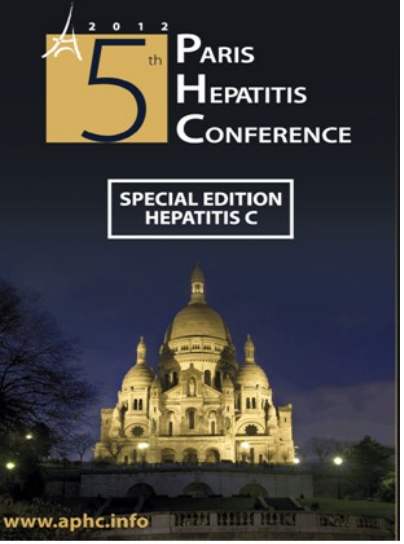


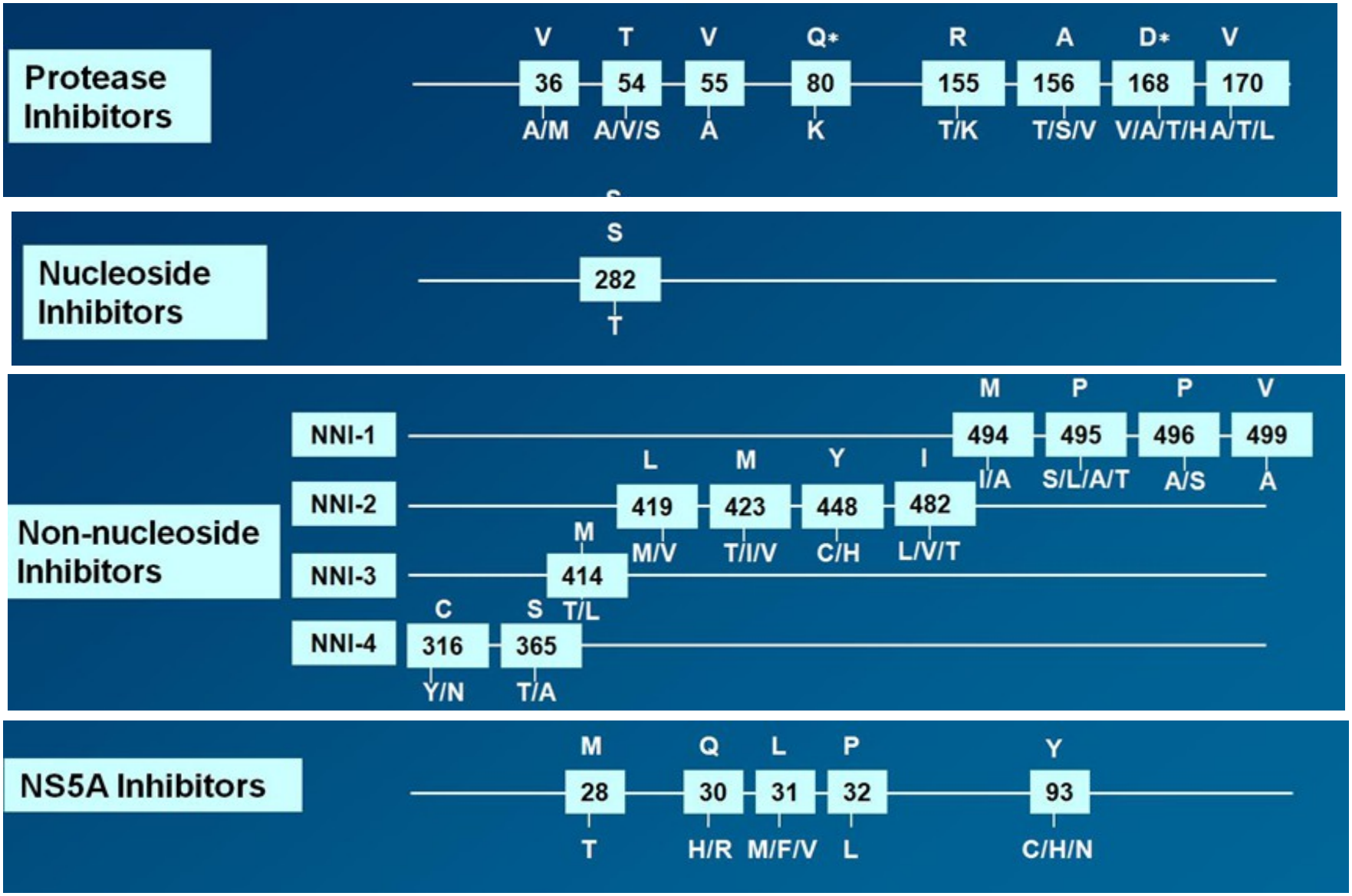
Is HCV Resistance a reel issue at the time of new DAA combination?

Philippe HALFON
Marseille, France

Is HCV Resistance a reel issue at the time of new DAA combination?

- HCV Resistance :Basic concept
- HCV Resistance assessement
- Resistance issues in P+R+PI 1rst generation DAA
- Resistance issues in P+R+2nd and other DAA
- Resistance issues in IFN-free Regimen
- Management of HCV resistance

Main HCV Resistance Mutations to DAA



Prevalence of natural polymorphisms that may influence DAA susceptibility across HCV genotypes/subtypes

Drug family	Key mutations associated with DAA resistance*	1a	1b	2	3	4	DAA affected by specific polymorphisms
NS3 protease inhibitors (no. NS3 sequences: 1612†)	T54A/S	1.4% S	1% S	0	0	5.5% S	Telaprevir, boceprevir
	V55A	1.2% A	0	0	0	0	Boceprevir
	Q80K	39.7% K	0	0	0	0	Simeprevir
	D168A/H/T/V/Q	0	0	0	99.2% Q	0	Simeprevir
NS5B non- nucleoside analogues (no. NS5B sequences: 1025†)	S15G	0	0	76.3% G	0	0	PSI35261 (NUC) PSI352938 (NUC)
	C316Y/N	0	36% N	0	0	0	ABT-333 (NNI-4) ABT-072 (NNI-4)
	M414T/L	0	0	0	0	34.2%L	Setrobuvir (NNI-3)
	L419M/V	0	0	2.7% V	0	0	VCH-759 (NNI-2)
	M423T/I/V	1.8% I	0	0	0	0	Filibuvir (NNI-2) VCH-759 (NNI-2) VHC-916 (NNI-2)
	I482L/V/T	0	0	100% L	100% L	100% L	VCH-759 (NNI-2)
	V494I/A	0	0	100% A	5.2%A	0	VCH-759 (NNI-2)
	V499A**	96.2% A	10.5%A	91% A	100%A	100%A	Tegobuvir (NNI-1) BI-7127 (NNI-1)
NS5A inhibitors (no. NS5a sequences: 3153†)	Q30H/R	0	0	0	0	51.3% R	Daclatasvir
	L31M/V/F	0	0	83.5 % M	0	92% M	Daclatasvir
	Y93C/H/N	0	2% H	0	1%H	5.4%H	Daclatasvir

*Only changes with a prevalence >1% are recorded. **V499A confers low-level resistance to NNI-1.

† NS3 protease, NS5B polymerase and NS5A sequences were obtained from Los Alamos database.

No mutations associated with resistance to NS5B nucleos(t)ide analogues are found as natural polymorphisms.

Poveda et al. Future Virol 2012

Based on *In vitro* assay (Replicon) :

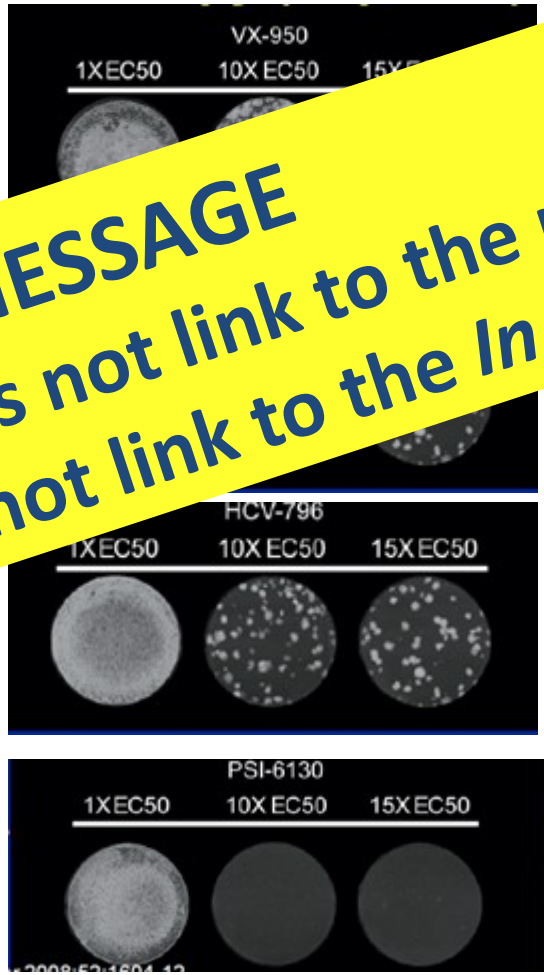
can we predicted the occurrence of drug resistance mutations?

In Vitro Resistance to DAA
14 days monotherapy (Replicon)

Protease Inhibitors

NS5 A Inhibitors

Nucleoside Inhibitors



0.01-0.1

10-1000

10-100

KEY MESSAGE

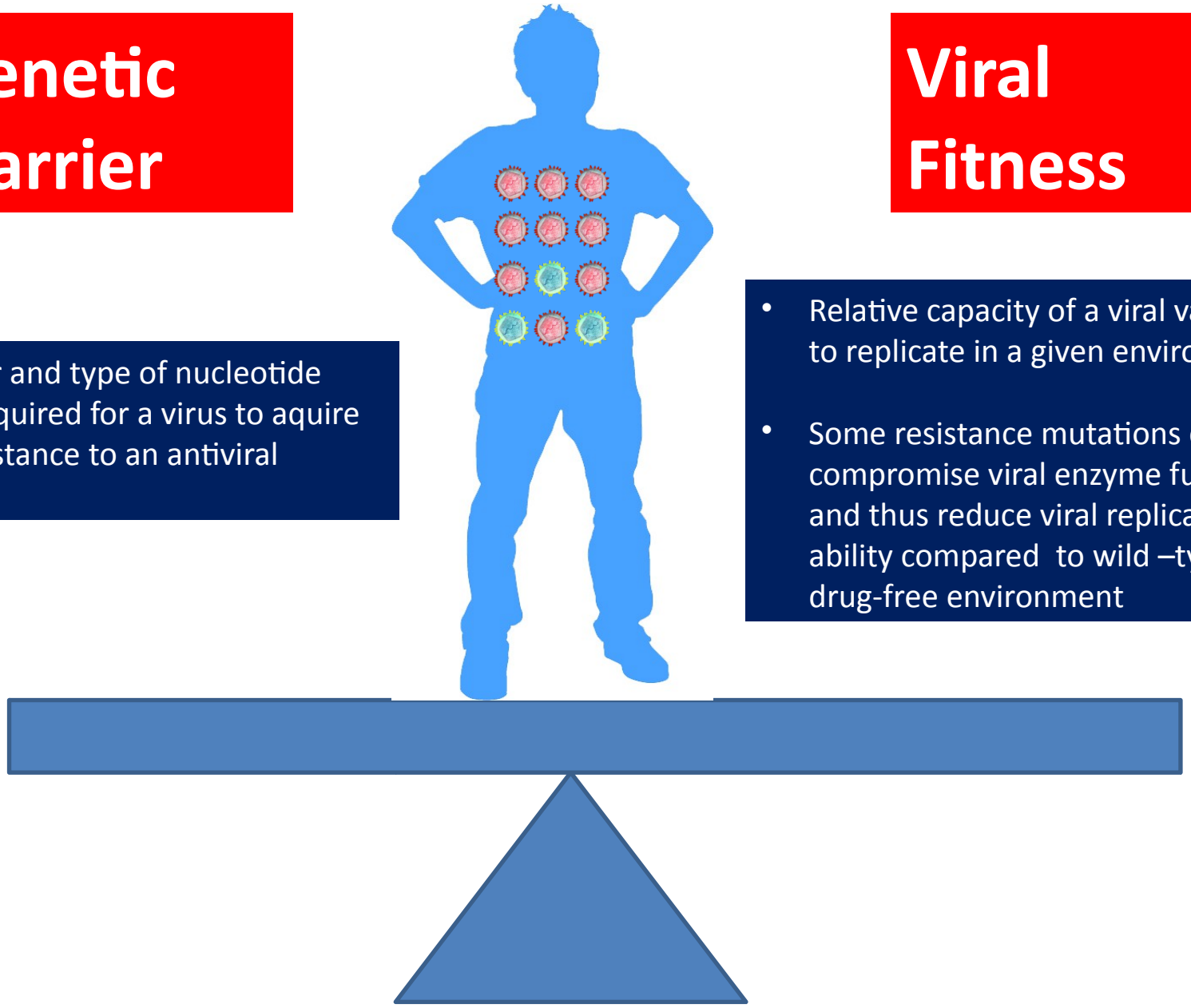
The genetic barrier is not link to the potency

The *In vitro* Potency is not link to the *In Vivo* activity

Adapted from McCown et al. AAC 2008

Genetic barrier

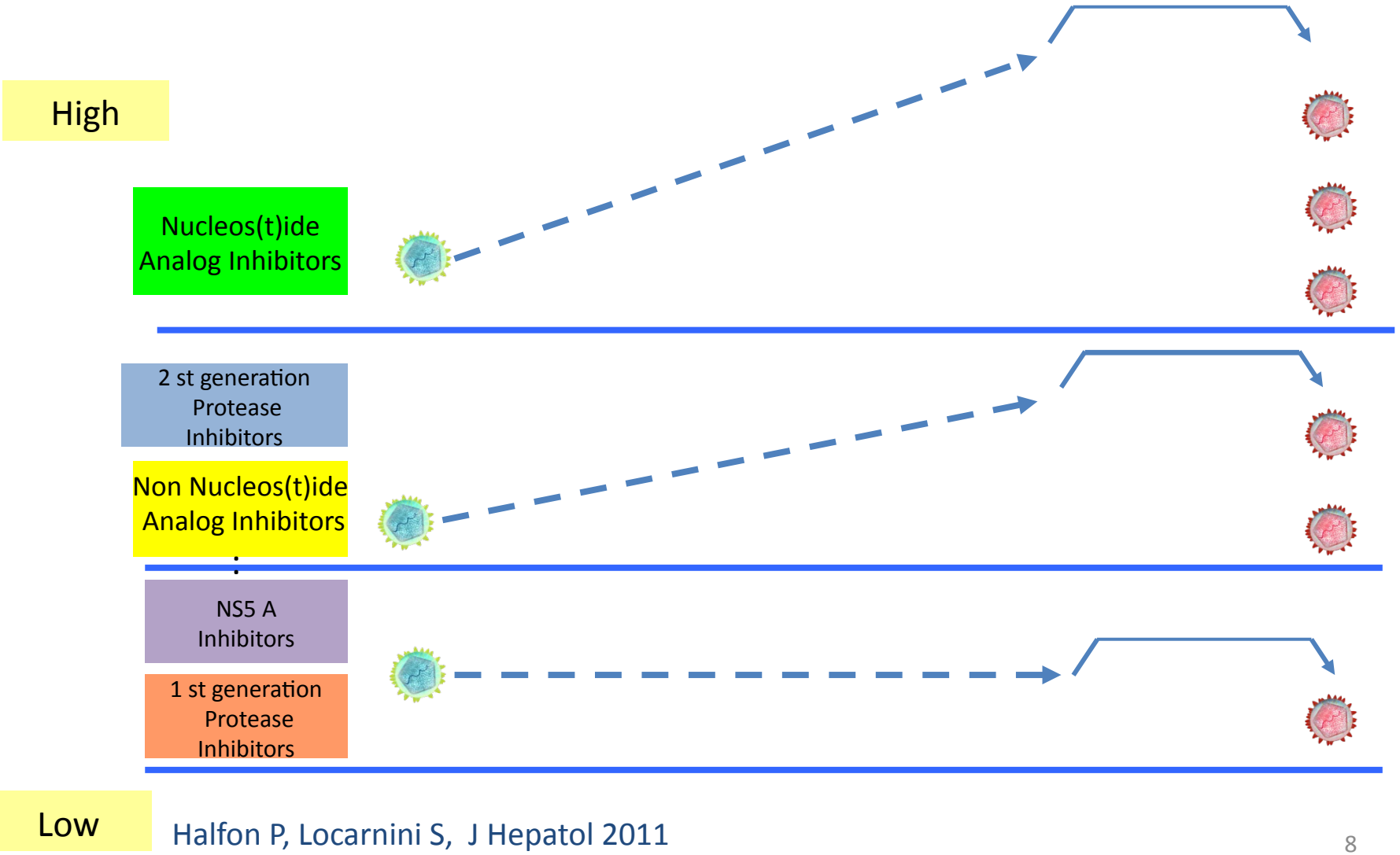
- Number and type of nucleotide Changes required for a virus to aquire clinical resistance to an antiviral regimen



Viral Fitness

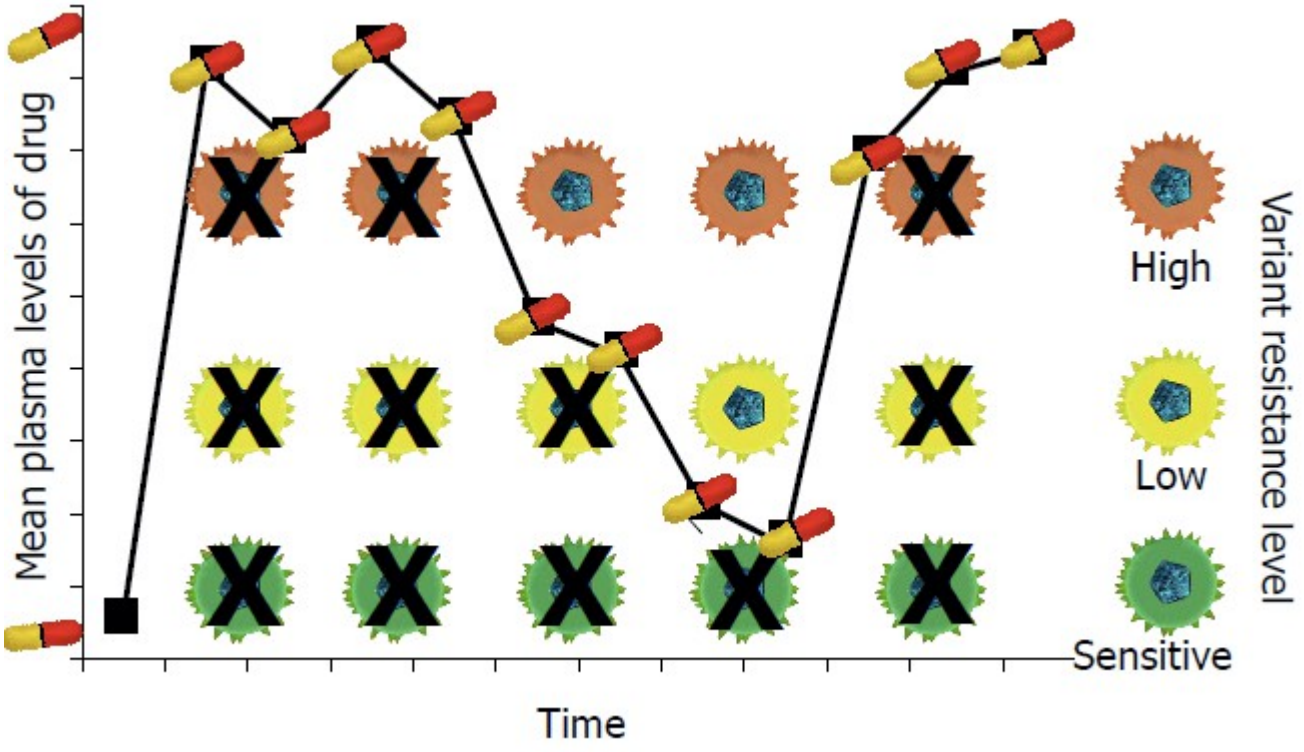
- Relative capacity of a viral variant to replicate in a given environment
- Some resistance mutations can compromise viral enzyme function and thus reduce viral replication ability compared to wild –type in a drug-free environment

Genetic Barrier for HCV Direct Antiviral Agents



Halfon P, Locarnini S, J Hepatol 2011

Importance of drug levels over time



Drug trough levels must be sufficient to suppress viral replication

HCV Resistance assessment :

No standardized assay and no RAVs clinical cut-off?

How to detect HCV Resistance?

"Detection depends on how carefully you look for it"

Assays used to assess a patient's resistance profile

Genotypic assays

Examine the genetic sequence of the virus and identify variants

Different assays have different levels of sensitivity to detect resistant variants

- **Population sequencing: simple but may not detect variants at low levels (<20%)**
- **Clonal sequencing: can detect variants at 5% frequencies**
- **Deep sequencing: can detect variants at very low levels but is costly**

Phenotypic assays

Assess the drug concentration required to inhibit viral replication *in vitro* by 50%
(IC50; enzyme/replicon assay)

Outputs include fold change in sensitivity versus a reference strain (e.g. wild-type)

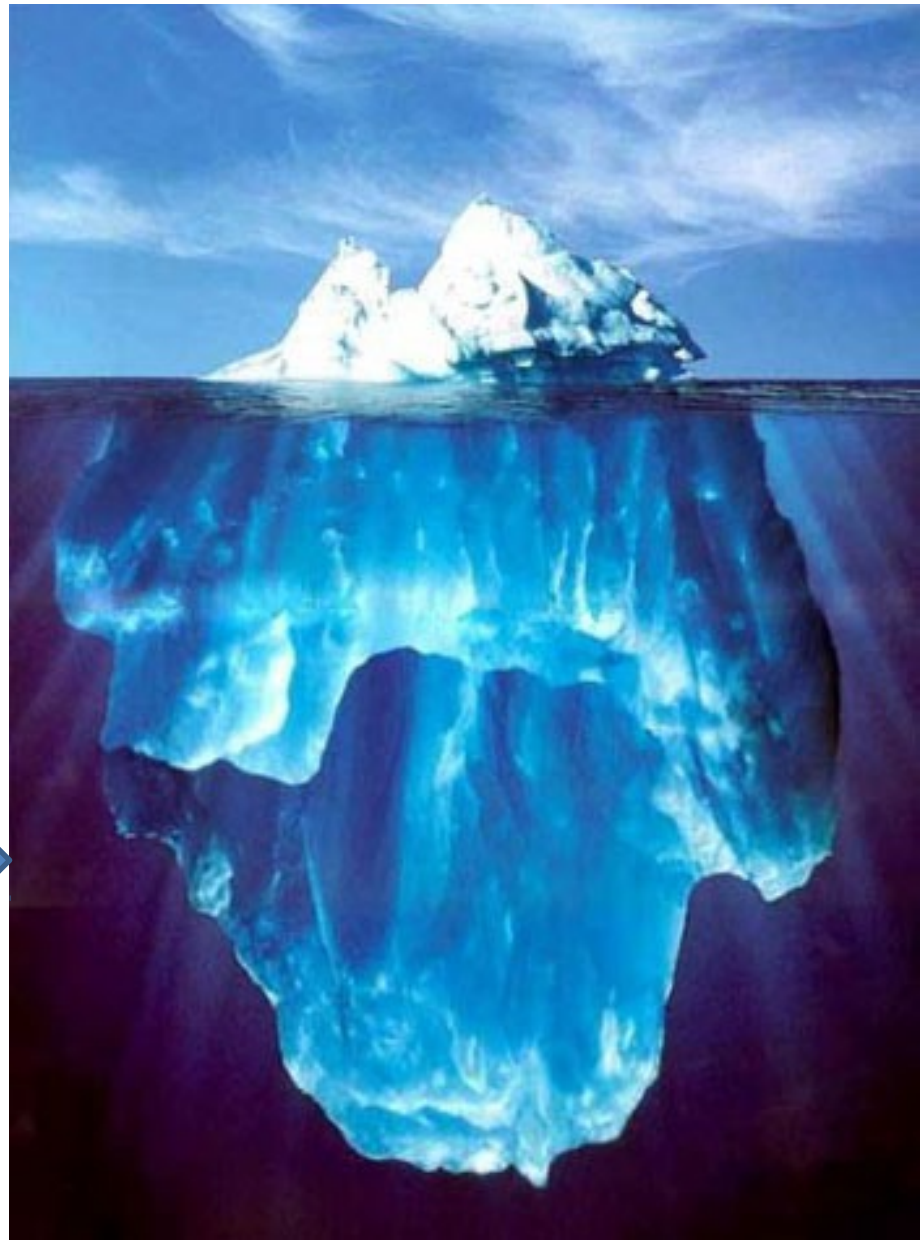
Biological and/or clinical cut-offs may allow interpretation of clinical significance

Clinical significance of RA minority mutants detection

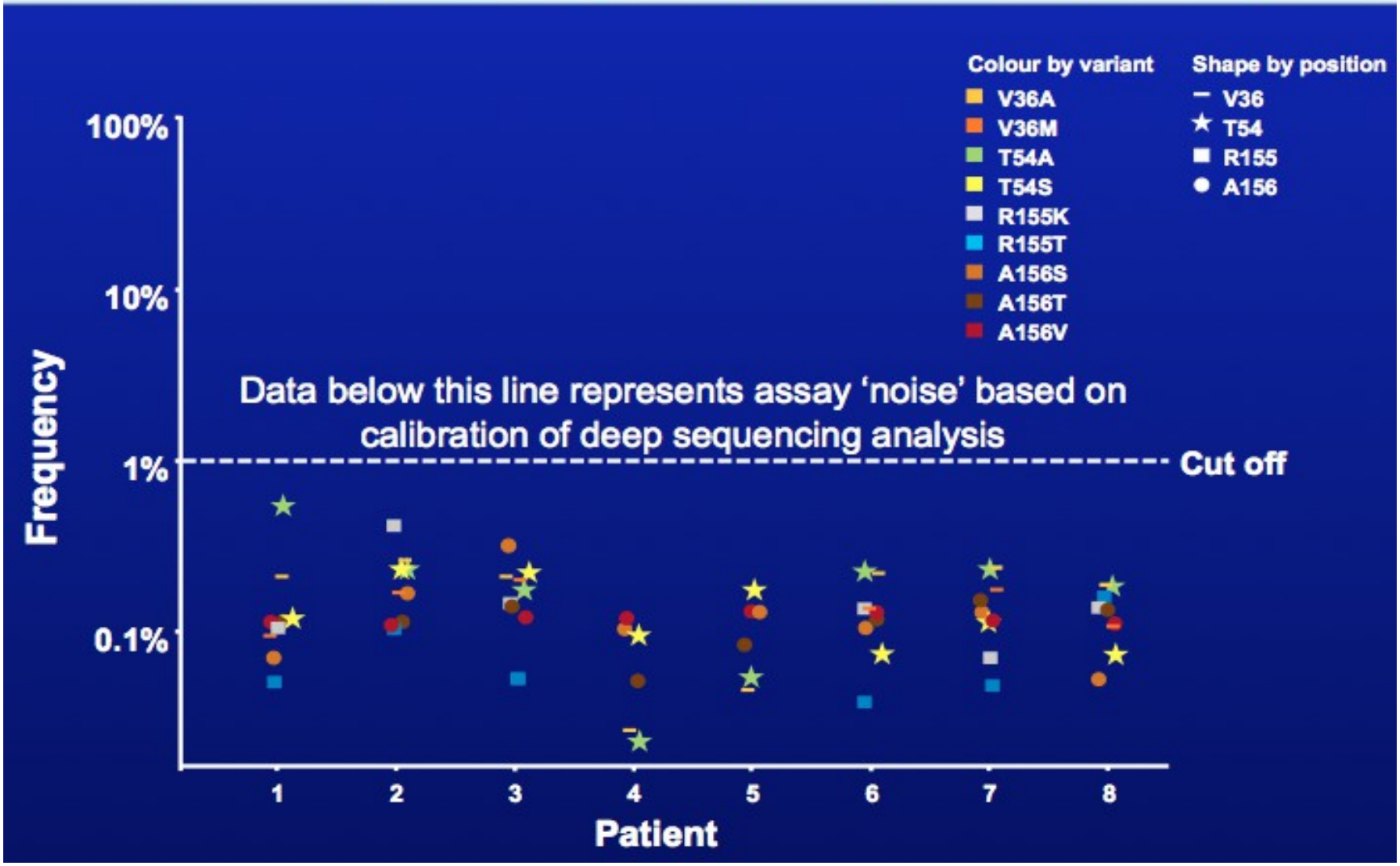
Sequencing : 15-20%

Pyrosequencing : 1-10 %

NGS : < 1%



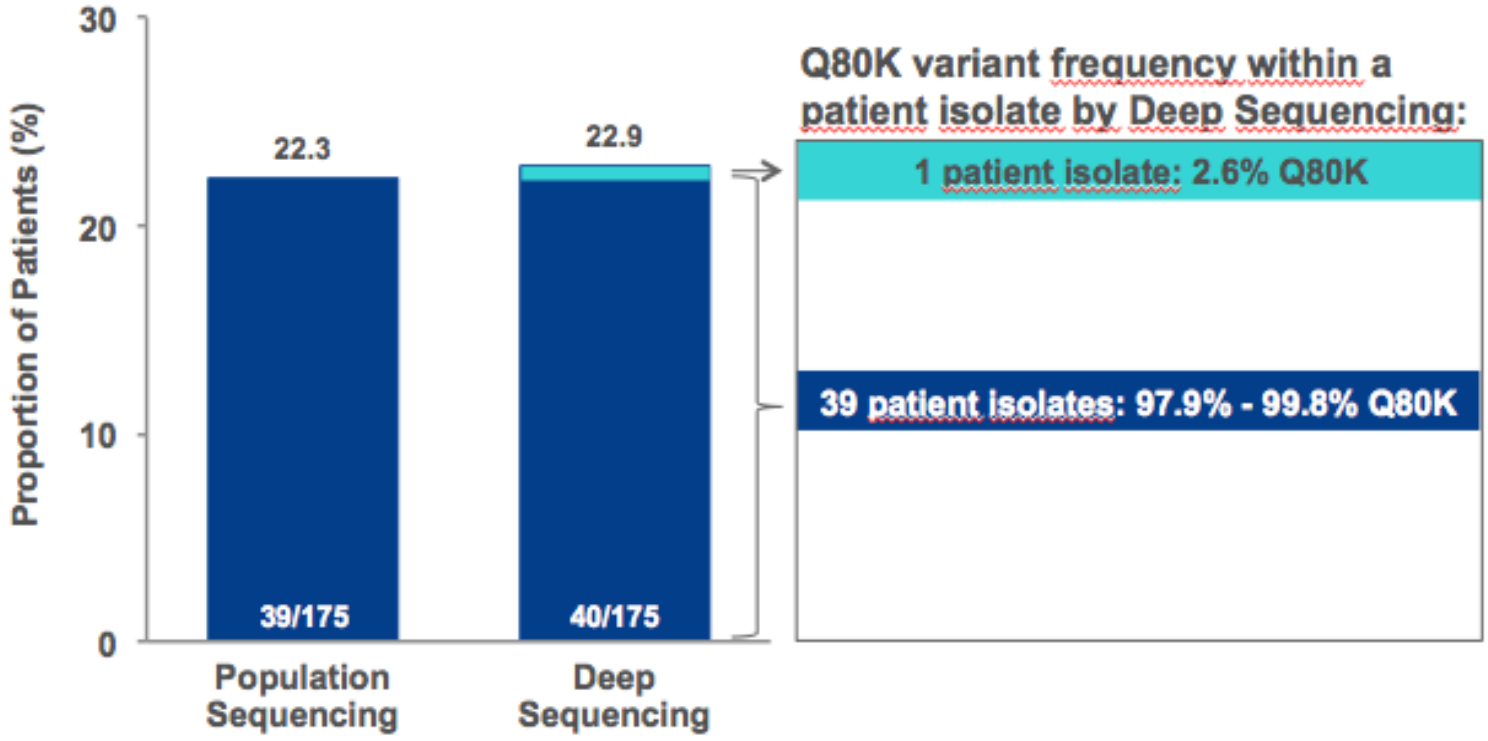
Absence of TVR-resistant Variants at Baseline in Study C219 (Illumina® deep sequencing data)



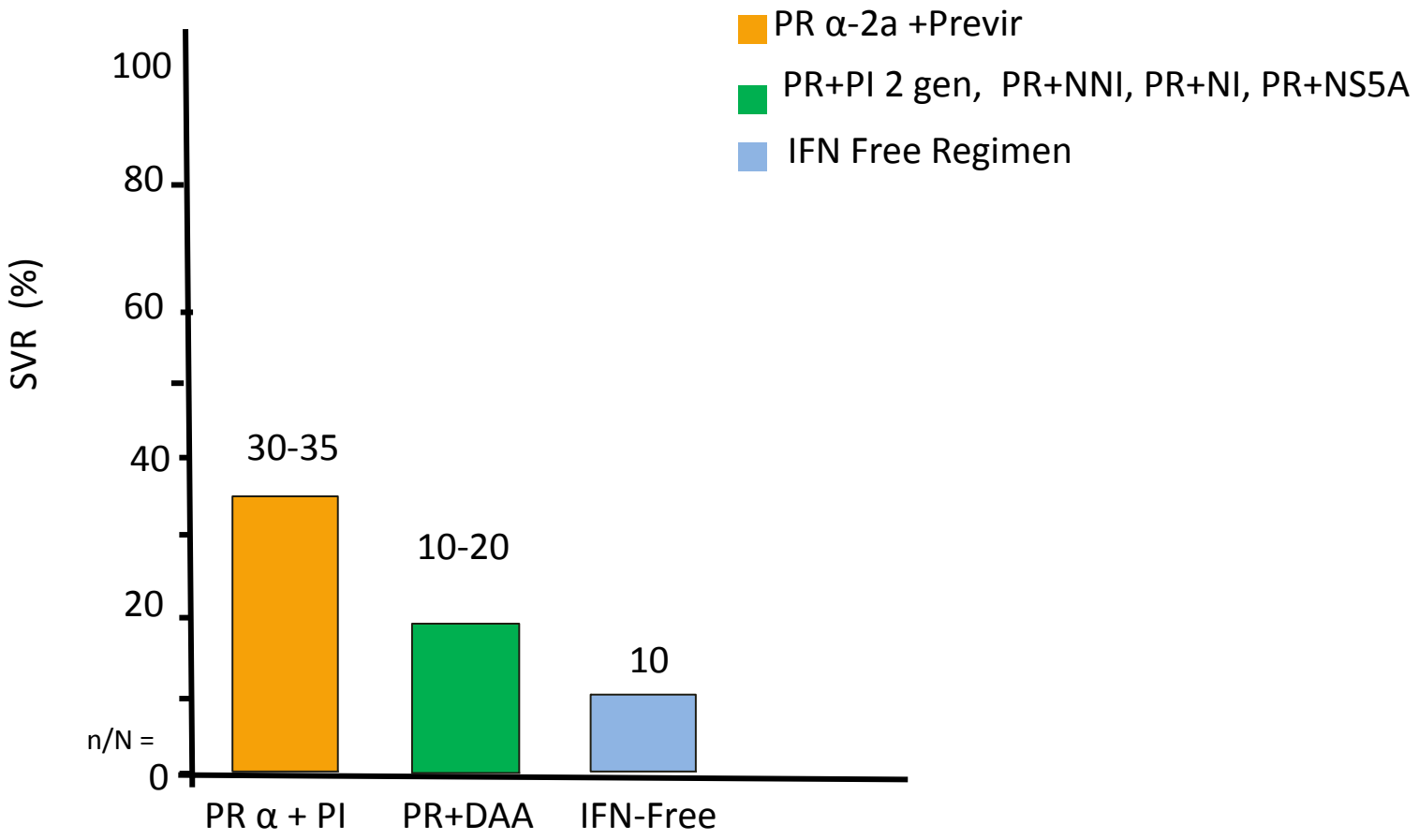
Sarrazin et al. AASLD 2011

Polymorphism Q80K at Baseline by Population and Deep Sequencing

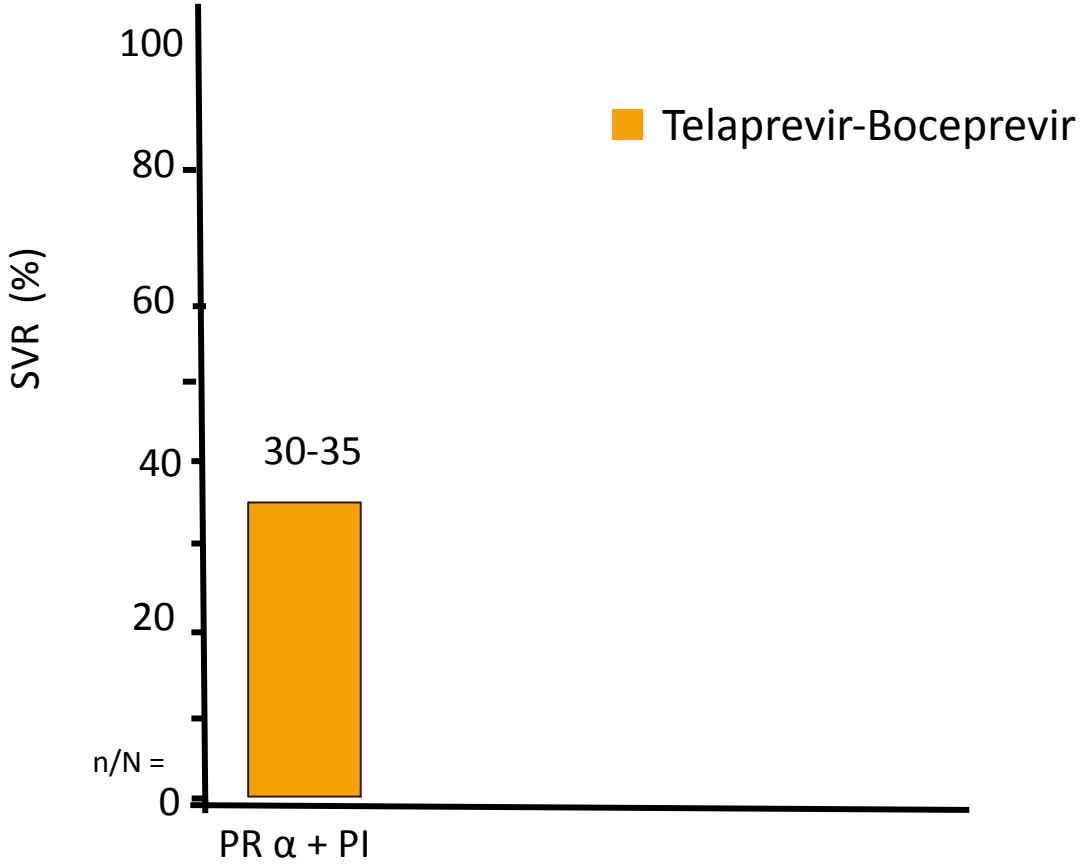
Phase 2b Studies – Selection (N = 175)



Magnitude of Treatment Failure using DAA



Triple therapy using First generation of protease inhibitors + Peg-Ribavirine



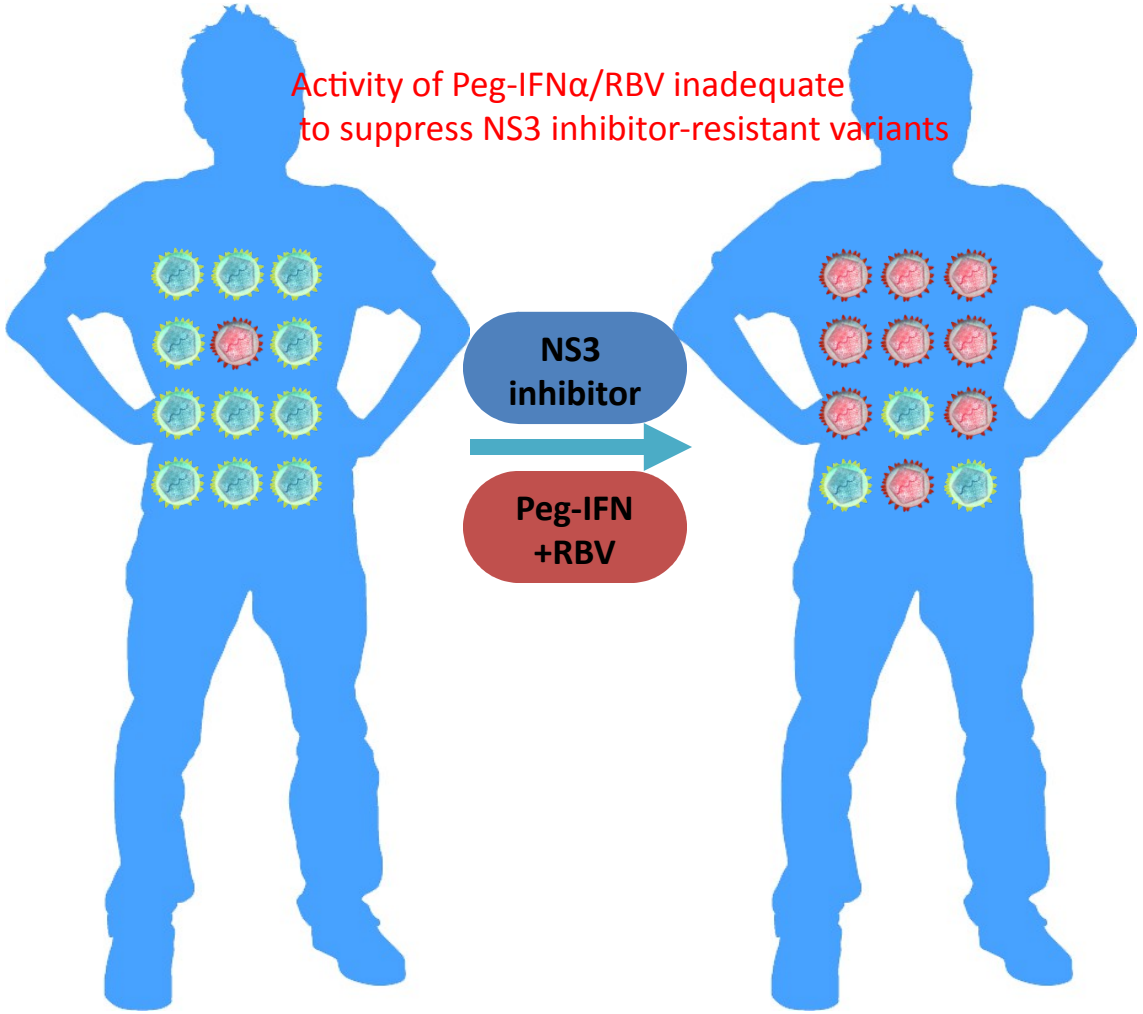
Failure to the treatment :
HCV Resistance or Treatment Discontinuation?

Resistance Emerges as a Result of Treatment Failure

Activity of Peg-IFNα/RBV inadequate to suppress NS3 inhibitor-resistant variants

Wild-type virus

Mutant



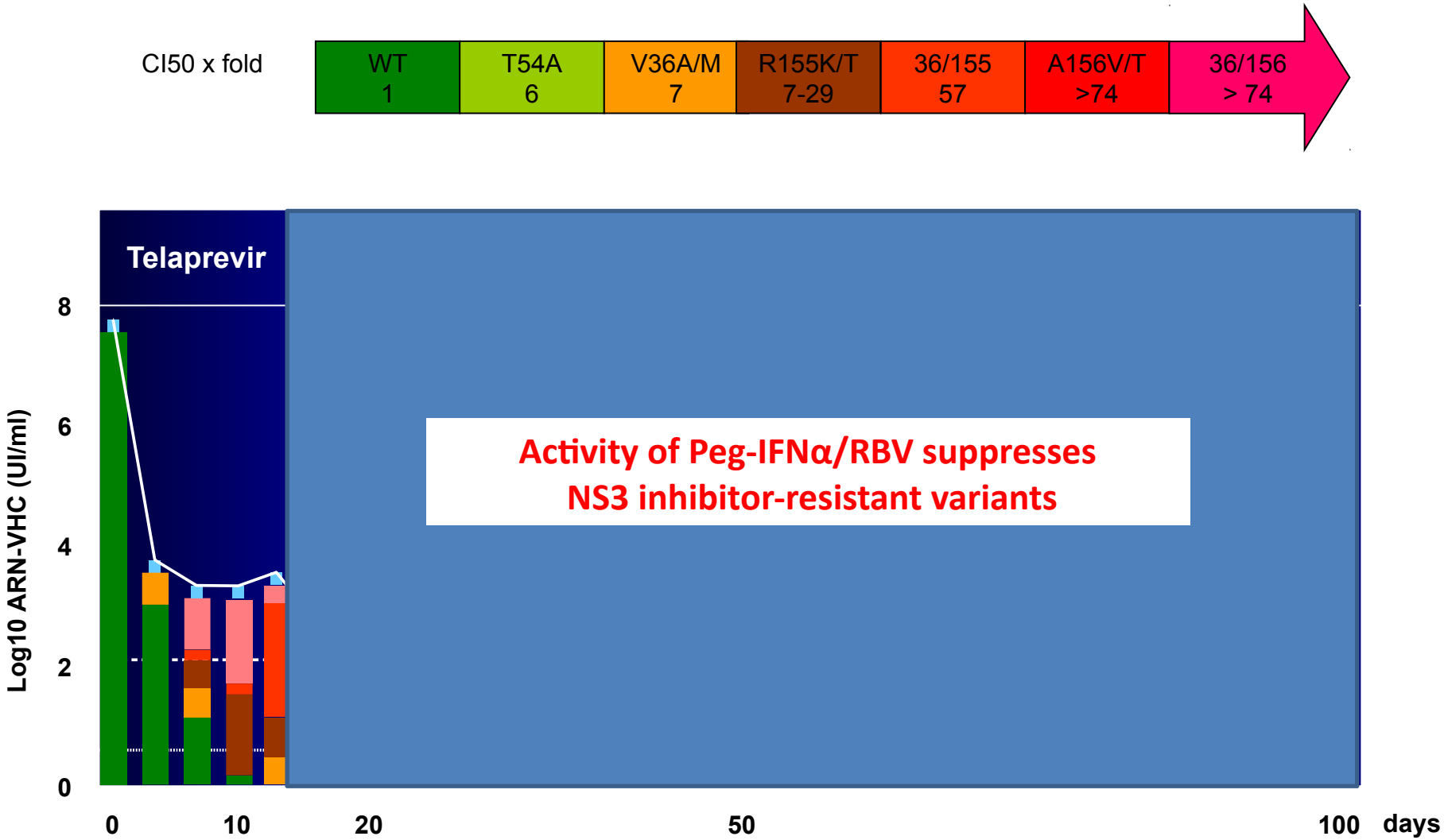
Treatment Failure: 30%

~15% discontinuations

~15% virologic failure
- **~5% breakthrough**
- **~10% relapse**

McHutchison JG, et al. N Engl J Med 2009;360:1827–38
Hézode C, et al. N Engl J Med 2009;360:1839–50; Marcellin P, et al. Hepatol 2009;50(Suppl. 4):395A
Adapted from Kwo P, et al. J Hepatol 2009;50(suppl 1):S4

Resistant virus is rapidly selected with Telaprevir alone



Kieffer et al.Hepatology 2007

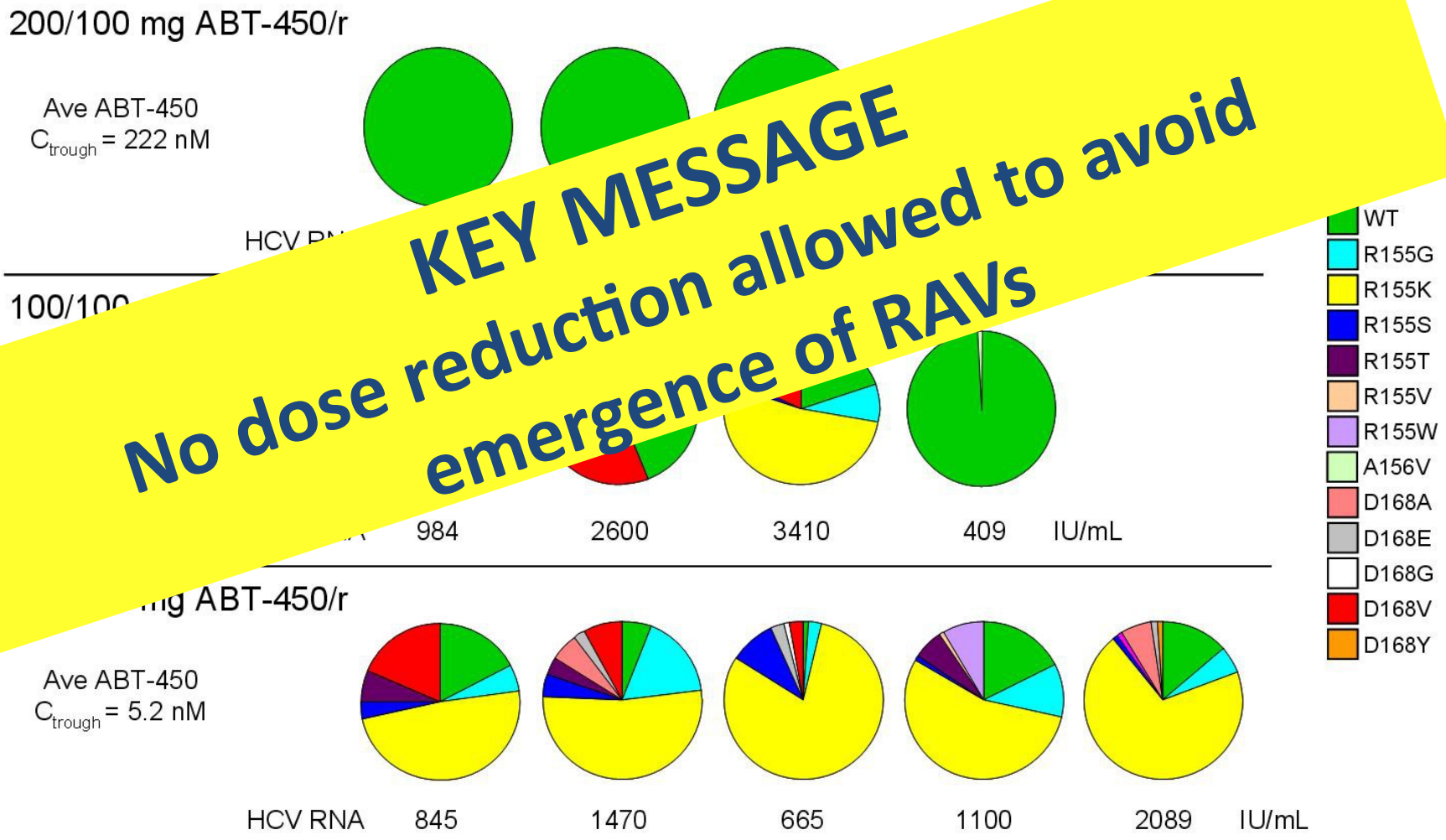
Low compliance → HCV resistance

- Important Side effects
- Treatment Duration 6 to 12 month
- High Pill burden
- Drug-drug Interaction
- Short therapeutic window : Ic 50/Cc 50

High fitness of the mutant not compensated with the drug exposure

Barrier to resistance: Role of pharmacology

Clonal sequence analysis from subjects dosed with ABT-450 for 3 da



KEY MESSAGE

No dose reduction allowed to avoid emergence of RAVs

Pilot-Matias TM *et al, 46th EASL*, Berlin, 2011, Abs#1107

Does Previous Response to PR influence the selection of RAV during a triple therapy PR+PI?

Peg-IFN treatment experienced patients can be retreated

Prior PR response	PR	Emergence of RAV
Relapse	22%	14 %
Partial	15%	40 %
Null	5%	68 %

Previous response influence the outcome of selection of RAV

Resistance Profiles in Non SVR patients

Variant	% of sequenced patients	
	Subtype 1a	Subtype 1b
WT	16%	46%
V36M	10%	3%
R155K	20%	0%
V36M+R155K	46%	0%
V36A	3%	16%
T54A	<1%	22%
A156S/T	3%	13%

Telaprevir Package Insert

Boceprevir Package Insert

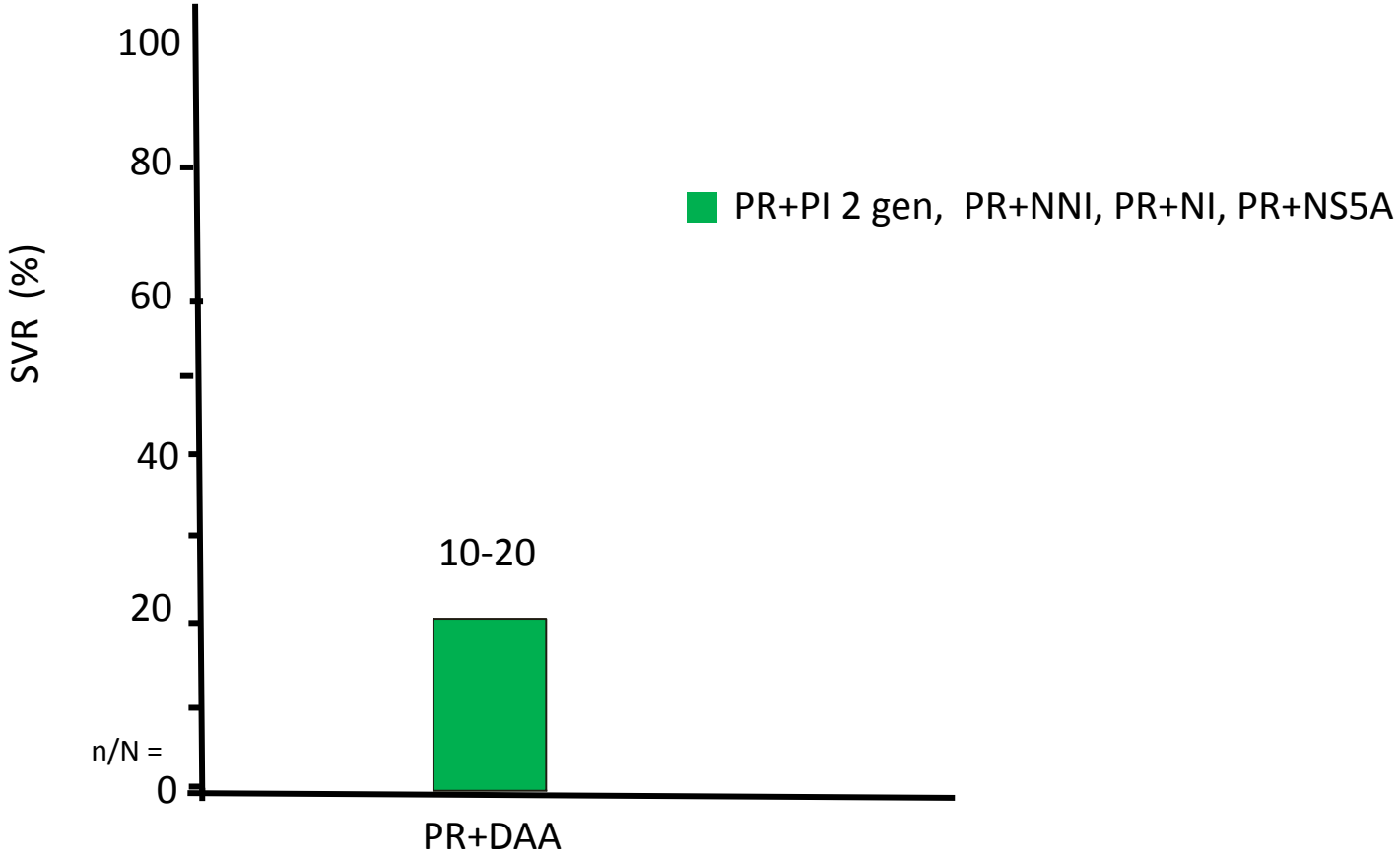
Amino acid positions within the NS3/4A protease associated with resistance mutations to different NS3 protease inhibitors

	V36A/M	T54A	V55A	Q80R/K	R155K/T/Q	A156S	A156V/T	D168A/V/T/H	V170A
Telaprevir (linear)			*						*
Boceprevir (linear)							*		
SCH900518 (linear)									
BILN-2061 (macrocyclic)									
ITMN191 (macrocyclic)						*	*		
MK7009 (macrocyclic)						*			
TMC435350 (macrocyclic)						*	*		
BI-201335 (linear)									
MK5172 (macrocyclic)									
GS-9256 (macrocyclic)									
ABT 450 (macrocyclic)									
BMS-791325 (macrocyclic)									

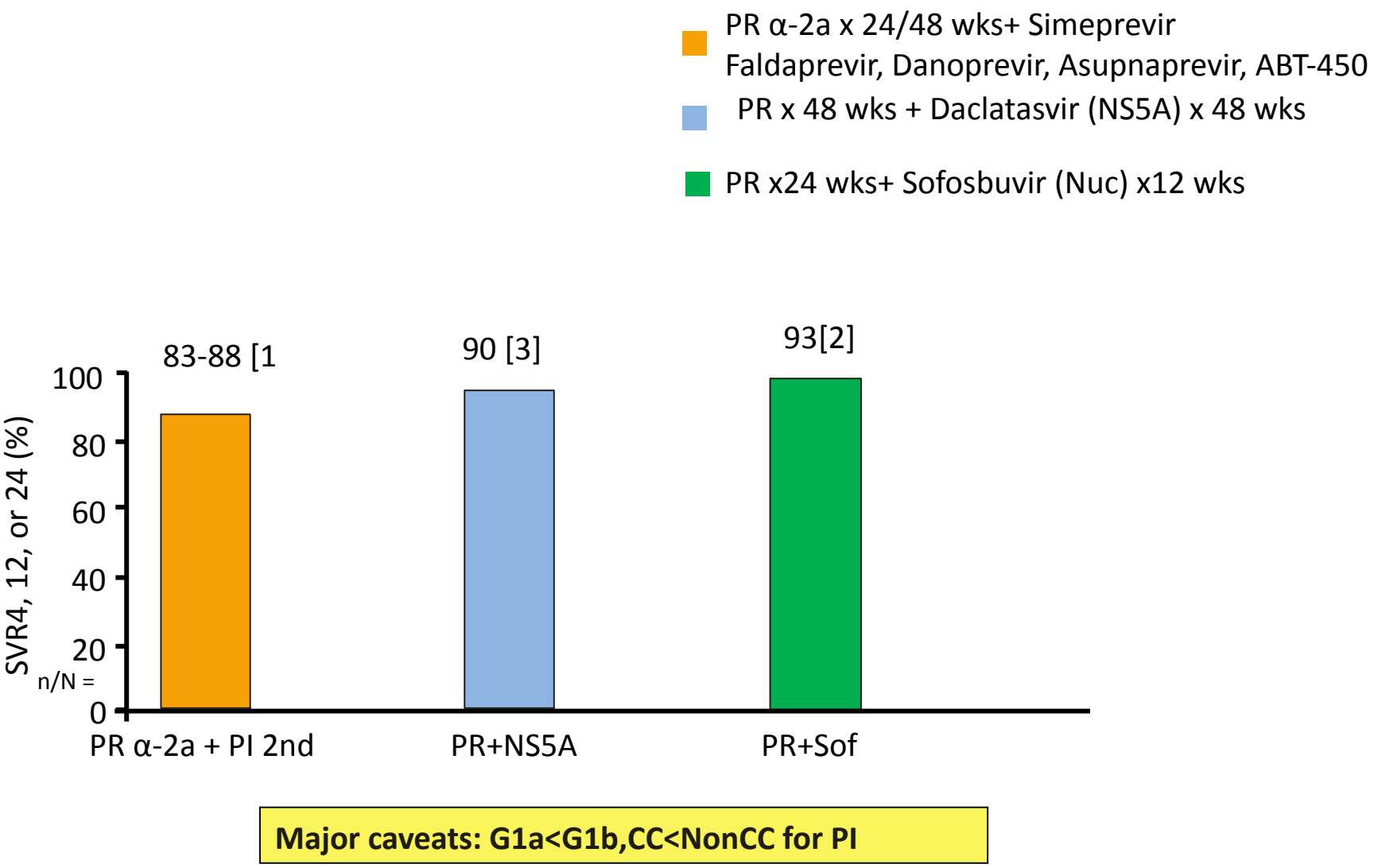
* Mutations associated with resistance in vitro only

HalfonP, Locarnini S et al J Hepatol 2011

Triple therapy using Peg-Ribavirine + 2nd of protease inhibitors, NS5A, and Nucleosides Inhibitors



Potent PegIFN alfa/RBV+ DAA Regimens in Treatment-Naive Genotype 1



Major caveats: G1a<G1b,CC<NonCC for PI

Do Baseline mutations polymorphism influence the SVR?

Difference between drugs within the same class

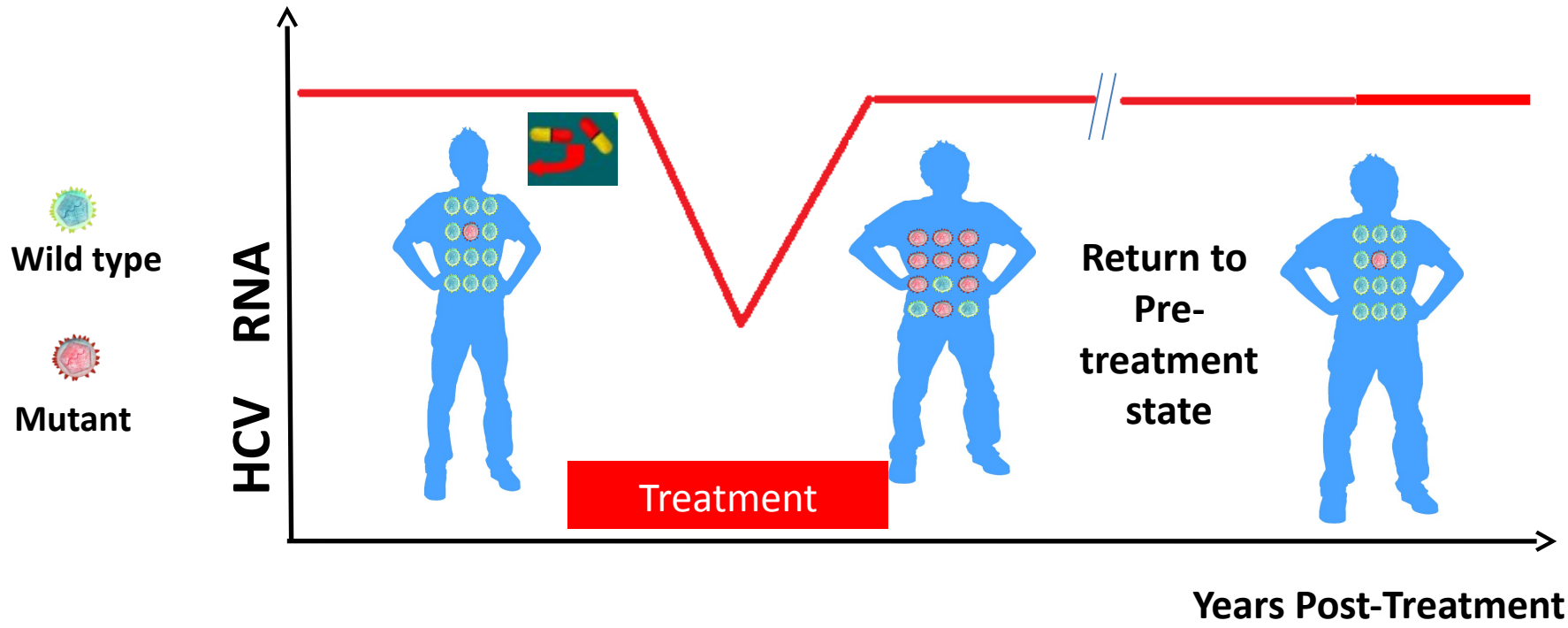
Simeprevir (OLYSIO™) indications and usage

The following points should be considered when initiating OLYSIO™ for treatment of CHC infection:

- OLYSIO™ must not be used as monotherapy
- OLYSIO™ efficacy in combination with PR is influenced by baseline host and viral factors
- OLYSIO™ efficacy in combination with PR is substantially reduced in patients infected with HCV GT 1a with an NS3 Q80K polymorphism at baseline compared to patients infected with HCV GT 1a without the Q80K polymorphism. Screening patients with HCV GT1a infection for the presence of virus with the NS3 Q80K polymorphism at baseline is strongly recommended. Alternative therapy should be considered for patients infected with HCV GT1a containing the Q80K polymorphism
- OLYSIO™ efficacy has not been studied in patients who have previously failed therapy with a treatment regimen that includes OLYSIO™ or other HCV protease inhibitors

Patient do not be re-treated with the same medication in the same regimen

Long term follow-up of patients with resistant variants after failing Treatment



- HCV population and clonal amino acid analyses in patient plasma suggest that PI-resistant viral populations ***may*** return to pre-treatment levels over time

Patient do not be re-treated with the same medication in the same regimen

but do patients be re-treated with HCV drugs from other DAA ?

Cross-Resistance

DAAs Compared with PEG-IFN/RBV

		DAA class								
HCV Target	Variant	NS3 Linear	NS3 Macrocyclic	NS5A inhibitor	NS5B nucleoside	NS5B Palm	NS5B Thumb	NS5B Finger	IFN	RBV
NS3 Protease	V36M	R	S	S	S	S	S	S	S	S
	T54A	R	S	S	S	S	S	S	S	S
	R155K	R	R	S	S	S	S	S	S	S
	A156T	R	R	S	S	S	S	S	S	S
	D168V	S	R	S	S	S	S	S	S	S
NS5A	L28V	S	S	R	S	S	S	S	S	S
	Y93H	S	S	R	S	S	S	S	S	S
NS5B	S282T	S	S	S	R	S	S	S	S	S
	C316Y	S	S	S	S	R	S	S	S	S
	M414T	S	S	S	S	R	S	S	S	S
	R422K	S	S	S	S	S	R	S	S	S
	M423T	S	S	S	S	S	R	S	S	S
	P495S	S	S	S	S	S	S	R	S	S

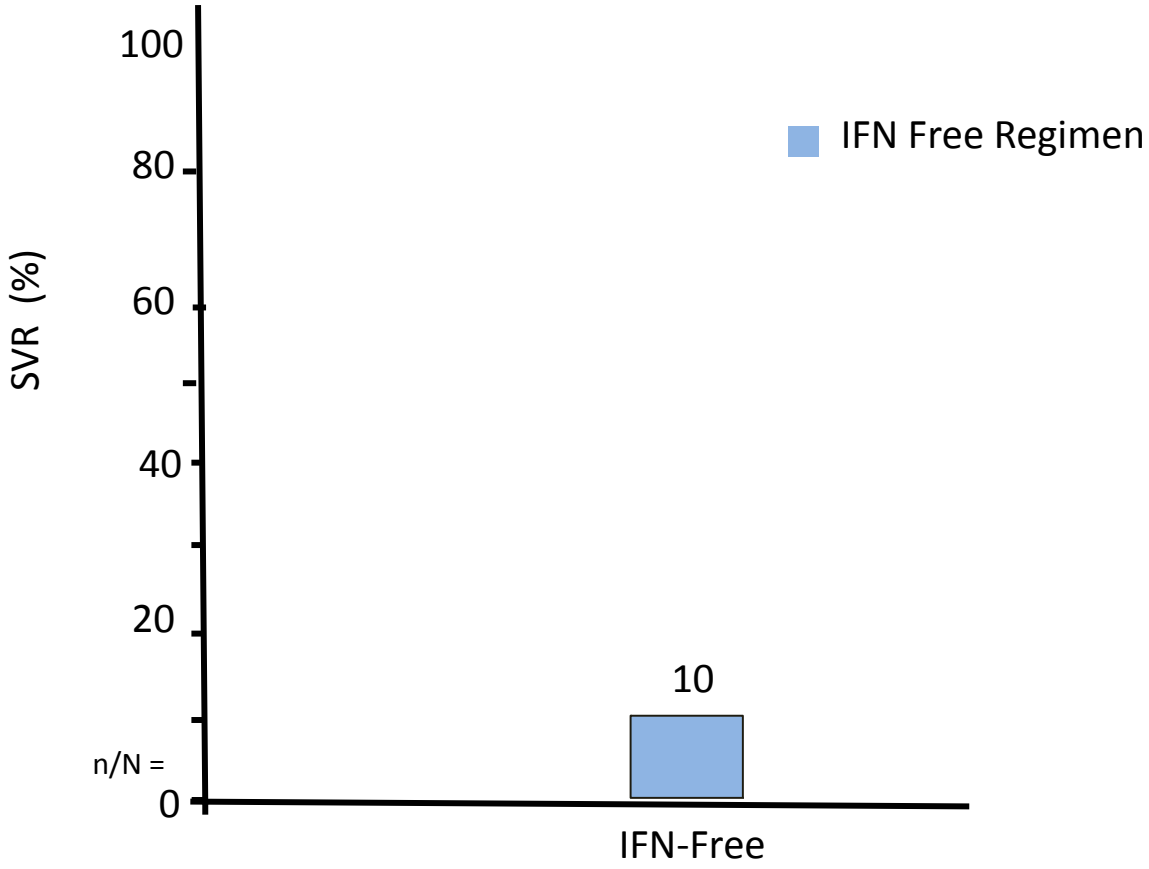
R = resistant = >4-fold increase in EC50; S = susceptible = <4-fold change in EC50; EC50 = 50% effective concentration (replicon assay)

DAA = direct-acting antiviral agent

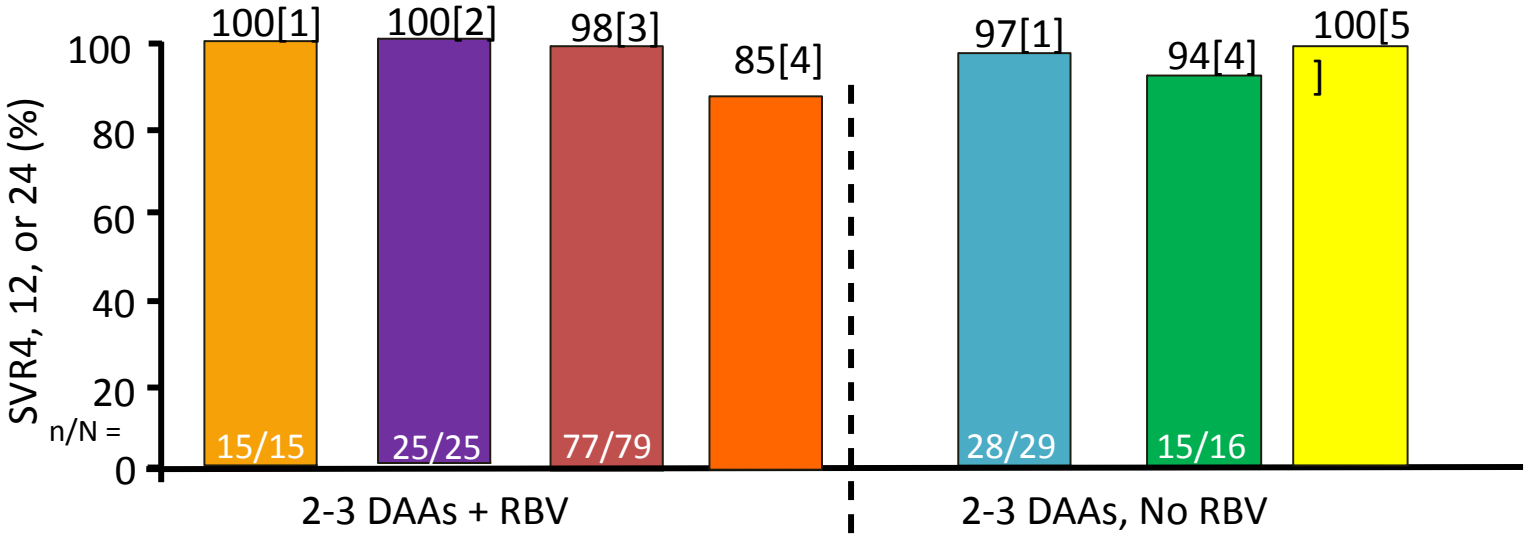
Adapted from Kieffer T, et al. J Antimicrob Chemother 2010;65:202–12

IFN Free Regimen ?

Magnitude of Treatment Failure using IFN Free Regimen ?



Potent IFN -FreeDAA Regimens in Treatment-Naive Genotype 1

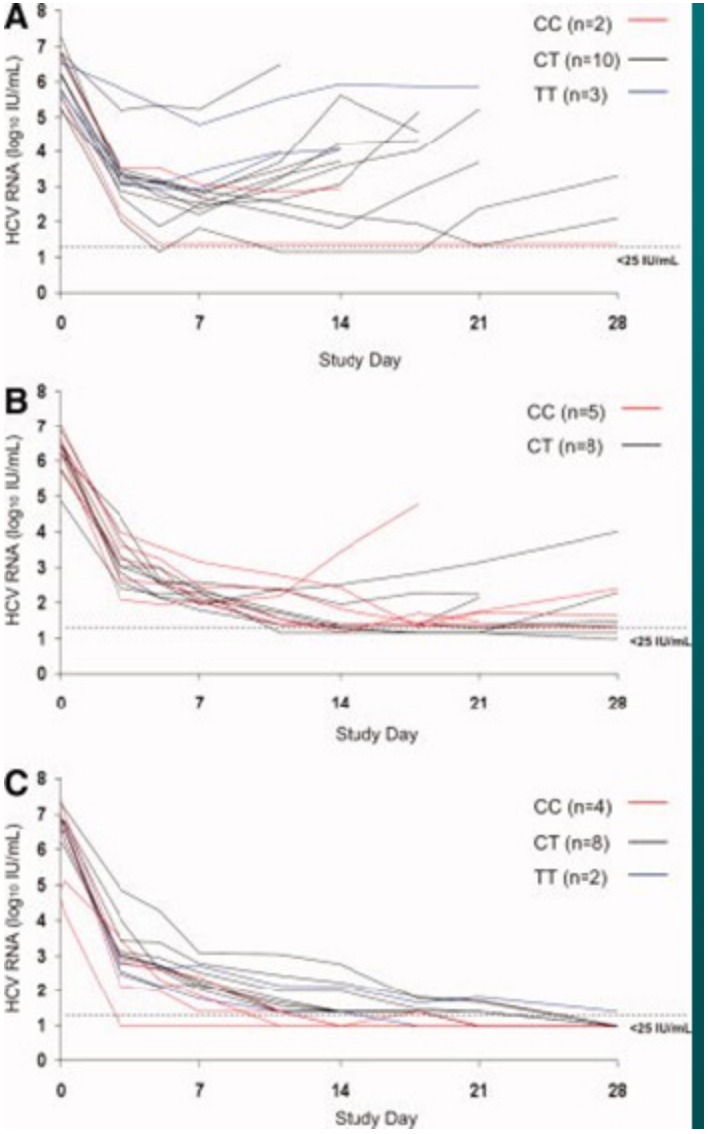


- Sofosbuvir (Nuc) + Daclatasvir (NS5A) + RBV x 24 wks
- Sofosbuvir (Nuc) + Ledispasvir (NS5A) + RBV x 12 wks
- ABT-450/r (PI) + ABT-333 (NNI) + ABT-267 (NS5A) + RBV x 12 wks
- Faldaprevir (PI) + Deleobuvir (NNI) + RBV x 24 wks (G1b)

- Sofosbuvir (Nuc) + Daclatasvir (NS5A) x 24 wks
- Daclatasvir (NS5A) + asunaprevir (PI) + BMS 791325 (NNI) x 12 wks
- Sofosbuvir (Nuc) +Simeprevir (PI)

Ribavirin-Free Regimen

During dual DAA treatment, Ribavarin and P/R increase the magnitude, extent and duration of viral reduction

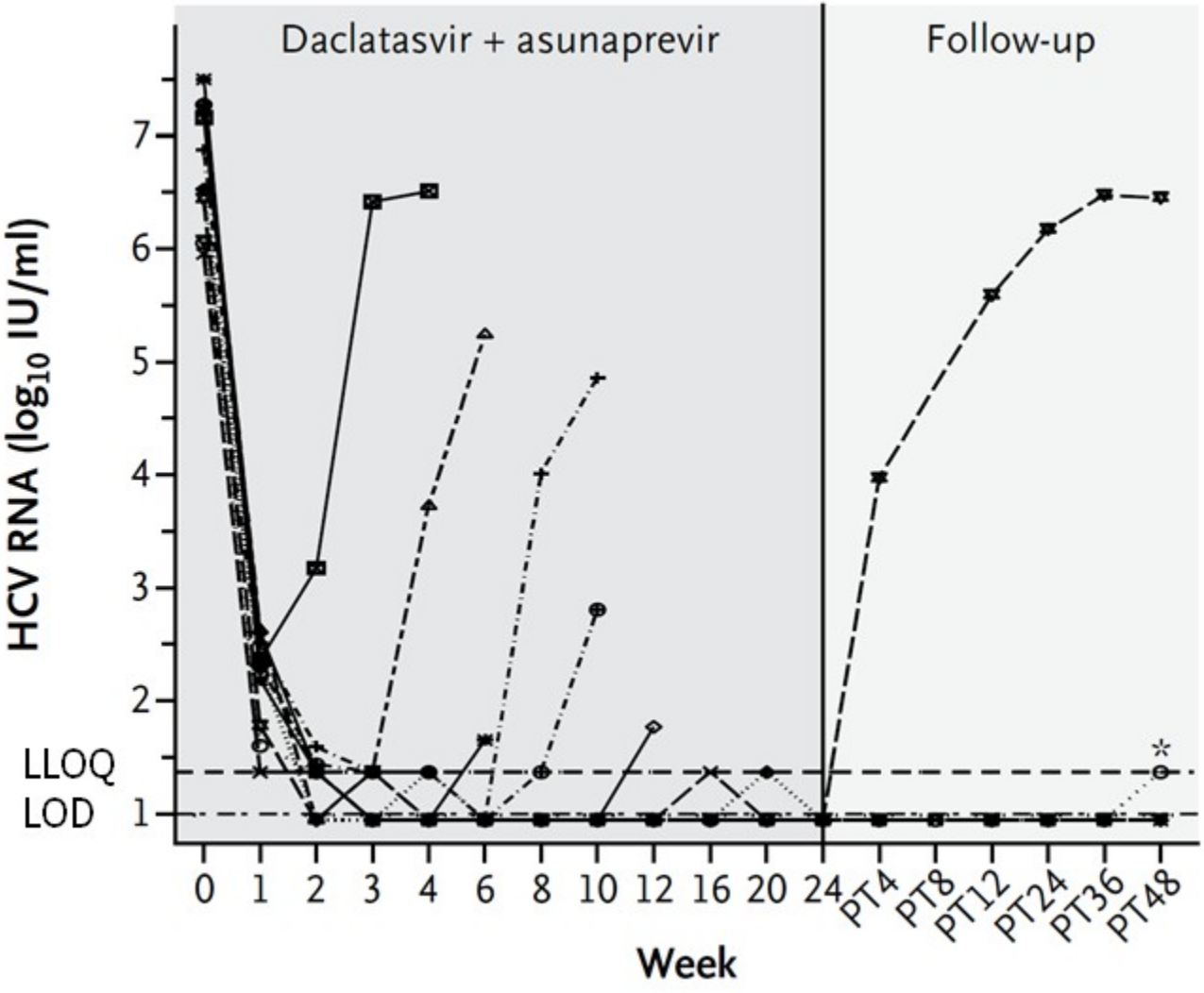


The addition of RBV enhanced antiviral activity, delayed the emergence/selection of resistance, and resulted in a greater proportion of patients achieving an RVR. Adding Peg-IFN plus RBV to the two antiviral agents further enhanced viral suppression, with 100% of patients reaching RVR

A) tegobuvir 40 mg BID and GS-9256 75 mg BID
B) tegobuvir 40 mg BID and GS-9256 75 mg BID plus RBV
C) tegobuvir 40 mg BID and GS-9256 75 mg BID plus Peg-IFN and RBV

Hepatology. 2012 Mar;55(3):749-58. doi: 10.1002/hep.24744. The protease inhibitor, GS-9256, and non-nucleoside polymerase inhibitor tegobuvir alone, with ribavirin, or pegylated interferon plus ribavirin in hepatitis C. Zeuzem S, Buggisch P, Agarwal K, Marcellin P, Sereni D, Klinker H, Moreno C, Zarski JP, Horsmans Y, Mo H, Arterburn S, Knox S, Oldach D, McHutchison JG, Manns MP, Foster GR.

IFN Free Regimen : combination of drugs have to be robust



Resistance to both drugs detected in all 7 subjects with breakthrough or relapse*:

NS5A

-$^{Q}30^{R}$

-$^{L}31^{MV}$

-$^{Y}93^{CN}$

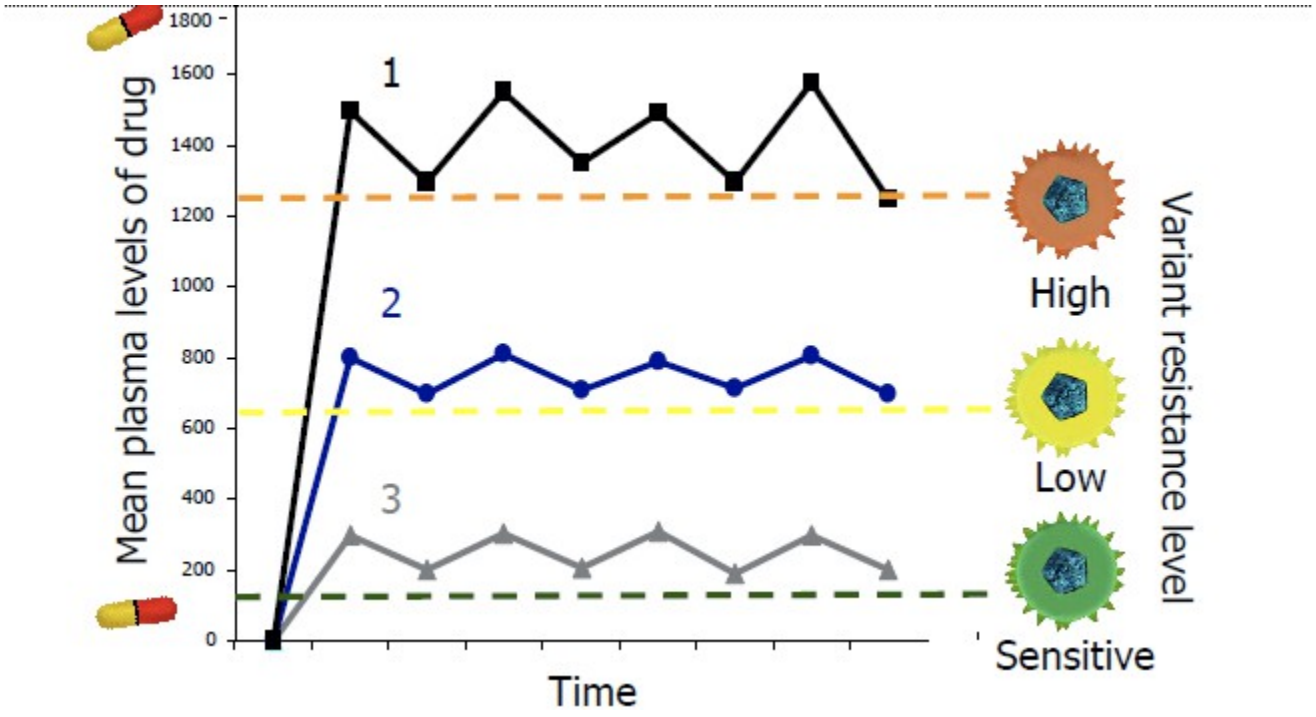
NS3

-$^{R}155^{K}$

-$^{D}168^{AETVY}$

*The relapser had NS3 R155K detected at Baseline, with emergence of NS5A Q30E at time of relapse

Clinical resistance occurs if drug levels are not sufficient to inhibit viral replication



Highly resistant viruses need very high drug levels (may not be achievable) to inhibit their replication

J Antimicrob Chemother
doi:10.1093/jac/dkt423

Journal of Antimicrobial Chemotherapy

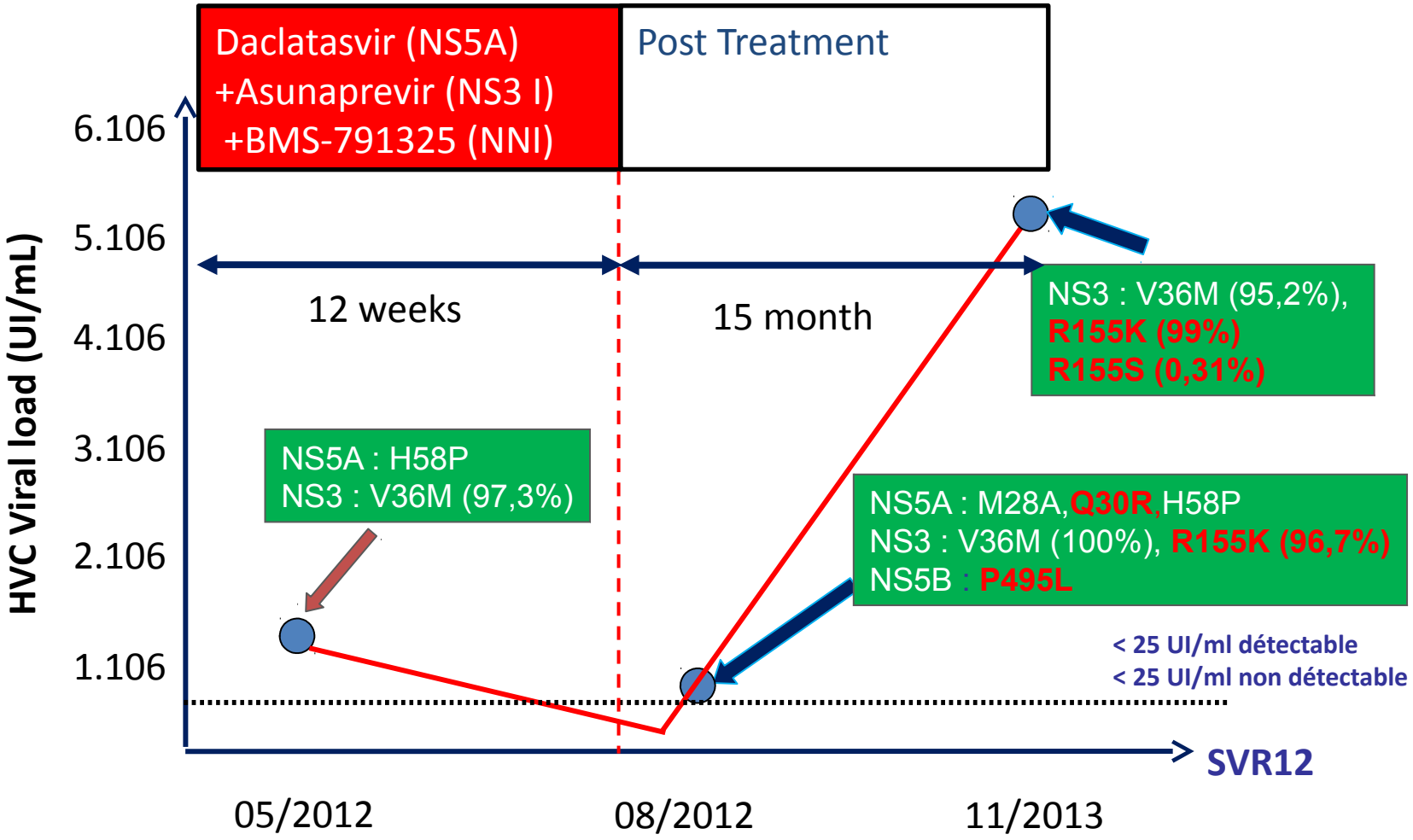
Modelling clinical data shows active tissue concentration of daclatasvir is 10-fold lower than its plasma concentration

Ruian Ke[1]*, Claude Loverdo[1], Hangfei Qi[2], C. Anders Olson[2], Nicholas C. Wu[3], Ren Sun[2–4]

Treatment	Dominant resistant mutants observed in the clinical trial reported by Fridell *et al.*[2]	Genotype	Patient(s)	Probability of resistance $\eta = 0.094$	Probability of resistance $\eta = 1$
10 mg once daily	**Y93H**[a]	1a	E	**0.684**	**0.236**
	L31V	1a	F	0.999	0.947
	L31M + Y93H	1b	G	0.669	0.499
	L31V + Y93H	1b	G	0.720	0.572
30 mg once daily	Q30E	1a	I, J, K	0.933	0.893
	Y93H[a]	1a	J	**0.556**	**0.021**[b]
60 mg once daily	**Q30H + Y93H**[a]	1a	M	**0.869**	**0.450**
	M28T	1a	N	0.006[b]	0.000[b]
	Q30E	1a	N, O	0.927	0.782
	Q30R	1a	P	0.102	0.000[b]
100 mg once daily	M28T	1a	R	0.0005[b]	0.000[b]
	Q30R + H58D	1a	S	1.00	1.000
	L31V + Q54H + Y93H[a]	1b	T	**0.981**	**0.113**

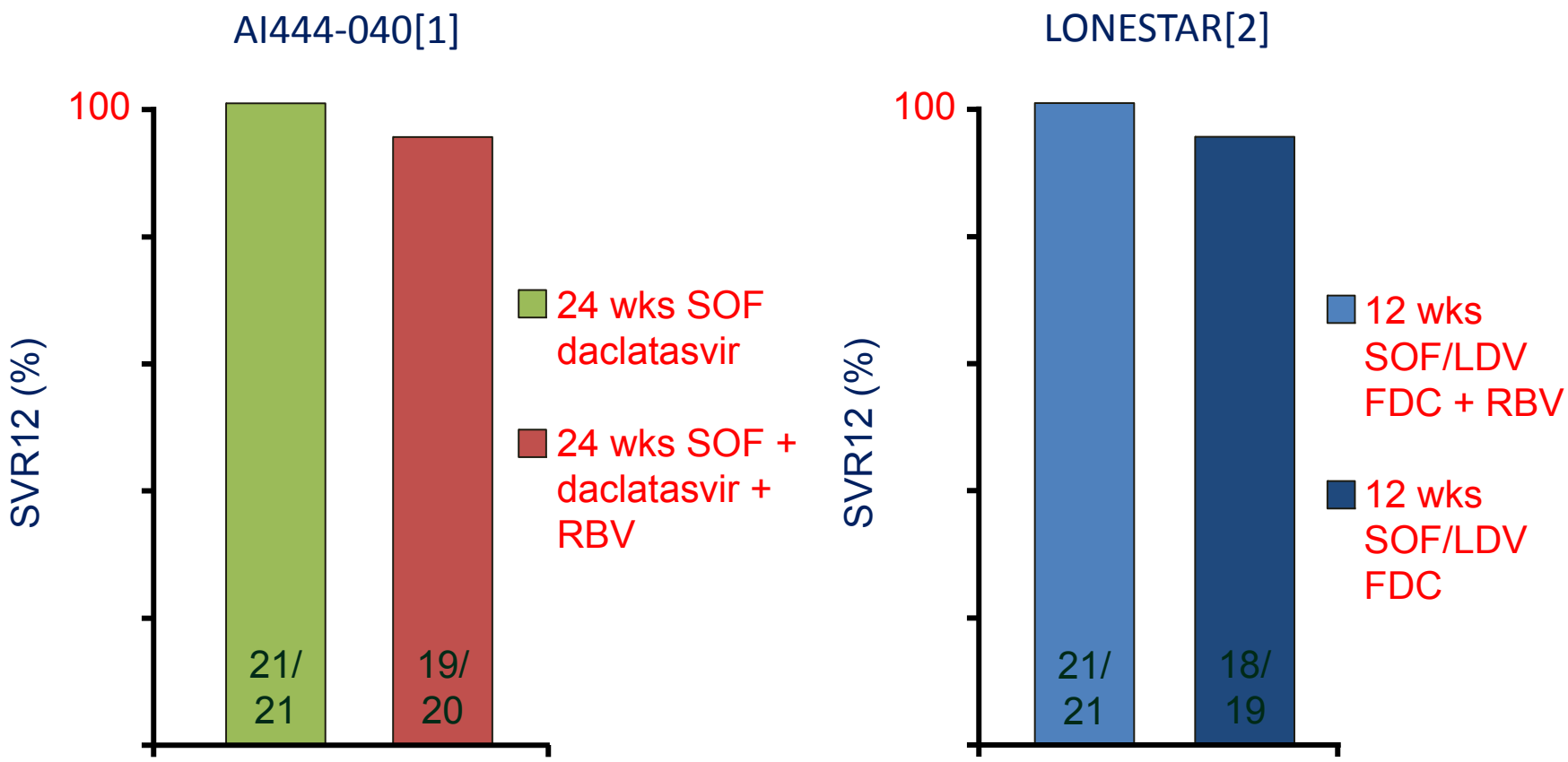
- The modelling results show that the active tissue concentration of daclatasvir is 9% of the concentration measured in plasma (95% CI 1%–29%).
- Using plasma concentrations as surrogates for clinical recommendations may lead to substantial underestimation of the risk of resistance

How we manage Patients Who Did Not Respond to PI Therapy ?



High fitness of the R155K mutation persisting > 1 year

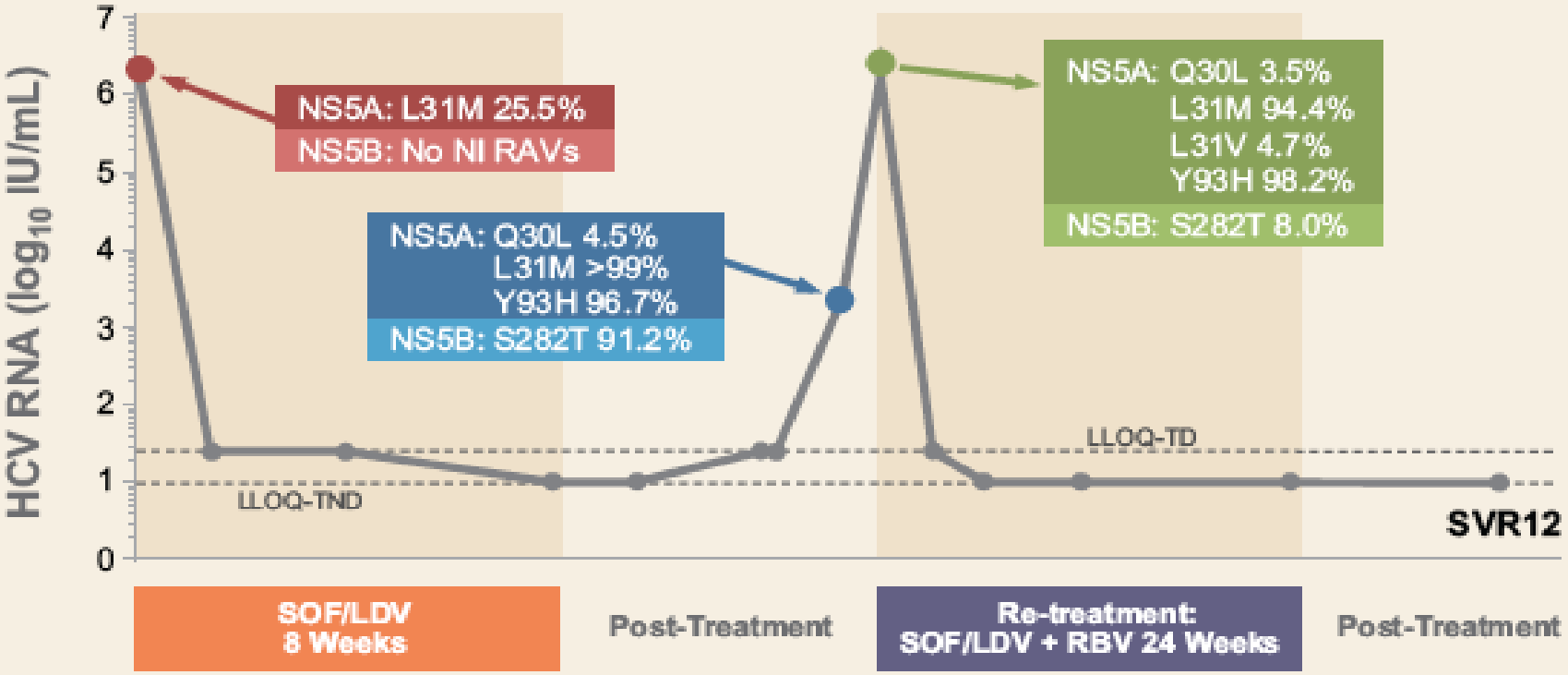
Options for Patients Who Did Not Respond to PI Therapy



*1 patient in triple-drug arm had missing data at Wk 12 posttreatment; this patient had undetectable HCV RNA at Wks 4 and 24 posttreatment.

1. Sulkowski MS, et al. EASL 2013. Abstract 1417. 2. Lawitz E, et al. AASLD 2013. Abstract 215.

Successful Re-treatment of Patient Who Failed 8 Weeks of SOF/LDV



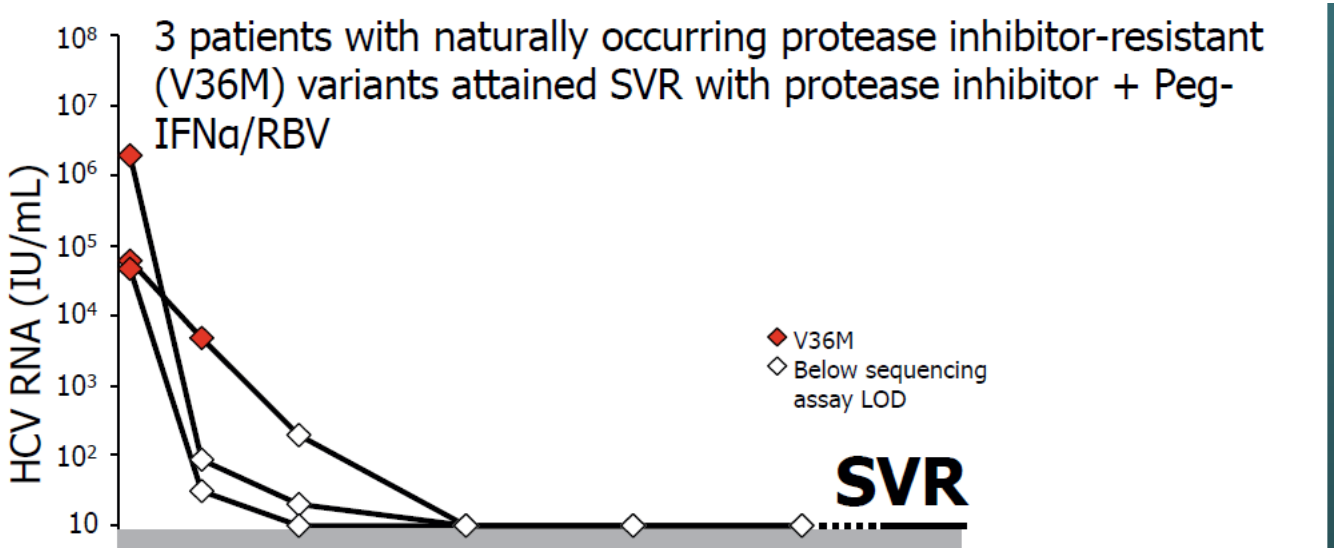
LLOQ, lower limit of quantitation; NI, nucleoside inhibitor; TD, target detected; TND, target not detected.

References

1. Lawitz E, et al. Lancet. 5 Nov 2013. DOI: 10.1016/S0140-6736(13)62121-2; **2.** Gane E, et al. AASLD 2013, abstract 73; **3.** Lawitz E, et al. EASL 2011, poster 1219; **4.** Cheng G, et al. EASL 2012, poster 1172; **5.** Gane E, et al. EASL 2013, abstract 2671.

Resistant variants can be eliminated with a combination drug regimen

Target	Variant	NS3 Covalent: Slow Reversible	NS3 Non-covalent: Linear and Macrocyclic	NS5A inhibitor	NS5B nucleoside	NS5B Palm	NS5B Thumb 1	NS5B Thumb 2	Peg-IFN	RBV
NS3	V36M	R	S	S	S	S	S	S	S	S



Adapted from Bartels DJ. , et al. J Infec Dis, 2008;198(6); 800-7

Maximize response, Minimize resistance
How Overcome virologic resistance?

- Adherence-friendly regimen
- Shorter regimen
- Minimal drug-drug interactions
- Potent viral suppression
- Good tolerability
- Combination regimens

Resistance to HCV DAAs: What is the threat level?

Resistance to HCV DAAS : what is the threat level?

Combinaison of DAA should suppress any replication under antiviral pressure in majority of cases in the future

- HCV resistance have to be survey using IFN free regimen combination, particulary with drugs without high potency or , DDI or not well tolerated
- Investigation of NGS have to be explored using combination of DAA
- Ribavirin will continue to have old bones in the future of HCV therapy…


That's all Folks!