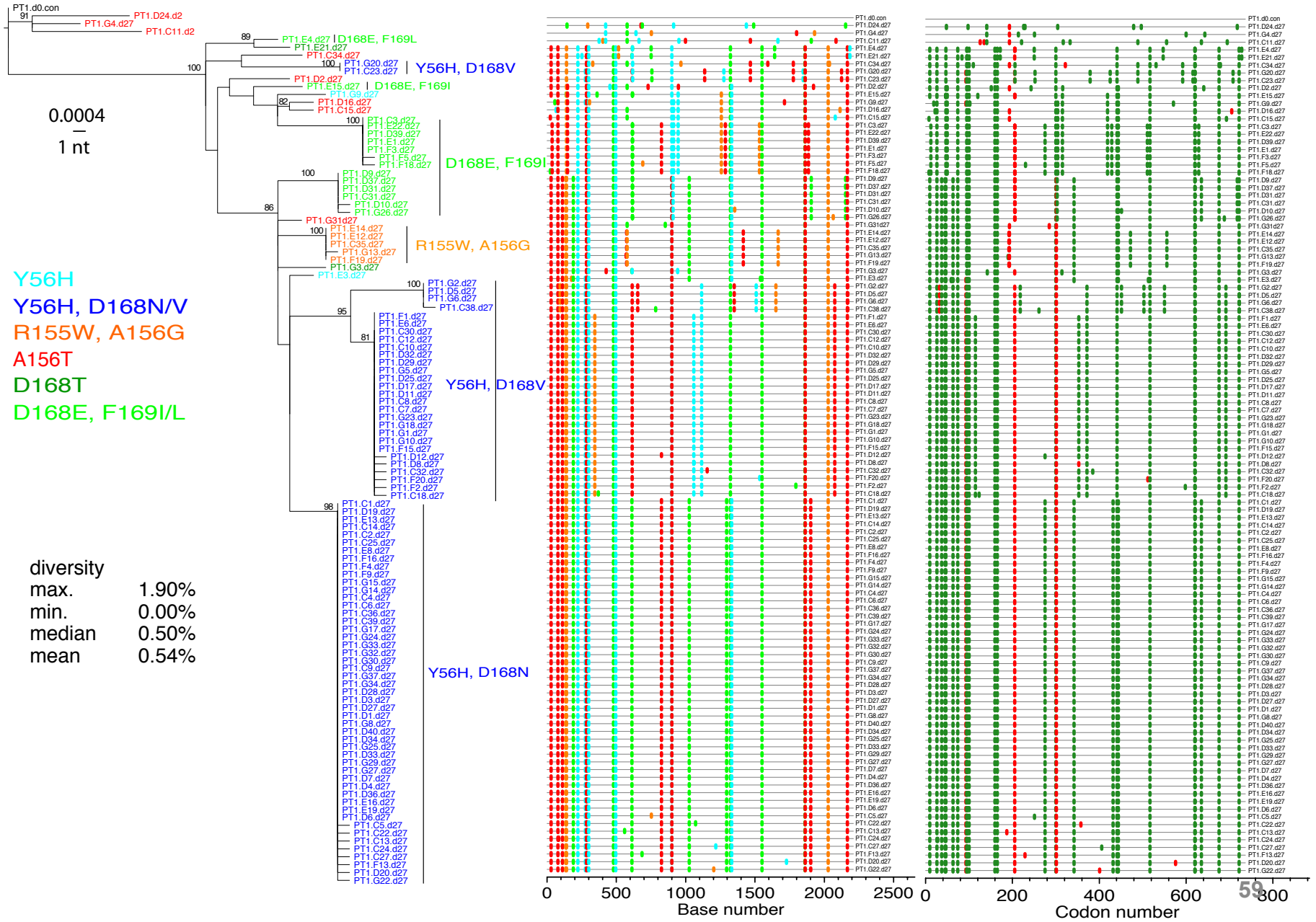
max.	2.17%
min.	0.00%
median	0.86%
mean	0.89%





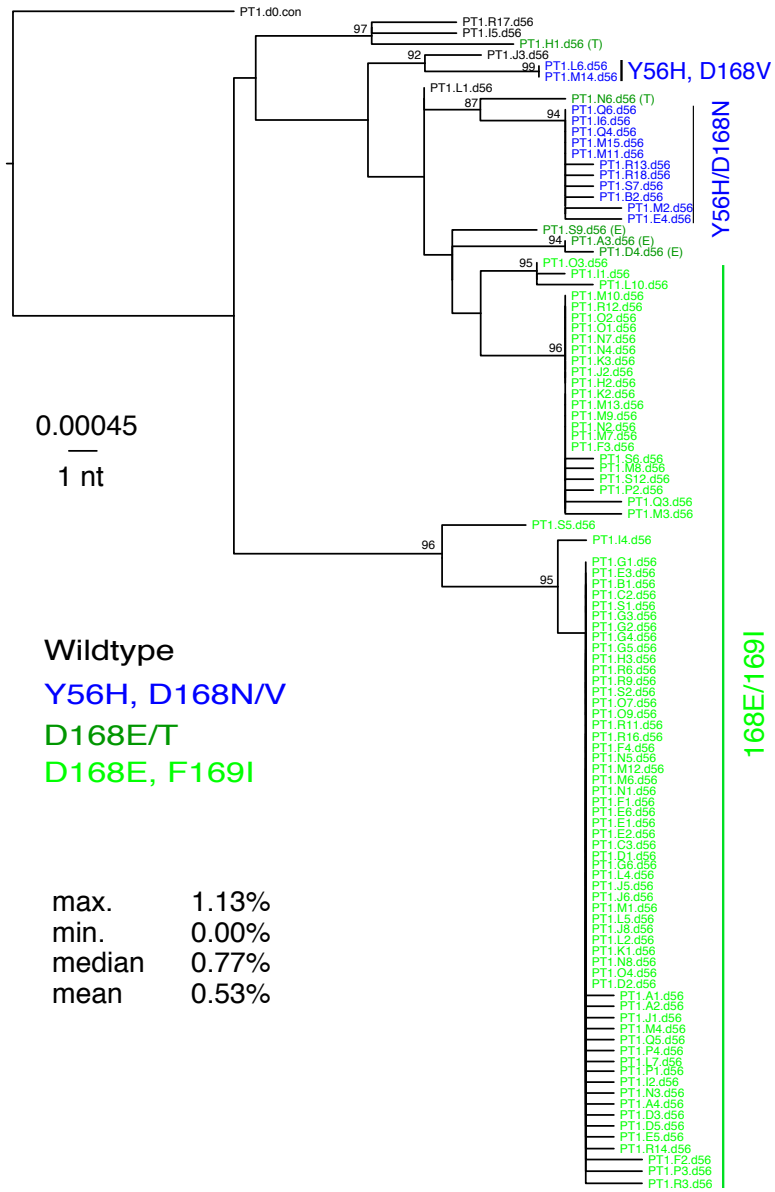
Patient 1 - day 27 (VL: 1,341,852 IU/ml)

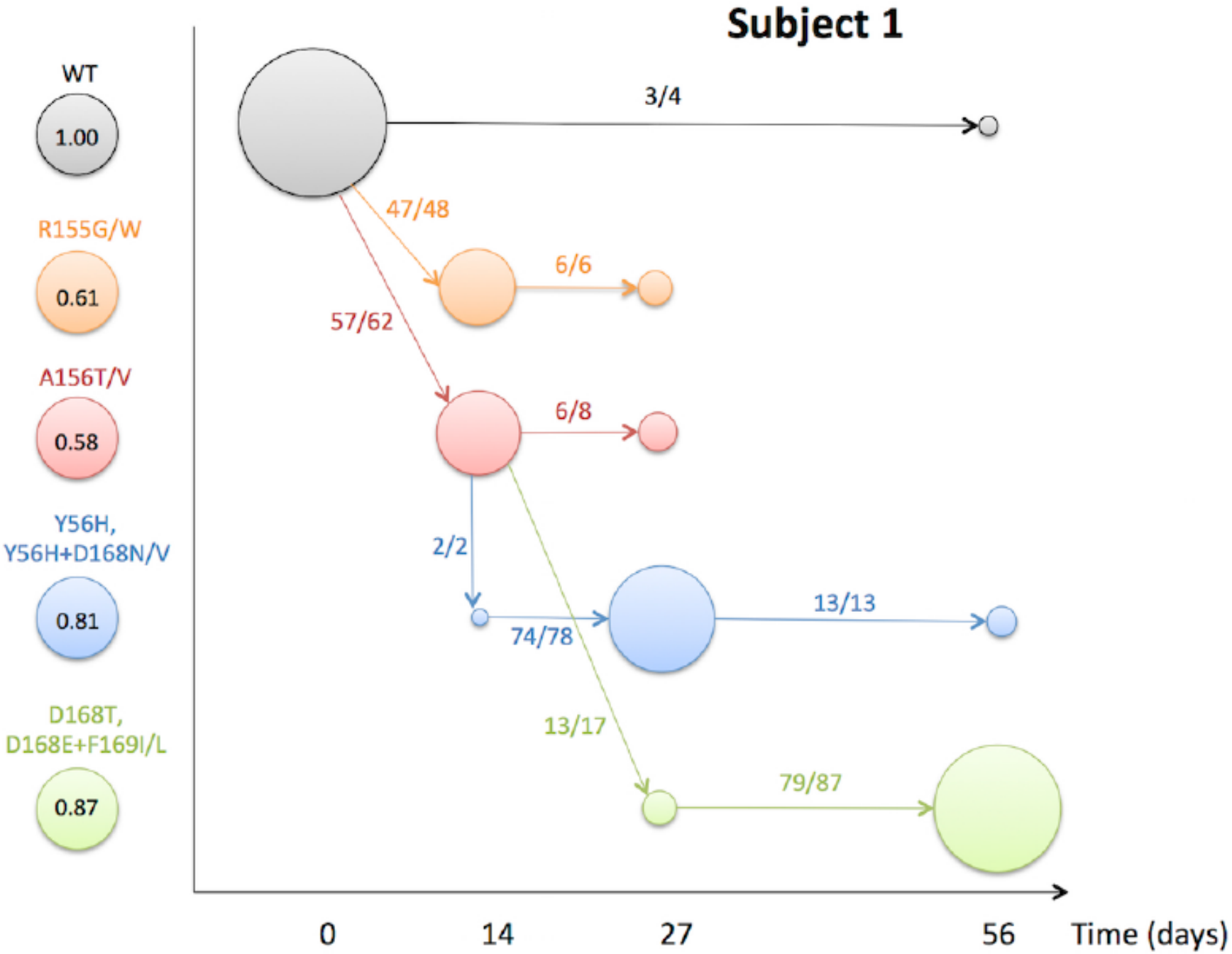
nseq = 111



Patient 1 - day 56 (VL: 1,011,478 IU/ml)

nseq = 106

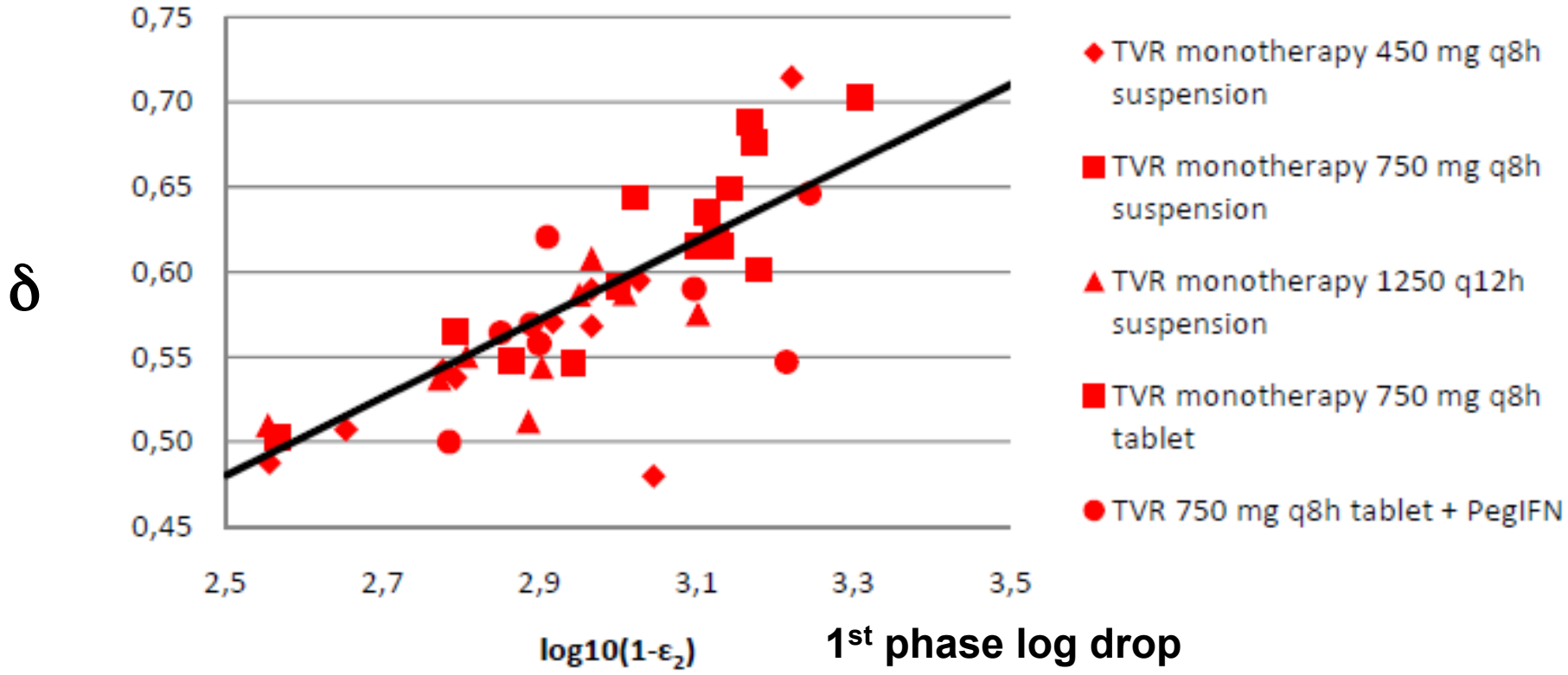



Subject 1
WT
1.00
R155G/W
0.61
A156T/V
0.58
Y56H,
Y56H+D168N/V
0.81
D168T,
D168E+F169I/L
0.87
3/4
47/48
6/6
57/62
6/8
2/2
74/78
13/13
13/17
79/87
0
14
27
56
Time (days)

Observations from the data

- 7 days of treatment with MK5172 rapidly selects resistant strains.
- There exists rapid turnover of different mutant strains over the 56 day study period. Most of these turnover events are the result of expansion of a few resistant clones, need replication space.
- In 4 out of 5 patients, the resistant mutants remain as dominant strains over 56 day study period.

Second phase slope, δ, higher than seen with IFN and correlates with antiviral efficacy, ε



r=0.79, p< 0.001 Guedj et al Hepatol 2010

Model with multiple strains of HCV

$$\frac{dT}{dt} = \lambda - d \cdot T - \sum_{i=1}^{4} \beta \cdot T \cdot V_i + \rho_T \cdot T \cdot \left(1 - \frac{T + \sum_{i=1}^{4} I_i + N}{T_{max}}\right) + \sum_{i=1}^{4} k_{cure} \cdot (-log_{10}(1 - \varepsilon_i)) \cdot I_i$$

$$\frac{dI_i}{dt} = \beta \cdot T \cdot V_i - \delta \cdot I_i + k_{super} \cdot \sum_{j=1}^{4} M_{i,j} - k_{cure} \cdot (-log_{10}(1 - \varepsilon_i)) \cdot I_i$$

$$\frac{dV_i}{dt} = (1 - \varepsilon_i) \cdot r_i \cdot p \cdot I_i - c \cdot V_i$$

$$\varepsilon_i = \frac{D \cdot \exp(-w \cdot \max(t - 7, 0)) / EC_{50,i}}{1 + D \cdot \exp(-w \cdot \max(t - 7, 0)) / EC_{50,i}}$$

$$M_{i,j} = \begin{cases} \Delta \cdot \beta \cdot I_j \cdot V_i & \Delta \geq 0 \\ \Delta \cdot \beta \cdot I_i \cdot V_j & \Delta < 0 \end{cases} \quad where\ \Delta = (1 - \varepsilon_i) \cdot r_i - (1 - \varepsilon_j) \cdot r_j$$

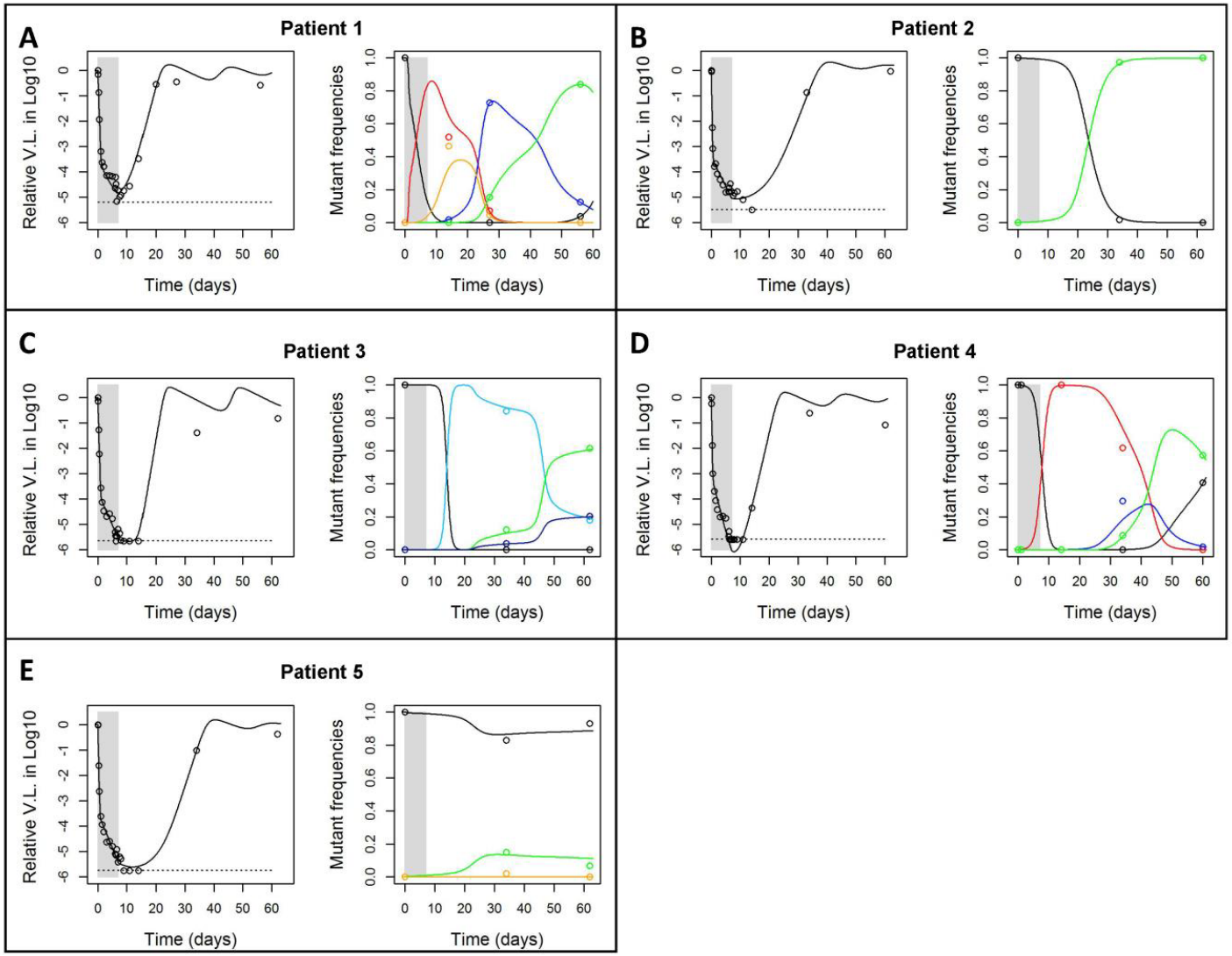
T: target cells I_i: cells infected by the ith viral strain V_i: the ith viral strain

Model Comparisons

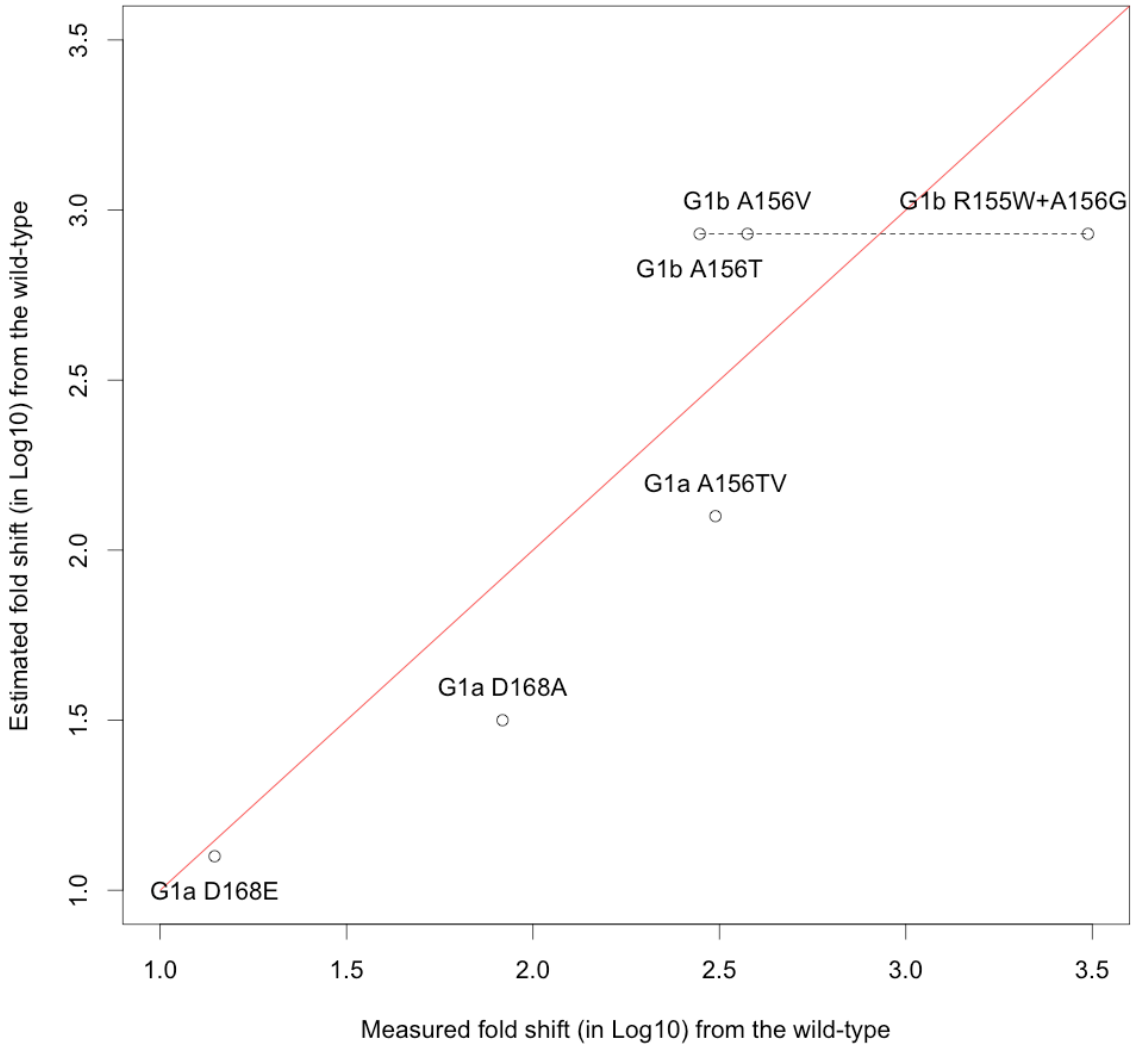
Table 1. Summary of the model characteristics and the fitting results, i.e. sums of squared residuals (SSR) and the AICc scores, of each model for each subject. Bolded AICc scores denote the best model fit among all models for the 5 subjects.

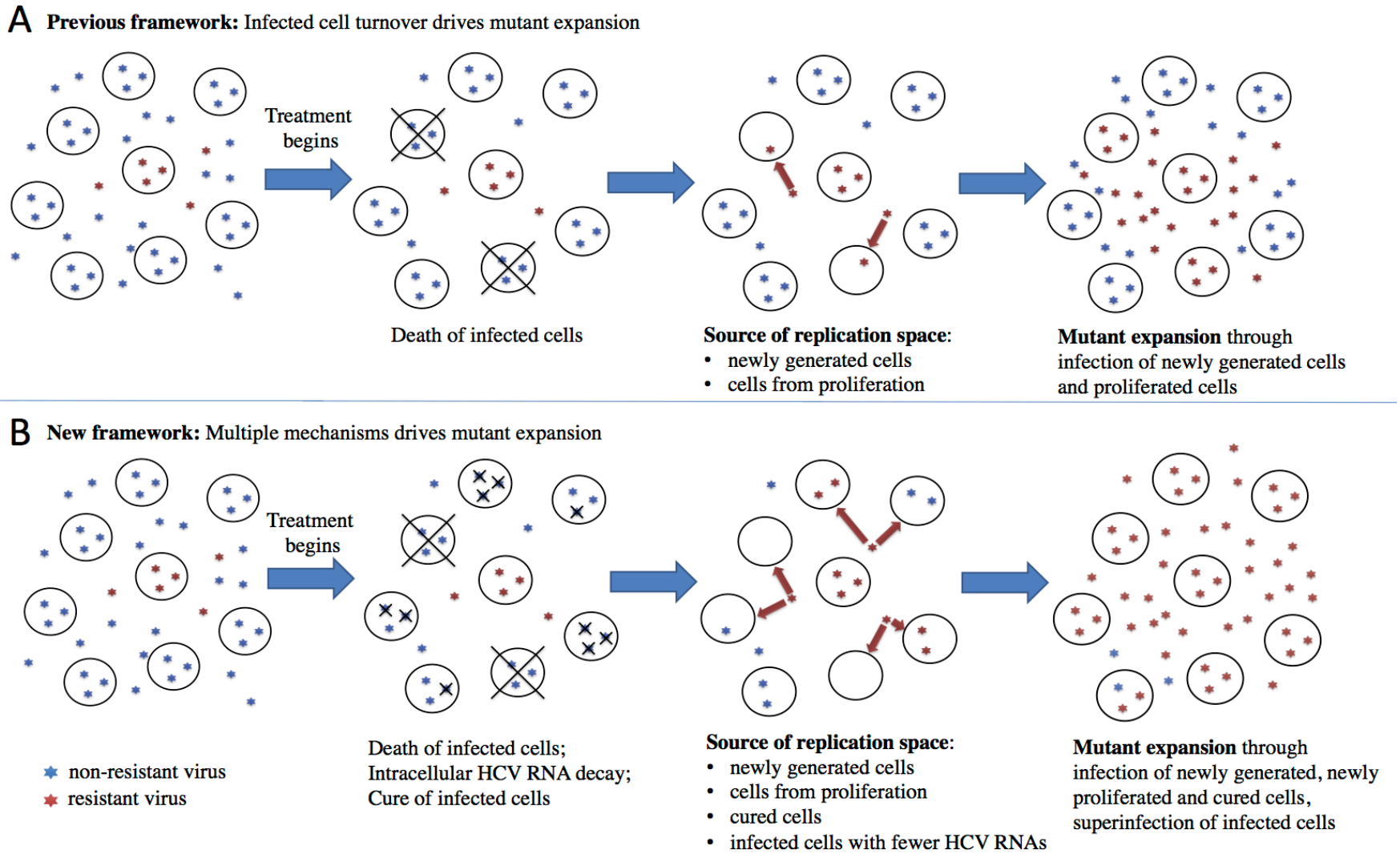
Model Characteristics								
Parameter	**Baseline model with δ=0.14 day^{-1}**		**Cure model**		**Superinfection model**		**Full model**	
k_{cure}	0.0		Fitted		0.0		Fitted	
k_{super}	0.0		0.0		Fitted		Fitted	
Fitting Results								
Subject	**SSR**	**AICc**	**SSR**	**AICc**	**SSR**	**AICc**	**SSR**	**AICc**
1	10.2	-16.2	5.9	-32.8	10.1	-12.0	2.4	**-63.8**
2	6.4	-22.1	2.1	**-53.2**	6.4	-17.8	1.8	-51.8
3	8.5	-8.1	2.3	**-47.6**	7.0	-9.5	2.1	-44.5
4	22.7	5.0	6.7	-42.6	16.2	-5.1	1.9	**-90.6**
5	7.6	-24.9	0.9	**-86.5**	7.6	-21.2	0.9	-83.6
Total AICc		-66.3		-262.7		-65.6		**-334.3**

Best-fit of model



Model Predictions of EC_{50}




A Previous framework: Infected cell turnover drives mutant expansion
Treatment begins
Death of infected cells
Source of replication space:
• newly generated cells
• cells from proliferation
Mutant expansion through infection of newly generated cells and proliferated cells
B New framework: Multiple mechanisms drives mutant expansion
Treatment begins
non-resistant virus
resistant virus
Death of infected cells;
Intracellular HCV RNA decay;
Cure of infected cells
Source of replication space:
• newly generated cells
• cells from proliferation
• cured cells
• infected cells with fewer HCV RNAs
Mutant expansion through infection of newly generated, newly proliferated and cured cells, superinfection of infected cells

Conclusions

- 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.

Collaborators

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- Ruian Ke, NCSU
- Jeremie Guedj, INSERM, PAris