Forum for Collaborative HIV Research

#3 – HCV Treatment Strategies to Reduce Drug Resistance

Slide set prepared by the
Forum for Collaborative HIV Research and
HCV Drug Development Advisory Group

The Slide Deck

- These slides may be used in user's own noncommercial presentations, but kindly do not change content and attribution. We ask that you honor this intent.
- These slides are not to be published or posted online without permission from the Forum for Collaborative HIV Research.

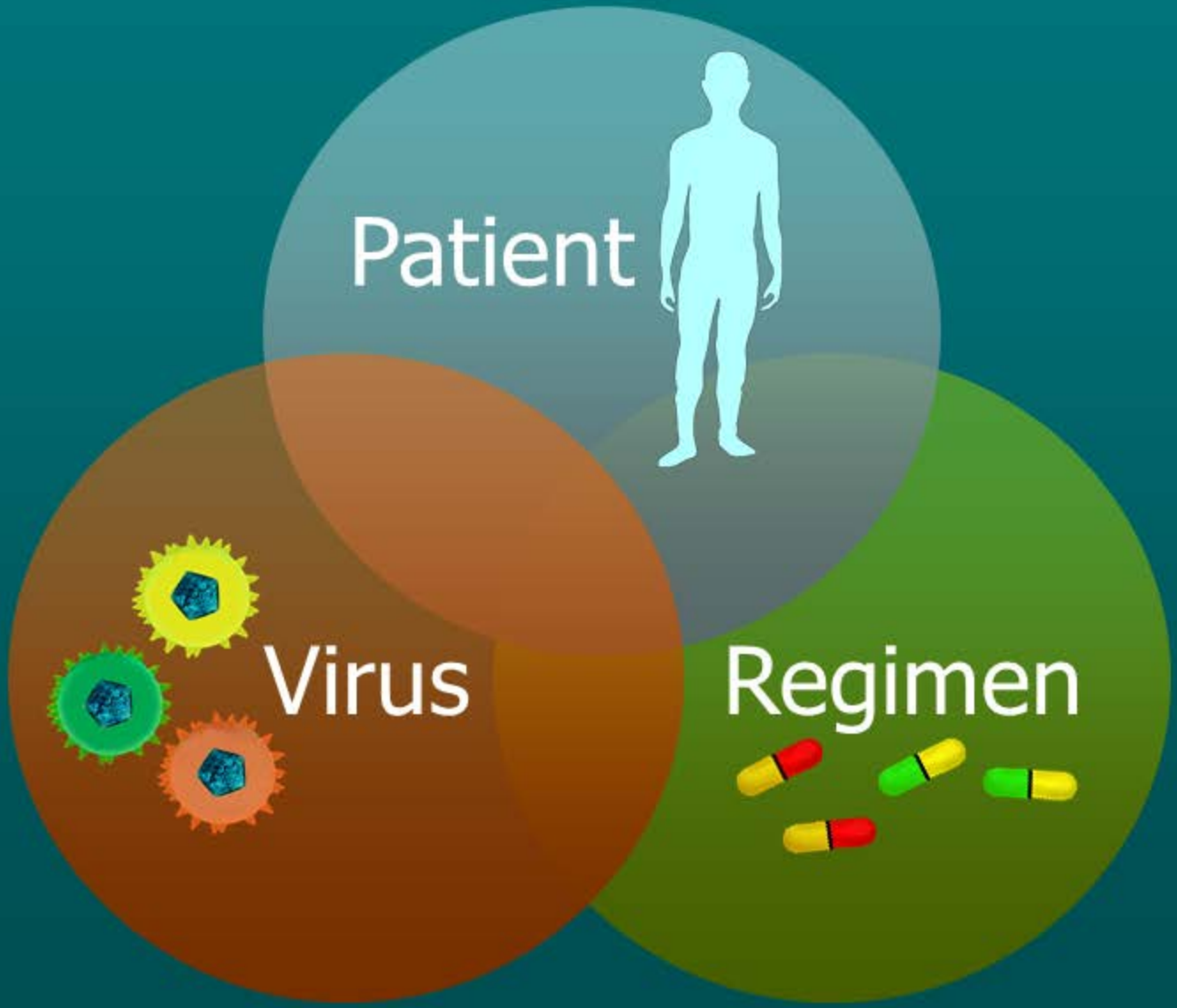
Slide Set #3 Table of Contents

Content	Slide #
Introduction	4-13
Peg-IFNα/RBV plus DAA regimens	14-24
Interferon-free regimens	25-37
Host IL-28B genotype	6, 10, 23, 27, 30, 32
DAA(s) plus Ribavirin only regimens	24, 30, 32, 33, 35-36
HCV subtype and genotype	8, 27, 31-32, 35-36
Index	38

Introduction

Many factors contribute to response

The symbol in the top-right hand corner of successive slides denotes whether the content refers to patient, virus, regimen or some combination thereof.



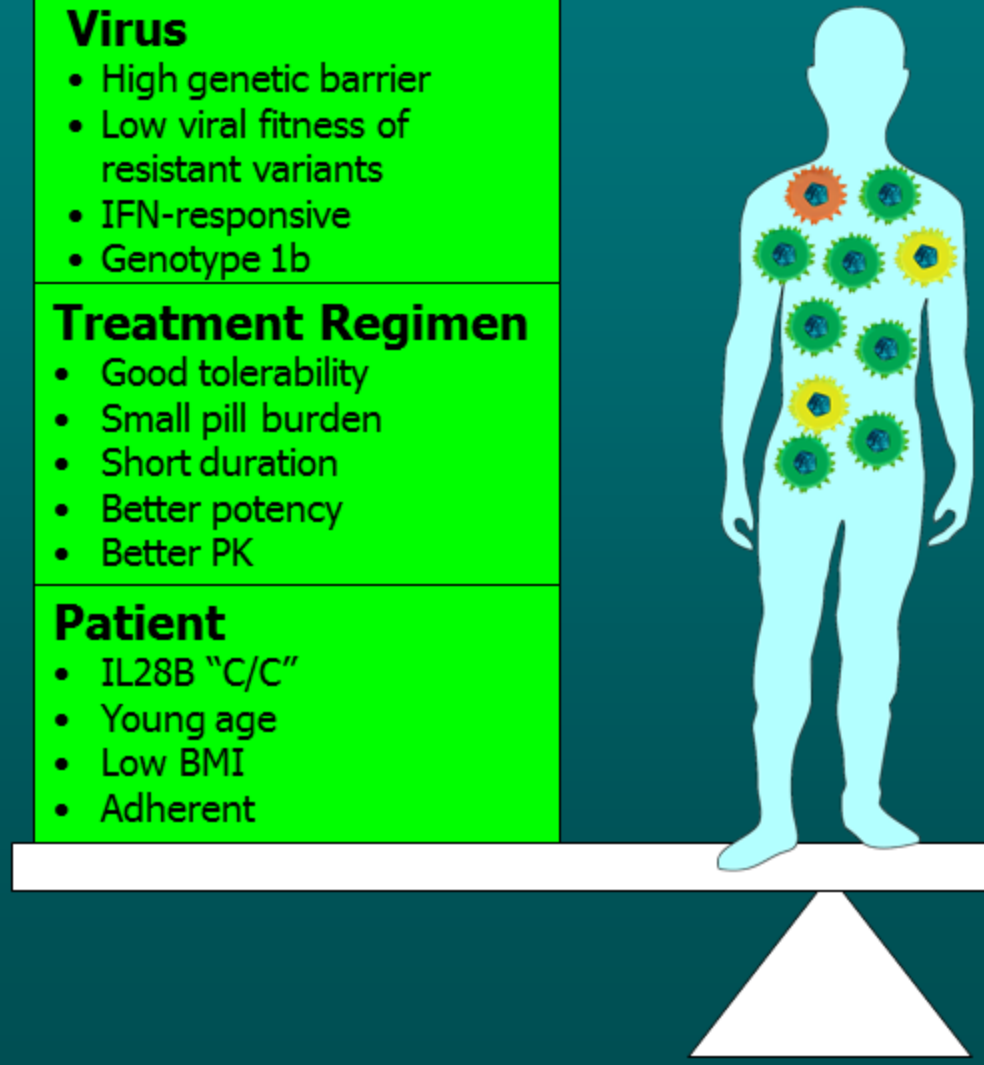
Sensitive virus

Resistant virus

Direct-acting antiviral (DAA)

Peg-IFNα/ ribavirin (P/R)

A balance of multiple factors contribute to SVR



Virus
- High genetic barrier
- Low viral fitness of resistant variants
- IFN-responsive
- Genotype 1b

Treatment Regimen
- Good tolerability
- Small pill burden
- Short duration
- Better potency
- Better PK

Patient
- IL28B "C/C"
- Young age
- Low BMI
- Adherent

Virus
- Drug resistance
- High viral load
- Genotype 1a

Treatment Regimen
- Poor tolerability
- Adverse events
- Drug interactions

Patient
- IL28B "T/T or C/T"
- HIV coinfection
- Substance abuse
- Insulin resistance
- Elderly
- High BMI
- Poor adherence
- Cirrhosis

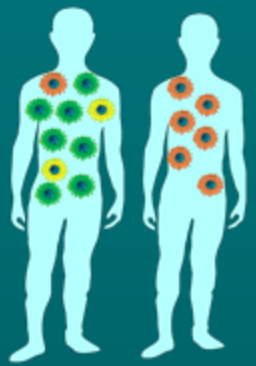
Resistance emerges as a result of treatment failure

Baseline contains WT (sensitive virus) + resistant variants in treatment naïve individuals

Treatment initiation

Direct-acting antiviral (DAA) plus Peg-IFNα/RBV (P/R)

P/R fails to suppress DAA-resistant variants



P/R suppresses DAA-resistant variants

- Breakthrough
- Relapse
- Discontinuations

SVR

Sensitive virus Resistant virus

McHutchison JG. , *et al. N Engl J Med,* 2009; 360(18): 1827-183
Sarrazin, C. & Zeuzem, S. *Gastro,* 2010;138:447-62
Hezode C. , *et al. N Engl J Med,* 2009; 360(18): 1839-1850 Kwo PY., et al *Hepatology,* 2008;48:1027A

Viral factors influencing treatment outcomes

Genetic barrier

- Number and type of nucleotide changes required for a virus to acquire clinical resistance to an antiviral regimen[1]

Viral fitness

- Relative capacity of a viral variant to replicate in a given environment
- Resistance mutations frequently compromise viral function and thus reduce viral fitness compared to wild-type in a drug-free environment

Genotype and subtype

- HCV genotype can impact response to Peg-IFNα/RBV, *e.g.* HCV genotype 1 (most common in the U.S.) is more difficult to treat with Peg-IFNα/RBV alone compared to other HCV genotypes
- Both HCV genotype and subtype (e.g. 1a vs 1b) can impact the activity and durability of HCV DAAs across several classes

Genetic barriers to resistance

- Relatively low barrier to resistance:
  - 1st generation protease inhibitors, non-nucleoside inhibitors of HCV RNA-dependent RNA polymerase, NS5A inhibitors
- Relatively high barrier to resistance:
  - nucleoside/nucleotide analogs and host targeted cyclophilin inhibitors
- Regimens should combine drugs that provide overall high barrier to resistance and hence greater chance of virological cure

Patient factors that influence treatment outcomes: IL28B genotype

- Certain single nucleotide polymorphisms (SNPs) upstream of *IL28B* gene are associated with the rate of SVR in patients treated with **Peg-IFNα/RBV**:

  SNP rs12979860: favorable allele=C, unfavorable allele=T

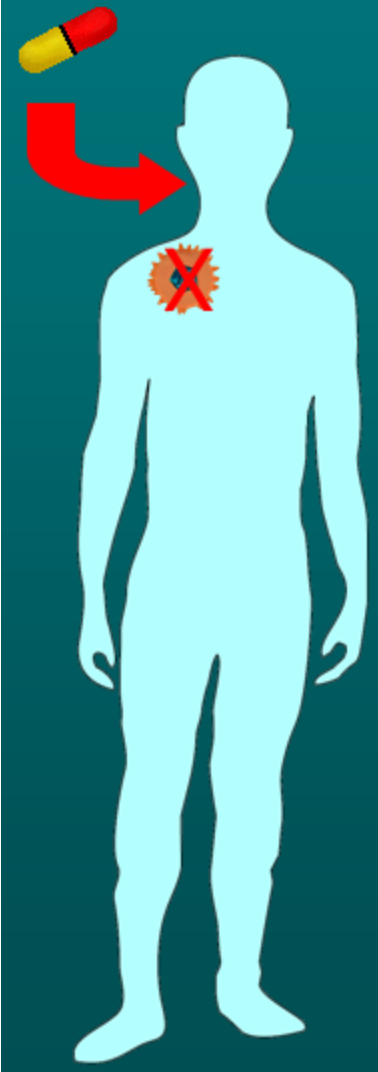
  SNP rs8099917: favorable allele=T, unfavorable allele=G

- IL28B genotype can have an impact on the efficacy of a Peg-IFNα/RBV/DAA regimen
- IL28B genotype may influence the activity of IFN-free, combination DAA regimens, although its impact is likely dependent on the drug classes combined along with their anti-HCV potency and durability

Ge D., *et al.*, *Nature*, 2009; 461:399-401
Tanaka Y., *et al. Nature Genetics*, 2009; 41:1105-1109
Suppiah V., *et al. Nature Genetics*, 2009; 41: 1100-1104

Pharmacological factors that affect outcomes

DAA



Higher potency and durability

- Create/use drugs with strong binding affinity and capacity to inhibit replication of pre-existing viral populations
- Drugs with a longer half-life may be more relevant to adherence than high drug levels
- Increase target organ exposure
- Take recommended dosage at recommended dosing intervals
- Food requirements which are easy to follow

Improved tolerability and adherence

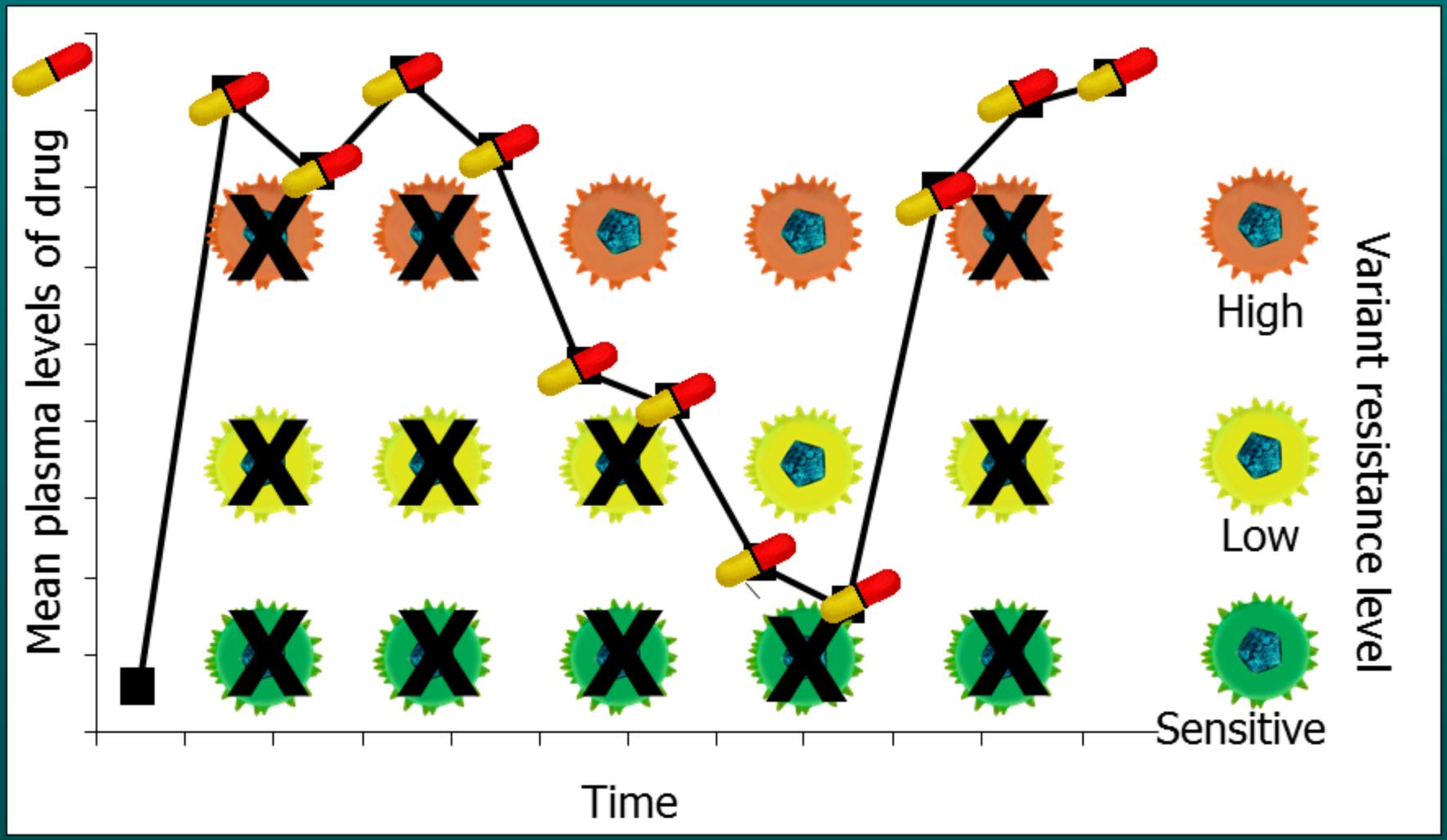
- Create/use drugs with minimal drug/drug interactions
- Create drugs with favorable safety profiles and convenient dosing schedules
- Develop better side effect management protocols

Combination drug regimens

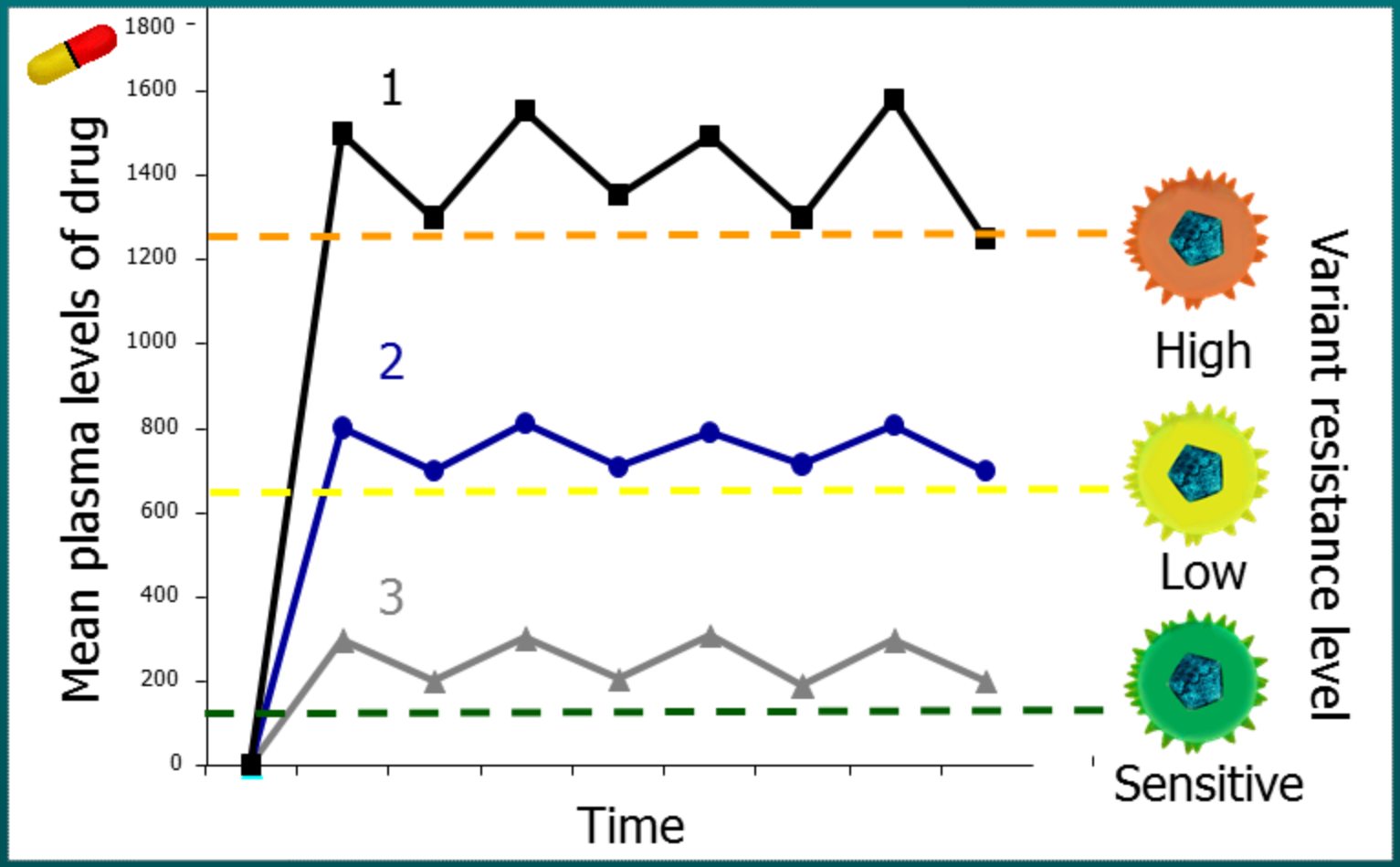
- Develop potent regimen of direct-acting antiviral drugs with or without Peg-IFN/RBV
- Regimens should combine drugs with different mechanisms of action and which provide overall high barrier to resistance and greater chance of virological cure

Regimen factors that affect outcomes

Drug trough levels must constantly be sufficient to suppress emergence of resistant variants



Resistance is not an all or none phenomenon

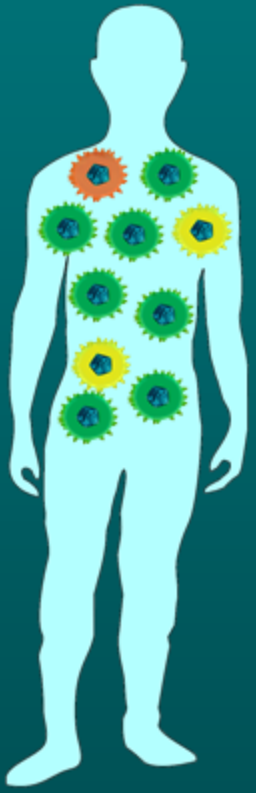


- Clinical resistance occurs if drug levels are insufficient to inhibit viral replication
- Highly resistant viruses need very high drug levels (may not be achievable) to inhibit replication

DAA plus P/R regimen

P/R experienced patients can be re-treated with DAA + P/R regiments

Peg-IFNα/RBV treatment experienced



New regimen of Protease Inhibitor + Peg-IFNα+RBV

Prior PR response	Retreatment SVR rate (approximate)	Resistance* Associated with Tx Failure
Relapse	86%	50%
Partial	60%	65%
Null	32%	80%

*% of subjects (approximate) who did not achieve SVR with protease inhibitor resistance substitutions by population sequencing

Definitions:

Prior relapser: HCV RNA not detected at end of treatment, but failed to achieve SVR

Prior partial responder: ≥2 log drop in HCV RNA at week 12 of prior therapy, but never achieved HCV RNA not detected during treatment

Prior null responder: Achieved <2log drop in HCV RNA at week 12 of prior therapy

See prescribing information of approved therapies for treatment recommendations and available data supporting re-treatment of specific patient populations

Contribution of Peg-IFNα and RBV to SVR

In a Peg-IFNα/RBV (P/R) plus protease inhibitor regimen for treatment-naïve patients, both Peg-IFNα and RBV enhance DAA antiviral durability by suppressing DAA-resistant variants

Baseline contains WT + resistant variants in treatment-naïve individuals

Sensitive virus

Resistant virus

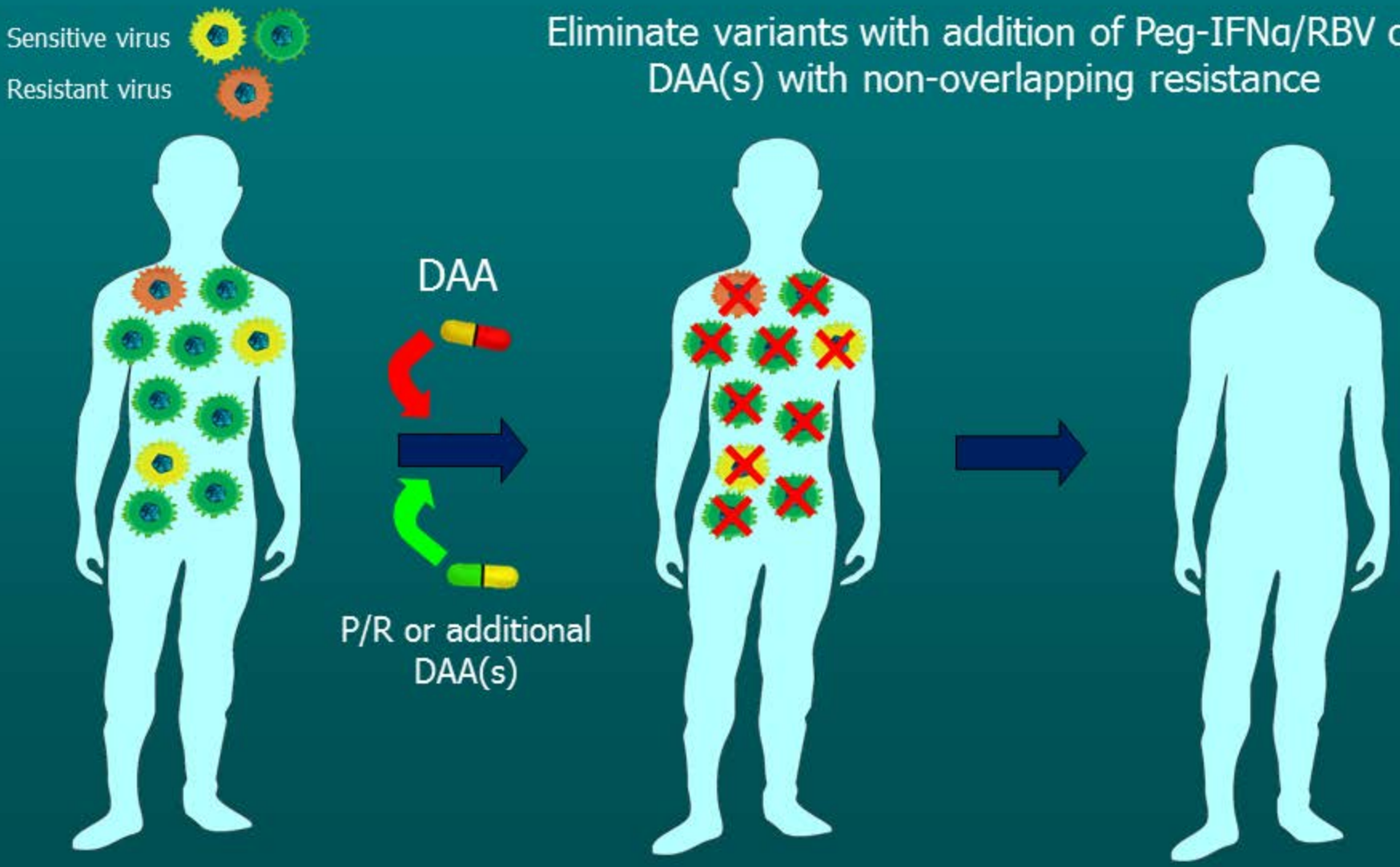
P/R + protease inhibitor → **SVR~70%**

P/R only → **SVR~46%**

Peg-IFNα (only) +protease inhibitor → **SVR~36%**

McHutchison J.G., et al. *N Engl J Med*, 2009; 360(18): 1827-1838
Hezode C., et al. *N Engl J Med*, 2009; 360(18): 1839-1850
Kwo PY., *et al. Hepatology*, 2008;48:1027A

Combination drug regimens increase the genetic barrier to resistance



Resistance profiles in non-SVR patients

Genotype and subtype

- Within each HCV subtype, different resistant variants can emerge during treatment
- Based on HCV genotype subtypes 1a vs 1b, the table below illustrates resistant variants that can emerge from Peg-IFNα/RBV plus telaprevir treatment

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%

Note: Information from a subset of patients in trials. Not a complete list of treatment-emergent substitutions observed in clinical trials. See drug Prescribing Information for a complete list.

Lack of cross-resistance between Peg-IFN/RBV and/or a combination of antiviral agents may provide an opportunity for elimination of resistant variants

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 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	L31V	S		R	S	S	S	S	S	S
	Y93H	S		R	S	S	S	S	S	S
NS5B	S282T	S		S	R	S	S	S	S	S
	C316Y	S		S	S	R	S	S	S	S
	M414T	S		S	S	R	S	S	S	S
	R422K	S		S	S	S	S	R	S	S
	M423T	S		S	S	S	S	R	S	S
	P495S	S		S	S	S	R	S	S	S

S = Susceptible (requires <4 fold shift in HCV replicon EC50)

R = Resistant (requires >4 fold increase in EC50)

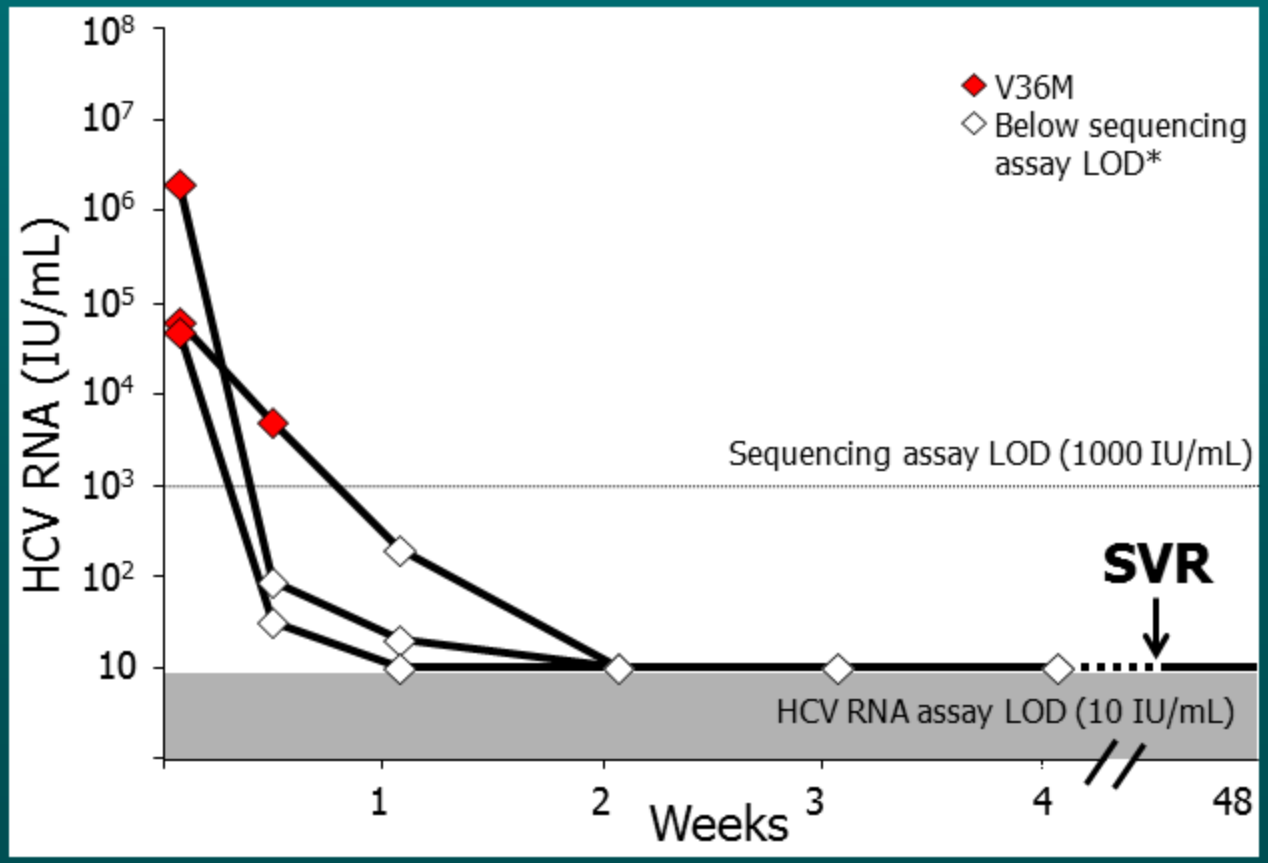
Note: This is not a comprehensive list of known HCV direct acting antivirals (DAA) resistance pathways. 4 fold shift represents arbitrary cutoffs for illustrative purposes only

Case Study: Elimination of resistant variants 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

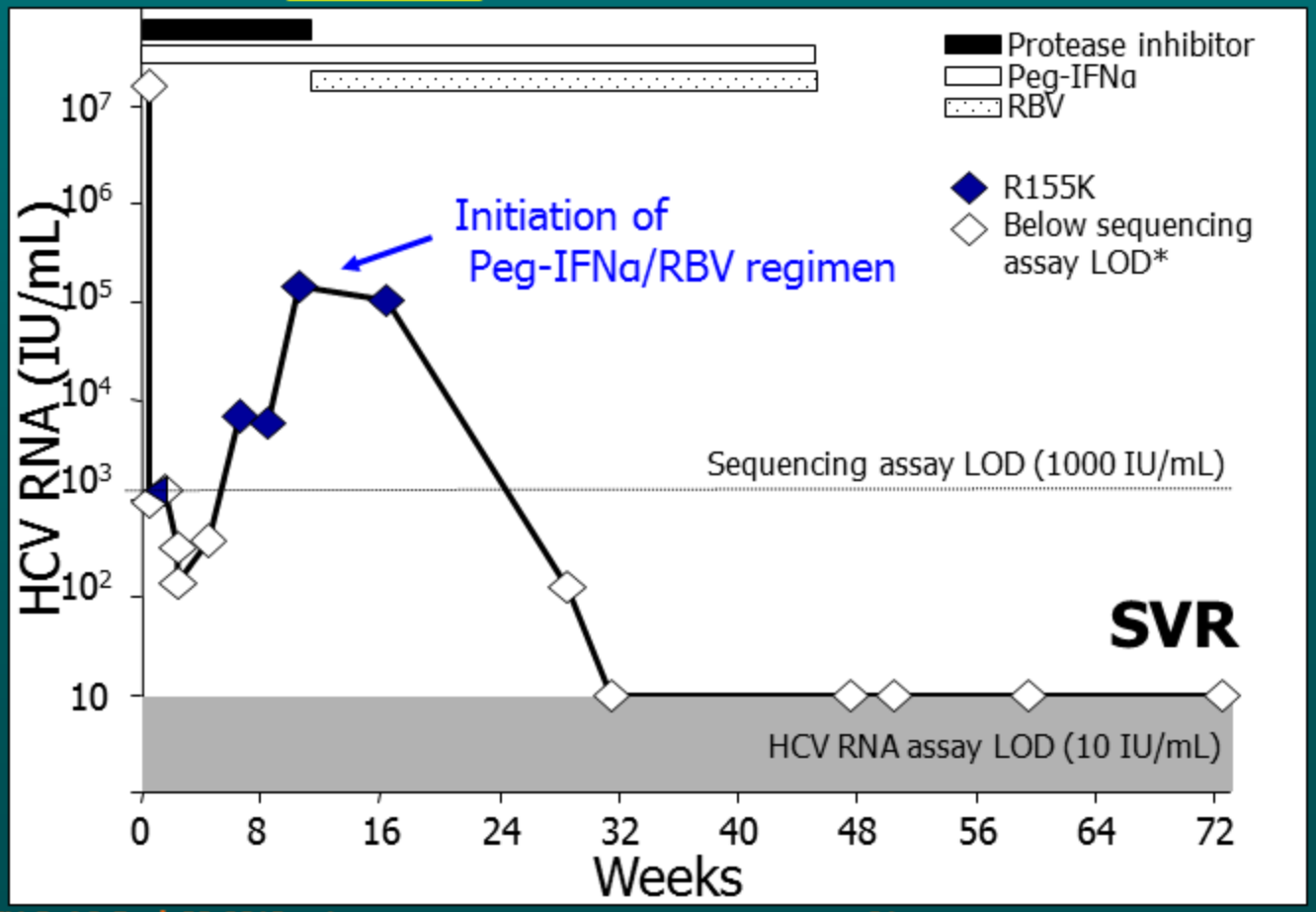
3 patients with naturally occurring protease inhibitor-resistant (V36M) variants attained SVR with combination of NS3 protease inhibitor + P/R

*White diamonds represent samples with an HCV RNA level below the LOD of the sequencing assay, for which no sequence data are available. For all other samples for which sequencing was successful, the red diamonds represent the variant observed at that time point.



Case Study: Patients with protease inhibitor-resistant variants can respond to Peg-IFNα/RBV

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

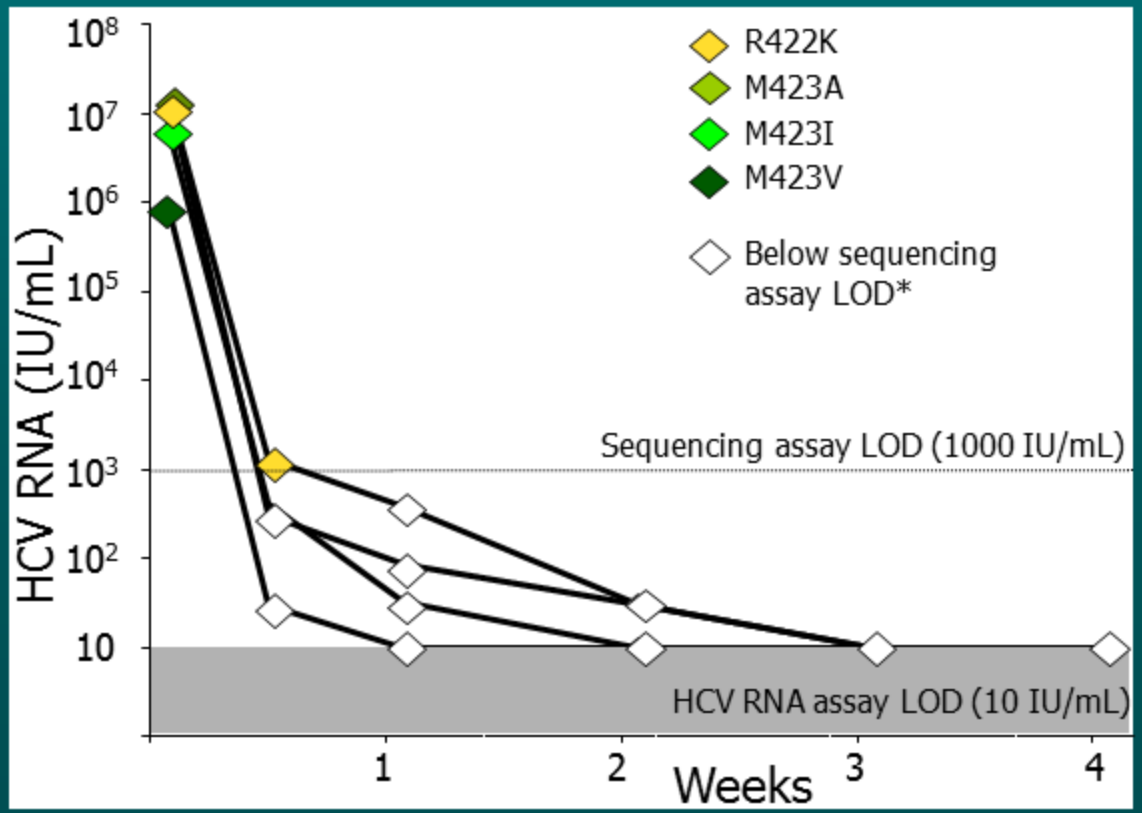


Patient with selected NS3 R155K variant achieved SVR with Peg-IFNα/RBV

*White diamonds represent samples with an HCV RNA level below the LOD of the sequencing assay, for which no sequence data are available. For all other samples for which sequencing was successful, the blue diamonds represent the variant observed at that time point.

Case Study: Patients with resistant variants to an NS5B inhibitor can respond to P/R plus PI

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
NS5B	R422K	S	S	S	S	S	S	R	S	S
	M423T	S	S	S	S	S	S	R	S	S



Naturally occurring polymerase inhibitor-resistant variants can eliminated with a protease inhibitor + Peg-IFNα/RBV regimen

*White diamonds represent samples with an HCV RNA level below the LOD of the sequencing assay, for which no sequence data are available. For all other samples for which sequencing was successful, the blue diamonds represent the variant observed at that time point.

Case Study: IL28B genotype effect on SVR in genotype-1 treatment-naïve patients

- Certain single nucleotide polymorphisms (SNPs) upstream of *IL28B* gene are associated with SVR in patients treated with P/R[1-3]:
  - SNP rs12979860: favorable allele=C, unfavorable allele=T

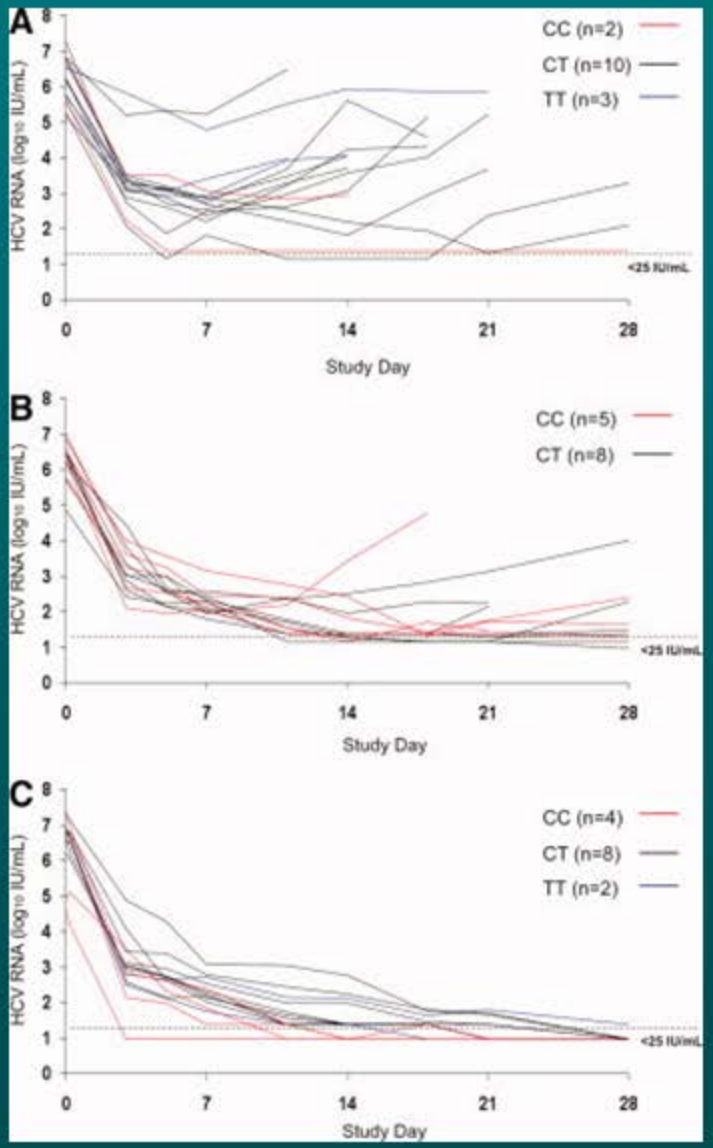
IL28B SNP rs12979860	PR (Ideal)		Telaprevir[5] (ADVANCE)		Boceprevir[6] (SPRINT-2)	
	ITT[4] Population	Adherent[3] Population	TVR/PR	PR control	BOC/PR*	PR control
CC	69%	~79%	90%	64%	80-82%	78%
CT	33%	~38%	71%	25%	65-71%	28%
TT	27%	~26%	73%	23%	55-59%	27%

*** Includes BOC/RGT and BOC/PR48 arms, ITT – intent to treat**

1 Tanaka Y., *et al. Nature Genetics*, 2009; 41:1105-1109
2 Suppiah V., *et al. Nature Genetics*, 2009; 41: 1100-1104
3 Ge D., *et al. Nature*, 2009; 461:399-401
4 Thompson A.J., *et al. Gastro, 2010; 139: 120-129*
5 INCIVEK™ [package insert]. Cambridge, Mass: Vertex Pharmaceuticals Inc; 2011
6 VICTRELIS™ [package insert]. Whitehouse Station, NJ: Merck & Co, Inc; 2011

Footnotes: 1) Data from ADVANCE and SPRINT-2 based only on subjects who consented to IL28B genotype analysis; 2) Results are confounded by variable treatment durations in active and control arms

Case Study: P/R increases the magnitude, extent and duration of viral reduction during dual DAA treatment



- Patients were treated with tegobuvir (NS5B inhibitor) and GS-9256 (protease inhibitor) with or without P/R
- The addition of RBV enhanced antiviral activity and resulted in a greater proportion of patients achieving an RVR.
- Addition of 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

Interferon-free, combination direct acting antiviral (DAA) regimens: Progress and challenges

- There is an urgent need for safe and effective treatment regimens that do not include Peg-IFN and/or RBV
- Numerous clinical trials investigating the efficacy of combination DAA regimens to replace Peg-IFN and/or RBV have been conducted or are in progress
- Challenge: **Anti-HCV potency without durability ≠ SVR**

Interferon-free, combination direct acting antiviral (DAA) regimens: Progress and challenges

- Lessons learned from recent trials:
  1. For a majority of patients, two potent but low resistance barrier DAAs are likely inadequate to achieve SVR
  2. Drug-drug interactions with DAA combination regimens may be more complicated than P/R and a single DAA
  3. Ribavirin may contribute to SVR in some DAA-only regimens
  4. HCV genotype/subtype can have an impact on treatment response
  5. IL28B genotype may influence the activity of different IFN-free combination DAA regimens, although its impact is likely dependent on the combined drug classes, their anti-HCV potency and durability
  6. Natural DAA resistance-associated polymorphisms may reduce the efficacy of some regimens, although this is an active area of investigation

  Only SVR proves efficacy
  - Virologic breakthrough can occur early (few days) or late (2-3 months)
  - Virologic relapse cannot be predicted based on initial HCV RNA response alone

Case Study 1: daclatasvir plus asunaprevir with or without P/R

Table 1. Baseline Demographic Characteristics of the Patients and Characteristics of the Disease.*

Characteristic	Group A (N=11)	Group B (N=10)
Age — yr		
Median	54.0	56.5
Range	36–61	38–63
Male sex — no. (%)	9 (82)	4 (40)
Race — no. (%)†		
White	9 (82)	7 (70)
Black	2 (18)	3 (30)
HCV genotype — no. (%)		
1a	9 (82)	9 (90)
1b	2 (18)	1 (10)
IL28B rs12979860 genotype — no. (%)		
CT or TT	10 (91)	9 (90)
CC	1 (9)	1 (10)
HCV RNA $\log_{10}$ — IU/ml	6.8±0.6	6.6±0.8
HCV RNA distribution — no. (%)		
<800,000	0	2 (20)
≥800,000	11 (100)	8 (80)
Baseline ALT — U/liter	70.5±57.65	57.9±29.94

Phase 2a (7 centers in U.S.)
Prior nonresponders to P + R
def: < 2 $\log_{10}$ drop during 12+ wks
Genotype 1, no cirrhosis

daclatasvir = BMS-790052
daily NS5A inhibitor
asunaprevir = BMS-650032
b.i.d. NS3/4A PI

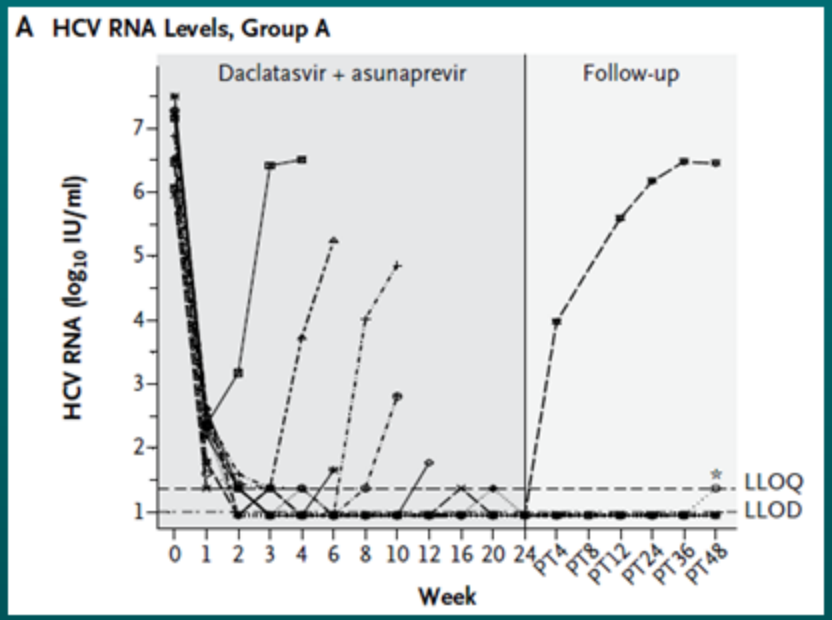
Group A (24 weeks):
daclatasvir + asunaprevir
P + R added for breakthrough

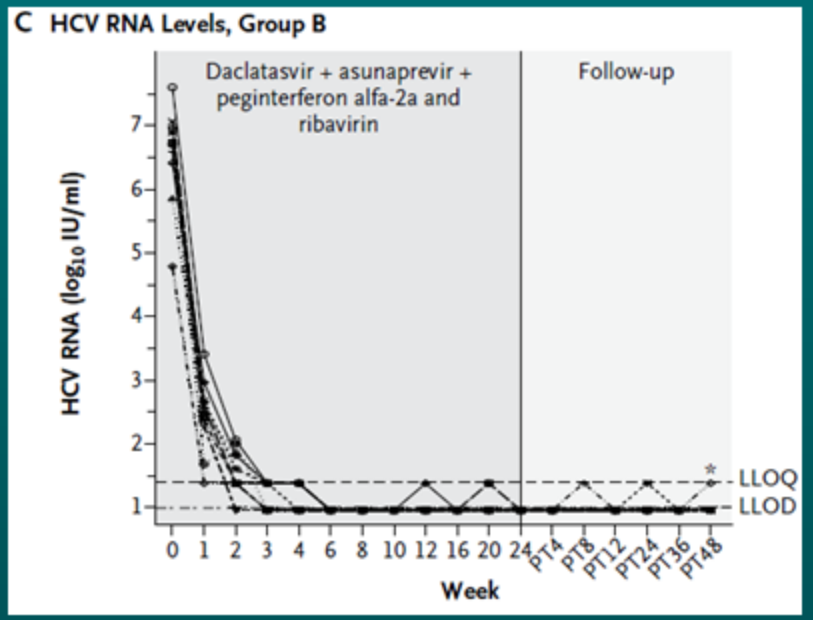
Group B (24 weeks):
P + R + daclatasvir + asunaprevir

Lok, A.S., et al., Preliminary study of two antiviral agents for hepatitis C genotype 1. The New England journal of medicine, 2012. 366(3): p. 216-24.
Chayama, K., et al., Dual therapy with the NS5A inhibitor BMS-790052 and the NS3 protease inhibitor BMS-650032 in HCV genotype 1b-infected null responders. Hepatology, 2011

Case Study 1: daclatasvir + asunaprevir with or without P/R

1. For some patients, two potent but low resistance barrier DAAs are likely inadequate to achieve SVR
6. Natural DAA resistance-associated polymorphisms may reduce the efficacy of some regimens, although this is an active area of investigation





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

NS5A: Q30R, L31MV, Y93CN
NS3-4A: R155K, D168AETVY

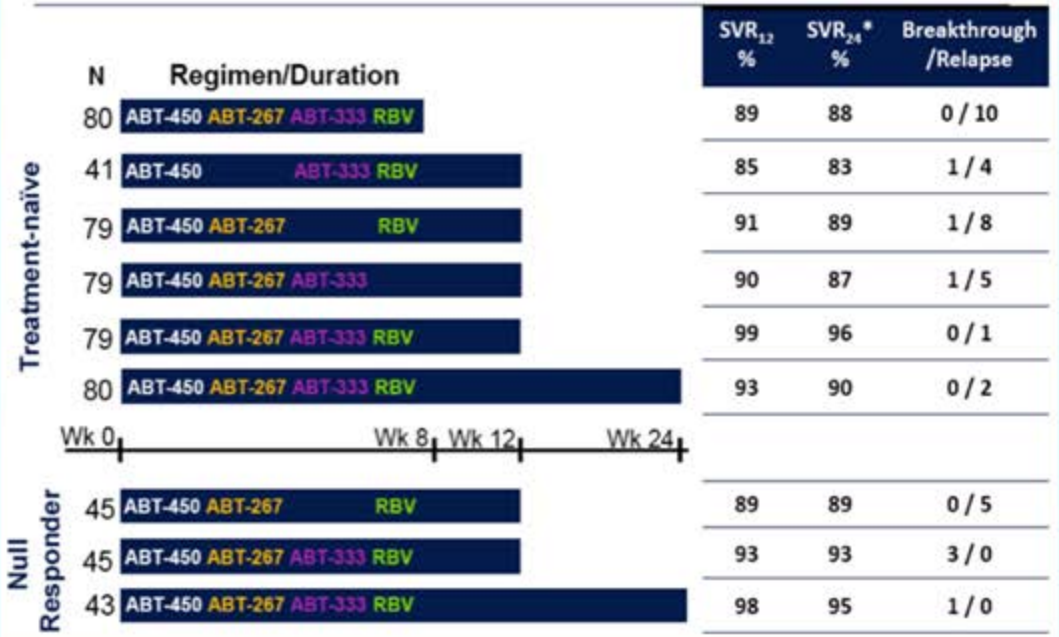
*The relapser had NS3 R155K detected at baseline, with emergence of NS5A Q30E at time of relapse

Lok, A.S., et al., Preliminary study of two antiviral agents for hepatitis C genotype 1. The New England journal of medicine, 2012. 366(3): p. 216-24.

Case Study 2: Aviator Study ABT-450/r (protease inhibitor), ABT-267 (NS5A inhibitor), ABT-333 (polymerase inhibitor) with or without RBV

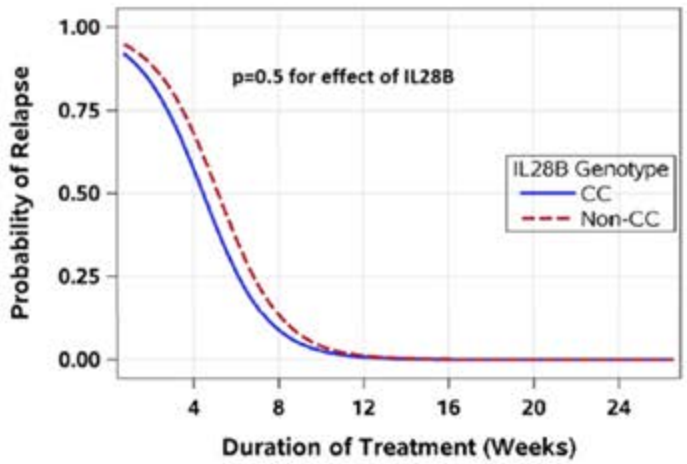
3. Ribavirin may contribute to SVR in some DAA-only regimens
5. IL28B genotype *may not* influence the activity of different IFN-free combination DAA regimens, although its impact is likely dependent on the combined drug classes, their anti-HCV potency and durability

Response Rates, All Groups, N=571



SVR_{12} %	SVR_{24}* %	Breakthrough /Relapse
89	88	0 / 10
85	83	1 / 4
91	89	1 / 8
90	87	1 / 5
99	96	0 / 1
93	90	0 / 2
89	89	0 / 5
93	93	3 / 0
98	95	1 / 0

No effect of IL28B Genotype on the Risk of Relapse



Case Study 2: Aviator Study - ABT-450/r (protease inhibitor), ABT-267 (NS5A inhibitor), ABT-333 (polymerase inhibitor) with or without RBV

4. HCV genotype/subtype can have an impact on treatment response
6. Natural DAA resistance-associated polymorphisms may reduce the efficacy of some regimens, although this is an active area of investigation

Table 3. Resistant Variants Present at Baseline and at the Time of Virologic Failure in Patients with a Null or Partial Response to Previous Therapy.*

Patient No.	Virologic Failure	HCV Genotype	NS3 Protease		NS5B Polymerase	
			Baseline	At Time of Failure	Baseline	At Time of Failure
1	Breakthrough during treatment	1a	None	R155K > D168A > D168V	None	G554S
2	Breakthrough during treatment	1a	None	D168A	None	M414T
3	Breakthrough during treatment	1a	None	D168V	None	C316Y > D559G
4	Breakthrough during treatment	1a	None	D168E > D168Y	None	G554S > S556G > M414V and G554S†
5	Breakthrough during treatment	1a	None	D168V	None	S556G
6	Breakthrough during treatment	1b	D168E > D168T‡	D168K	None	C316Y
7	Relapse after treatment	1a	None	D168Y > D168V > D168A	None	S556G > M414T
8	Relapse after treatment	1a	None	None	None	None
9	Relapse after treatment	1a	None	D168V	None	S556G

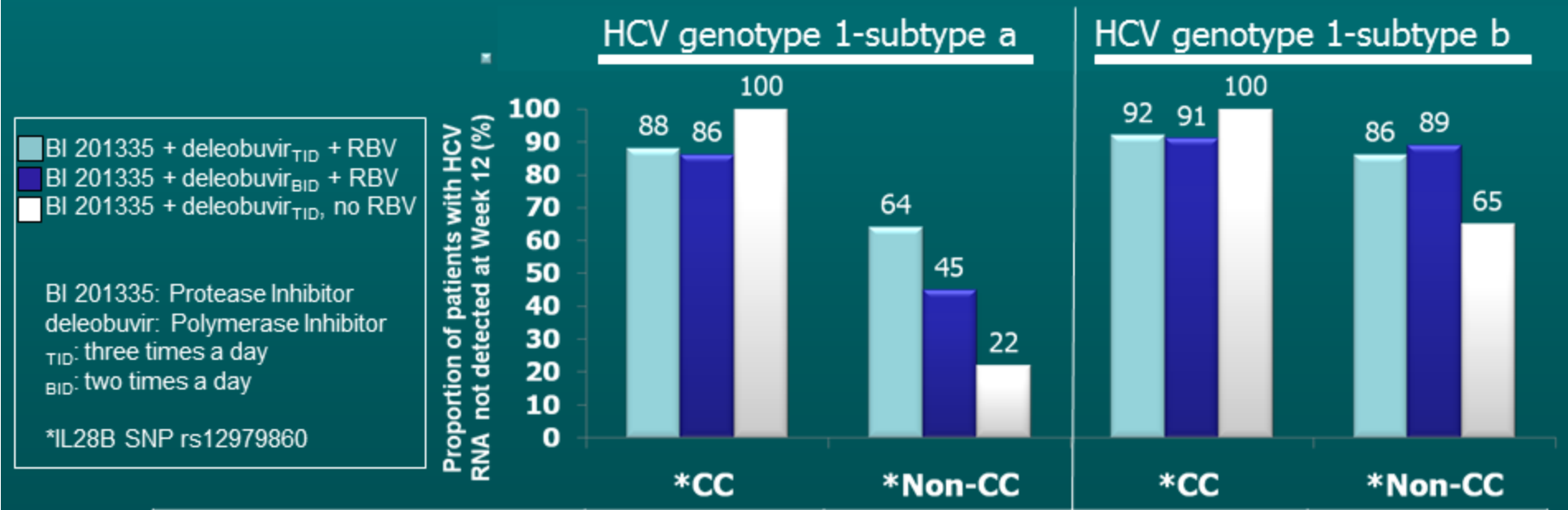
* Where a resistance-associated variant is indicated, it was present in 5% or more of clones from that sample. If a sample contained multiple variants, they are shown in order of prevalence from highest to lowest percentage of clones containing that variant, as indicated by the greater-than (>) sign. None denotes that no variants were found at amino acid positions known to be associated with resistance to ABT-333 or ABT-450.

† Individual clones contained both amino acid variants.

‡ The conversion of D168E or D168T to D168K in HCV genotype 1b requires only a single nucleotide change.

Case Study 3: BI201335 + deleobuvir (BI207127) with or without RBV

3. Ribavirin may contribute to SVR in some DAA-only regimens
4. HCV genotype/subtype can have an impact on treatment response
5. IL28B genotype may influence the activity of different IFN-free combination DAA regimens, although its impact is likely dependent on the combined drug classes, their anti-HCV potency and durability



BI201335 + deleobuvir$_{TID}$ +RBV	22/25	39/61	24/26	79/92
BI201335 + deleobuvir$_{BID}$ + RBV	6/7	10/22	10/11	32/36
BI201335 + deleobuvir$_{TID}$ no RBV	3/3	2/9	7/7	13/20

Zeuzem et al, 2011 AASLD Annual Meeting Boston, USA. Late-breaker#15

Case Study 4: ELECTRON Study – sofosbuvir (NS5B inhibitor), ledipasvir (NS5A inhibitor), GS-9669 (NS5B inhibitor) with RBV

3. Ribavirin *may not* contribute to SVR in some DAA-only regimens

sofosbuvir (sof): NS5B nucleotide inhibitor

ledipasvir (ldv): NS5A inhibitor

GS-9669: NS5B non-nucleotide inhibitor

12 week treatment duration

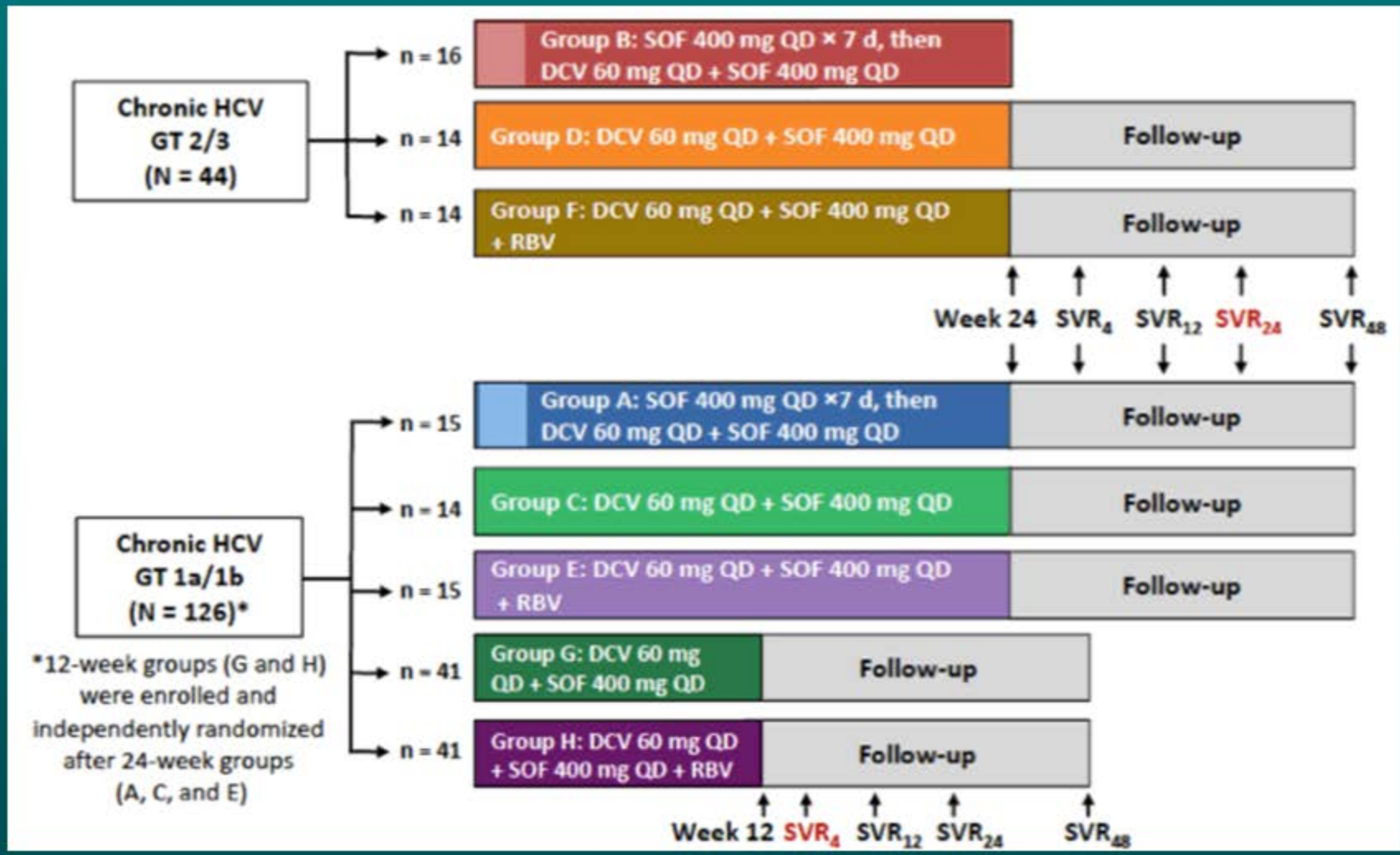
Patients with HCV RNA <LOD* over time, n/N (%)

	SOF + RBV		SOF + LDV + RBV		SOF + GS-9669 + RBV	
	Naïve (n=25)	Null (n=10)	Naïve (n=25)	Null (n=9)	Naïve (n=25)	Null (n=10)
Week 1	8/25 (32)	1/10 (10)	11/25 (44)	0/9 (0)	3/25 (12)	0/10 (0)
Week 2	17/25 (68)	7/10 (70)	22/25 (88)	4/9 (44)	15/25 (60)	2/10 (20)
Week 4	25/25 (100)	10/10 (100)	25/25 (100)	8/9 (89)	23/25 (92)	10/10 (100)
EOT	25/25 (100)	10/10 (100)	25/25 (100)	9/9 (100)	25/25 (100)	10/10 (100)
SVR4	22/25 (88)	1/10 (10)	25/25 (100)	9/9 (100)	23/25 (92)	10/10 (100)
SVR12	21/25 (84)	1/10 (10)	25/25 (100)†	9/9 (100)	23/25 (92)	10/10 (100)

*Analyzed by TaqMan® HCV Test 2.0 with limit of detection (LOD) of 15 IU/mL.
†Includes 1 patient who stopped all treatment due to a serious adverse event (AE) at Week 8; this patient subsequently achieved SVR12.

Gane, *et al*, *63rd AASLD*, Boston, 2012, Once Daily sofosbuvir Regimens in HCV Genotype 1-3: the ELECTRON Trial

Case Study 5: AI444-040 - sofosbuvir (polymerase inhibitor), daclatasvir (NS5A inhibitor) with or without RBV

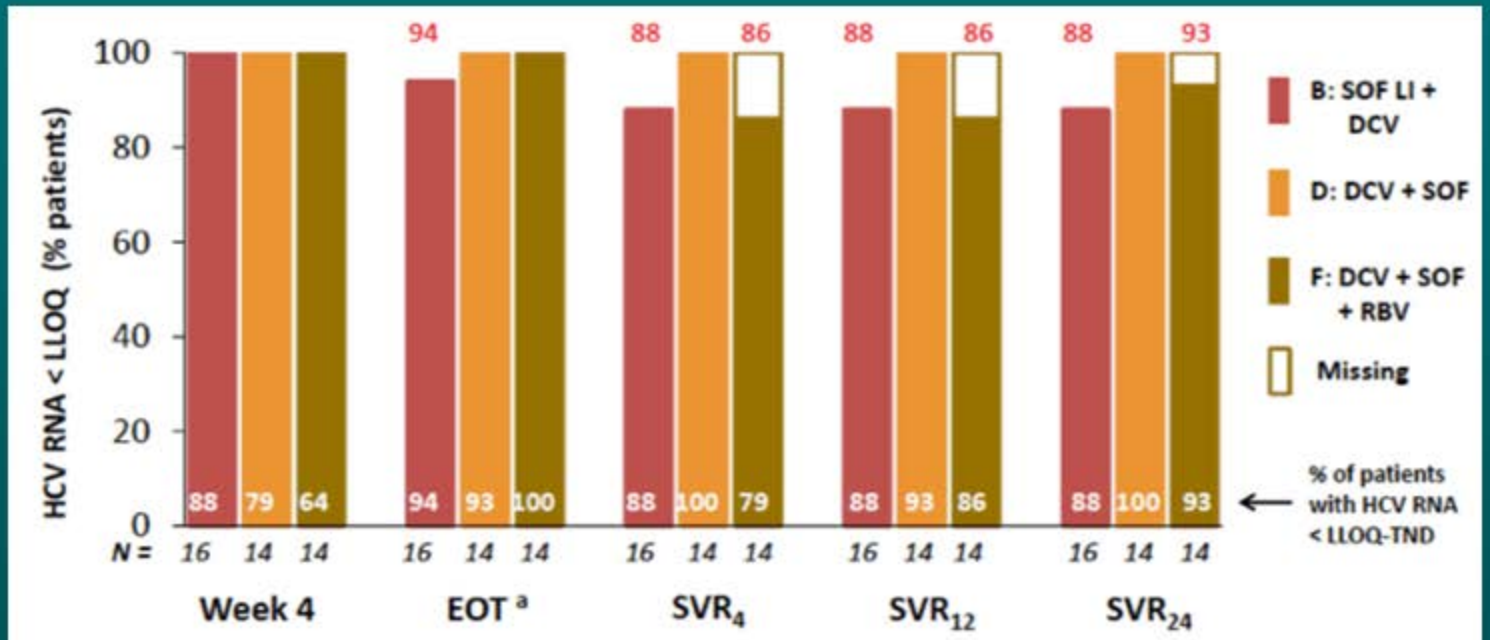


Sulkowski, *et al, 63rd AASLD*, Boston, 2012, High Rate of SVR with the All-oral Combination of daclatasvir, with or without RBV, in Treatment-naïve Patients Chronically Infected with HCV GT 1,2, or 3

Case Study 5: AI444-040 - sofosbuvir (polymerase inhibitor), daclatasvir (NS5A inhibitor) with or without RBV

3. Ribavirin *may not* contribute to SVR in some DAA-only regimens
4. HCV genotype/subtype *may not* have an impact on treatment response

Genotype 2/3



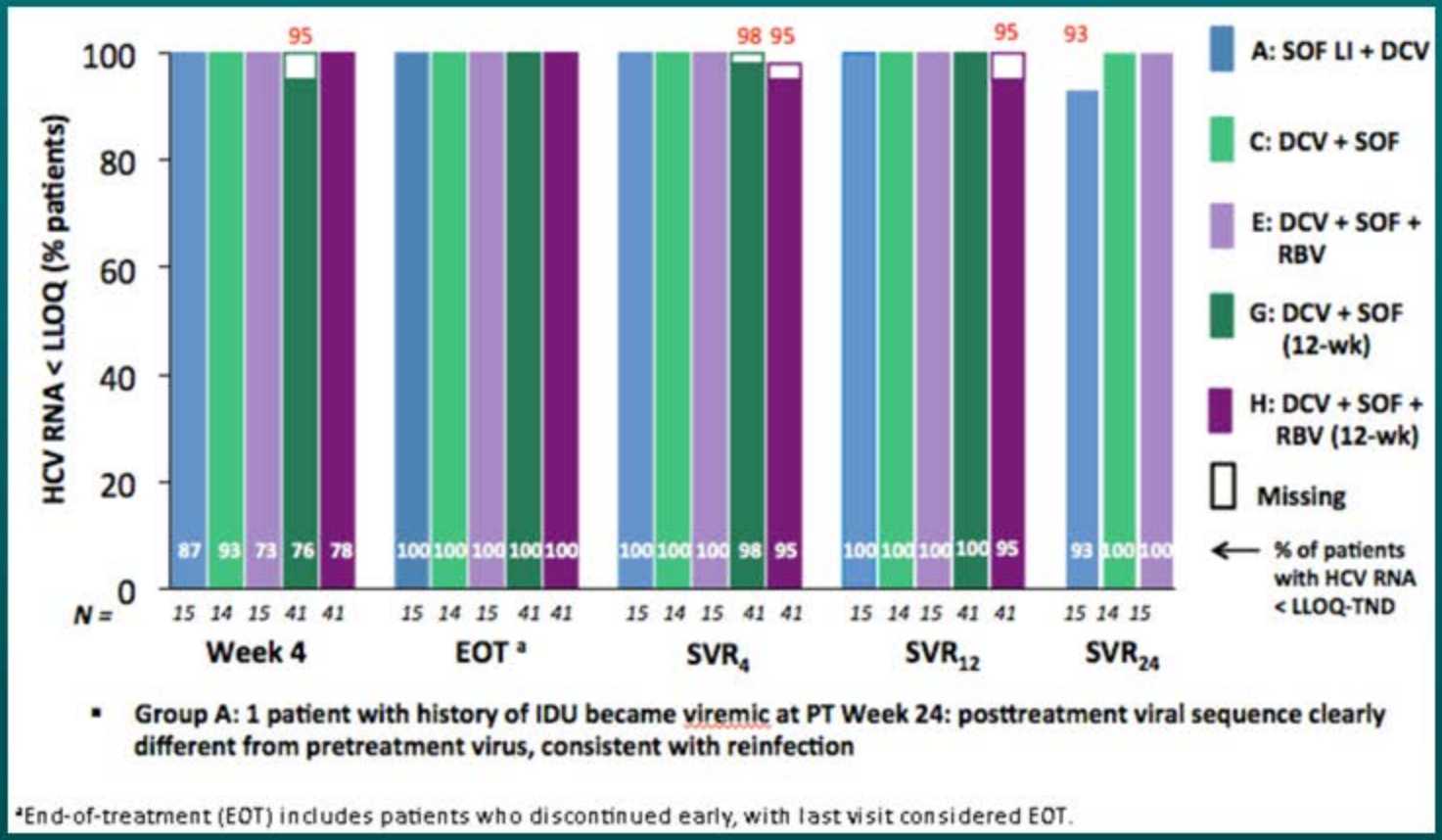
- **Group B**: 1 patient (GT3) relapsed; NS5A-A30K polymorphism (associated with DCV resistance) detected at baseline and PT Week 4. 1 patient (GT3) met protocol definition of virologic breakthrough; added pegIFN alfa/RBV – achieved SVR_{24}
- **Group F**: 2 lost to follow-up after EOT; 1 returned at PT Week 24 with HCV RNA < LLOQ-TND

[a] End-of-treatment (EOT) includes patients who discontinued early, with last visit considered EOT; TD, target detected; TND, target not detected.

Sulkowski, *et al, 63rd AASLD*, Boston, 2012, High Rate of SVR with the All-oral Combination of daclatasvir, with or without RBV, in Treatment-naïve Patients Chronically Infected with HCV GT 1,2, or 3

Case Study 5: AI444-040 - sofosbuvir (polymerase inhibitor), daclatasvir (NS5A inhibitor) with or without RBV

3. Ribavirin *may not* contribute to SVR in some DAA-only regimens
4. HCV genotype/subtype *may not* have an impact on treatment response

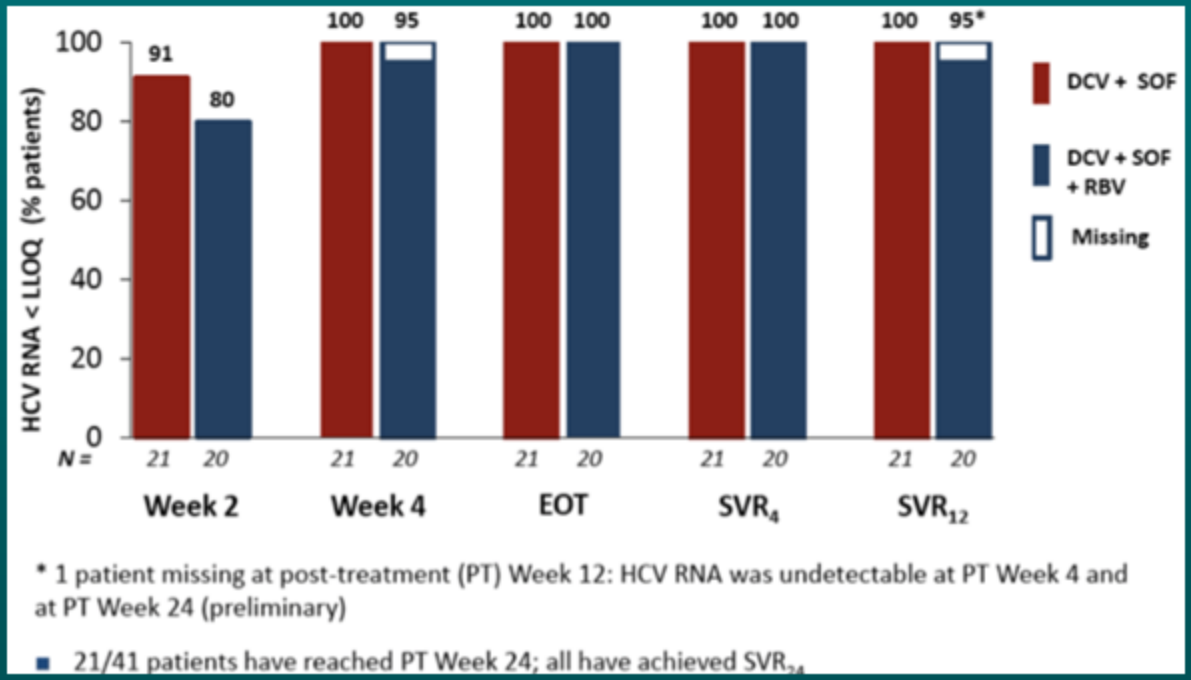


- **Group A: 1 patient with history of IDU became viremic at PT Week 24: posttreatment viral sequence clearly different from pretreatment virus, consistent with reinfection**

[a]End-of-treatment (EOT) includes patients who discontinued early, with last visit considered EOT.

Sulkowski, *et al, 63rd AASLD*, Boston, 2012, High Rate of SVR with the All-oral Combination of daclatasvir, with or without RBV, in Treatment-naïve Patients Chronically Infected with HCV GT 1,2, or 3

Case Study 5: AI444-040 - sofosbuvir (polymerase inhibitor), daclatasvir (NS5A inhibitor) with or without RBV



* 1 patient missing at post-treatment (PT) Week 12: HCV RNA was undetectable at PT Week 4 and at PT Week 24 (preliminary)

- 21/41 patients have reached PT Week 24; all have achieved SVR_{24}

- Certain combination DAA regimens may be effective for patients who did not achieve SVR with previous telaprevir/boceprevir treatment

Sulkowski, *et al, 46th EASL*, Amsterdam, 2013, SVR with daclatasvir plus sofosbuvir +/- RBV in Chronic HCV Genotype 1-infected patients who previously failed TVR or BOC

Slide Set #3 Index

Content	Slide #
Detection of Resistance	39
Terms used to guide treatment response	40-41

Changes in drug susceptibility: Detection of resistance

- Sequence analysis and phenotype analysis are used in combination to identify/discover resistance pathways

- **Sequence Analysis**: Detects specific amino acid substitutions relative to a pre-treatment or standard reference sequence
  - Can identify substitutions known to impact drug susceptibility
  - Can identify novel drug resistance pathways associated with treatment failure

- **Phenotypic Analysis**: Determines drug concentrations needed to inhibit viral replication
  - Effective concentration (EC): drug concentration required to inhibit viral replication by 50% or 90% (EC_{50} or EC_{90})
  - Less susceptible (resistant) viruses will require *more* drug to be inhibited, thus an *increase* in EC_{50} or EC_{90}

Terms used to guide treatment response in HCV infection (I)

Virologic response nomenclature for key decision points in trials (with and without lead-in treatment) is based on assay–specified lower limit of quantitation (LLOQ) cut-off

- **SVR12**: New primary endpoint in DAA trials defined as HCV RNA<LLOQ 12 weeks *after* the completion of treatment *
- **SVR24**: HCV RNA<LLOQ at least 24 weeks *after* treatment cessation *
  - *If Follow-up Week 12 or Follow-up Week 24 HCV RNA are reported as Target Detected, but not quantifiable (*e.g.*, SVR24U_{TD}), repeat analysis of the sample or a subsequent sample is recommended to confirm SVR was achieved
- **Failure of HCV therapy**: Persistence of HCV RNA in serum/plasma after therapy

Ghany, M.G., *et al. Hepatology*, 2009; 49:1335-74
Shiffman M.L. *Curr Gastro Reports*, 2006; 8:46-52

Terms used to guide treatment response in HCV infection (II)

Virologic response nomenclature for key decision points in trials (with and without lead-in treatment) is based on assay–specified lower limit of quantitation (LLOQ) cut-off

- **HCV RNA Quantifiable (Q)**: HCV RNA level above the LLOQ of a particular assay
- **HCV RNA Unquantifiable (U), Target Not Detected (TND):** HCV RNA level below the LLOQ of a particular assay *but no* HCV RNA detected
- **HCV RNA Unquantifiable (U), Target Detected (TD)**: HCV RNA level below the LLOQ of a particular assay *but* with HCV RNA detected
- **$W4U_{TND}$ without lead-in or LI_{4W}-$W8U_{TND}$ with lead-in(LI)**; (formerly RVR): Unquantifiable HCV RNA and target not detected at week 4 of DAA therapy
- **$W4\text{-}12U_{TND;}$** (formerly eRVR): Unquantifiable HCV RNA at weeks 4 and through week 12 of therapy with target not detected
- **$W12U_{TND;}$** (formerly cEVR): Unquantifiable HCV RNA at week 12 of therapy with target not detected

Ghany, M.G., *et al. Hepatology*, 2009; 49:1335-74
Shiffman M.L. *Curr Gastro Reports*, 2006; 8:46-52