Le resistenze agli antivirali sono un problema cogente?

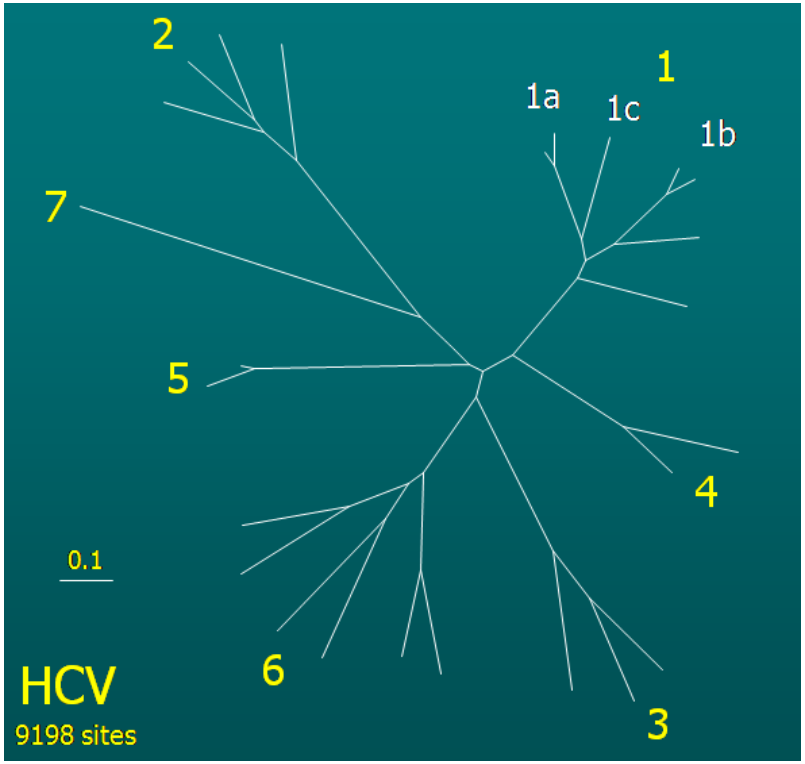
Francesca Ceccherini-Silberstein

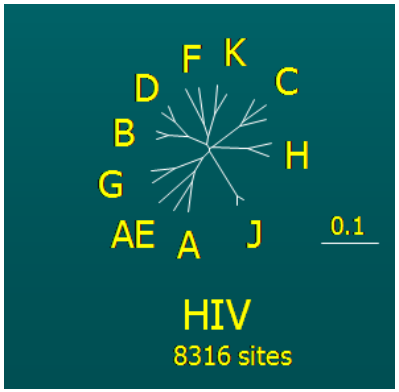
Università degli Studi di Roma "Tor Vergata"

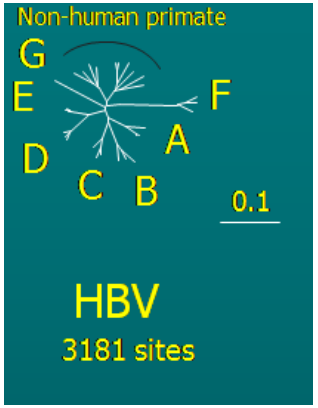
Cattedra di Virologia

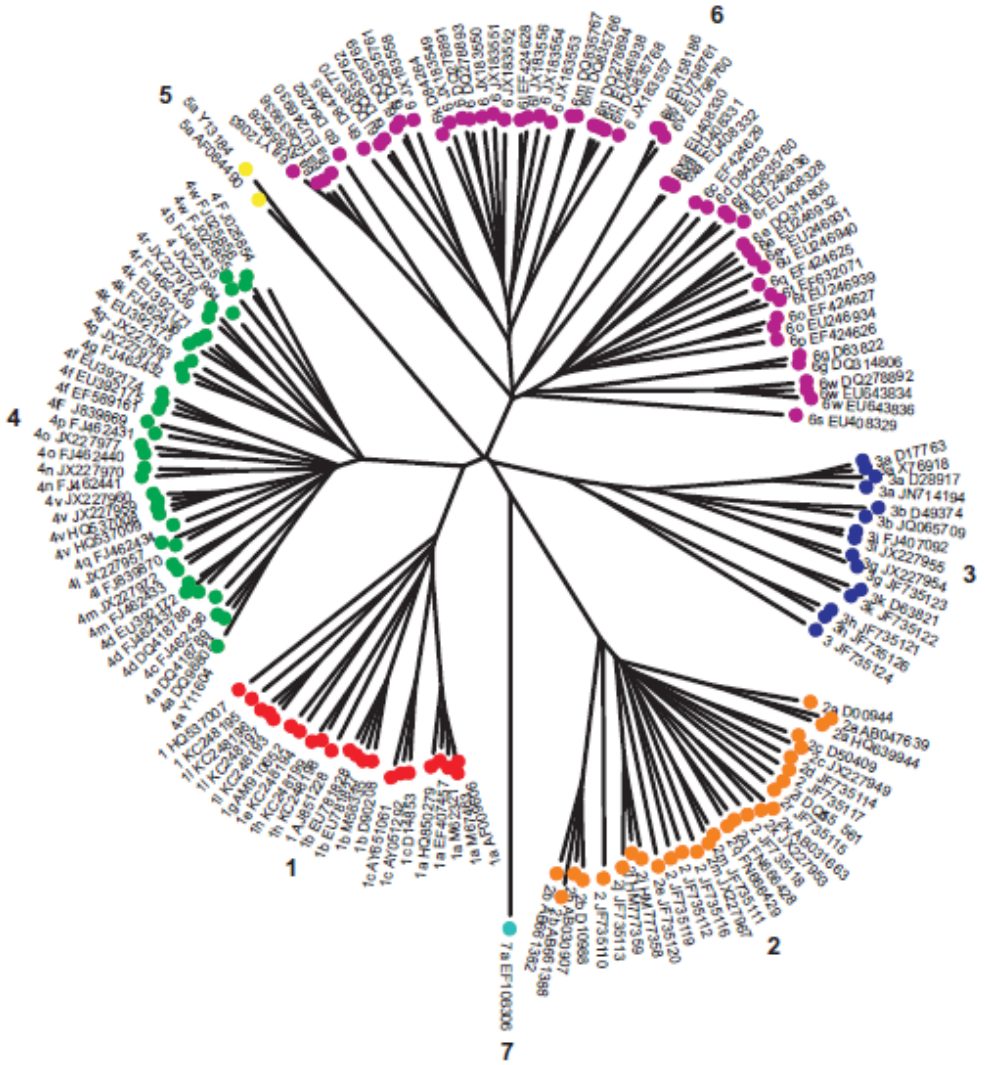
Vietri sul mare (Salerno) 17 Marzo 2016

HCV genetic variability is higher than HIV's and HBV's





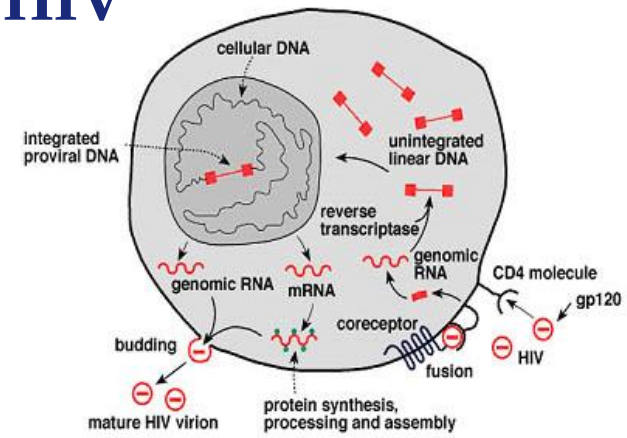




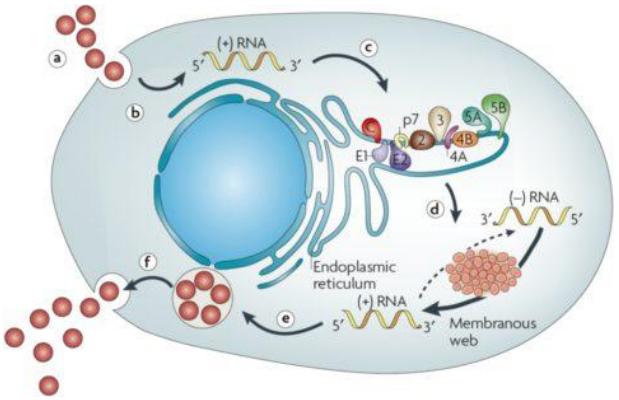
31%–33% nucleotide difference among the 7 known HCV genotypes and 20%–25% among the nearly 67 HCV subtypes (Smith et al., 2014).

Mutations occur frequently during the replication of HIV and HCV

HIV



HCV



It has been predicted that every nucleoside of the 3.2 kb HBV genome or the 10 kb HIV and HCV genomes theoretically can be substituted every day within a given infected patient

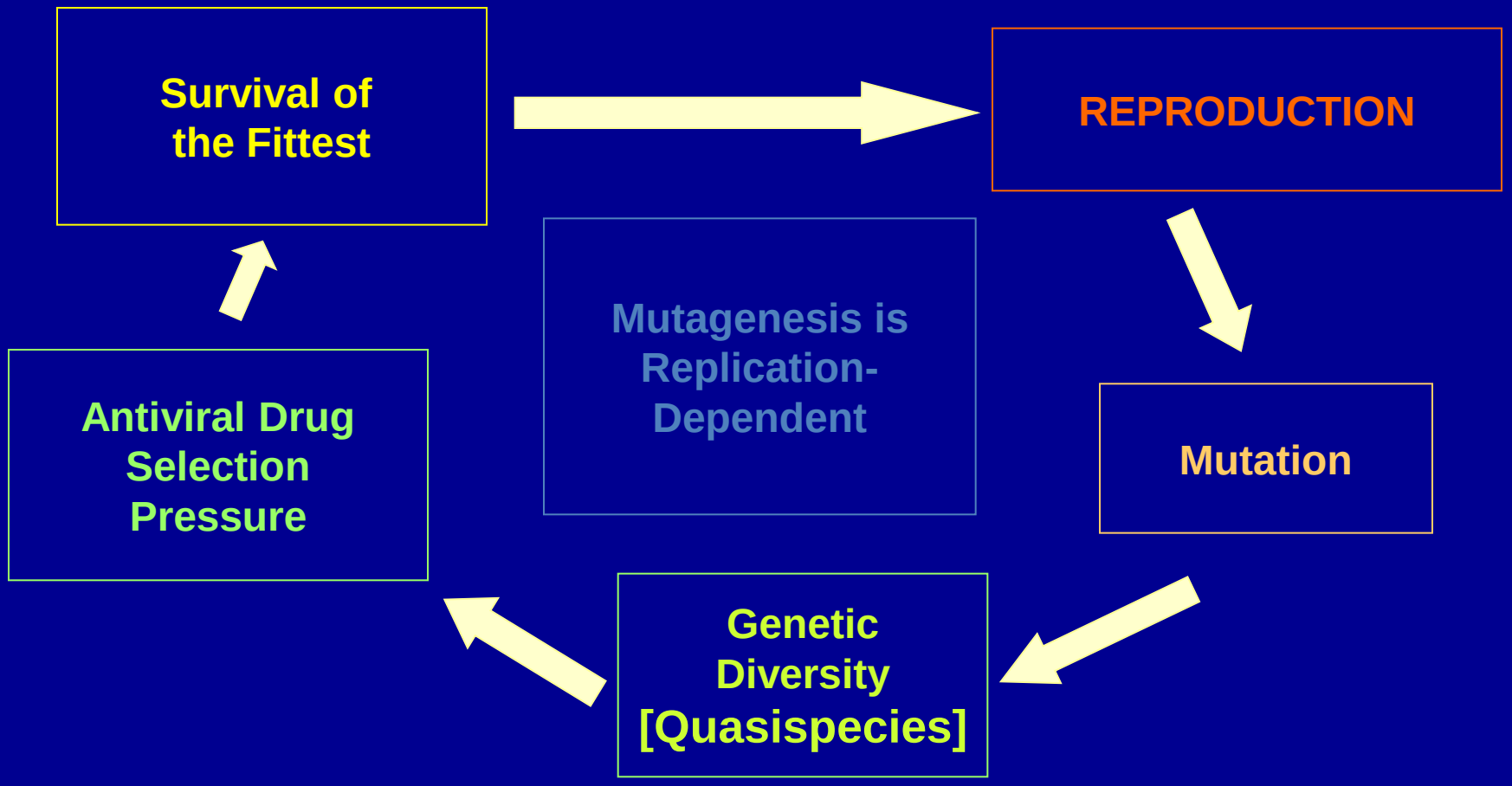
Table 1. Probabilities and rates of generation of various HCV mutants.

Time	Number of nucleotide changes	Probability	Number of virions generated per day	Number of all possible mutants	Fraction of all possible mutants created per day
Before therapy	0	0.91	9.1×10^{11}		
	1	0.087	8.7×10^{10}	2.9×10^{4}	1
	2	0.0042	4.2×10^{9}	4.1×10^{8}	1
	3	0.00013	1.3×10^{8}	4.0×10^{12}	3.4×10^{-5}
End of first day of therapy*	0	0.91	9.1×10^{6}		
	1	0.087	8.7×10^{5}	2.9×10^{4}	1
	2	0.0042	4.2×10^{4}	4.1×10^{8}	1.0×10^{-4}
	3	0.00013	1.3×10^{3}	4.0×10^{12}	3.4×10^{-10}

*Additional drug-resistant or compensatory mutation after a 5-$\log_{10}$ decrease in the HCV RNA production during tr

Rong L et al., Sci Transl Med 2010

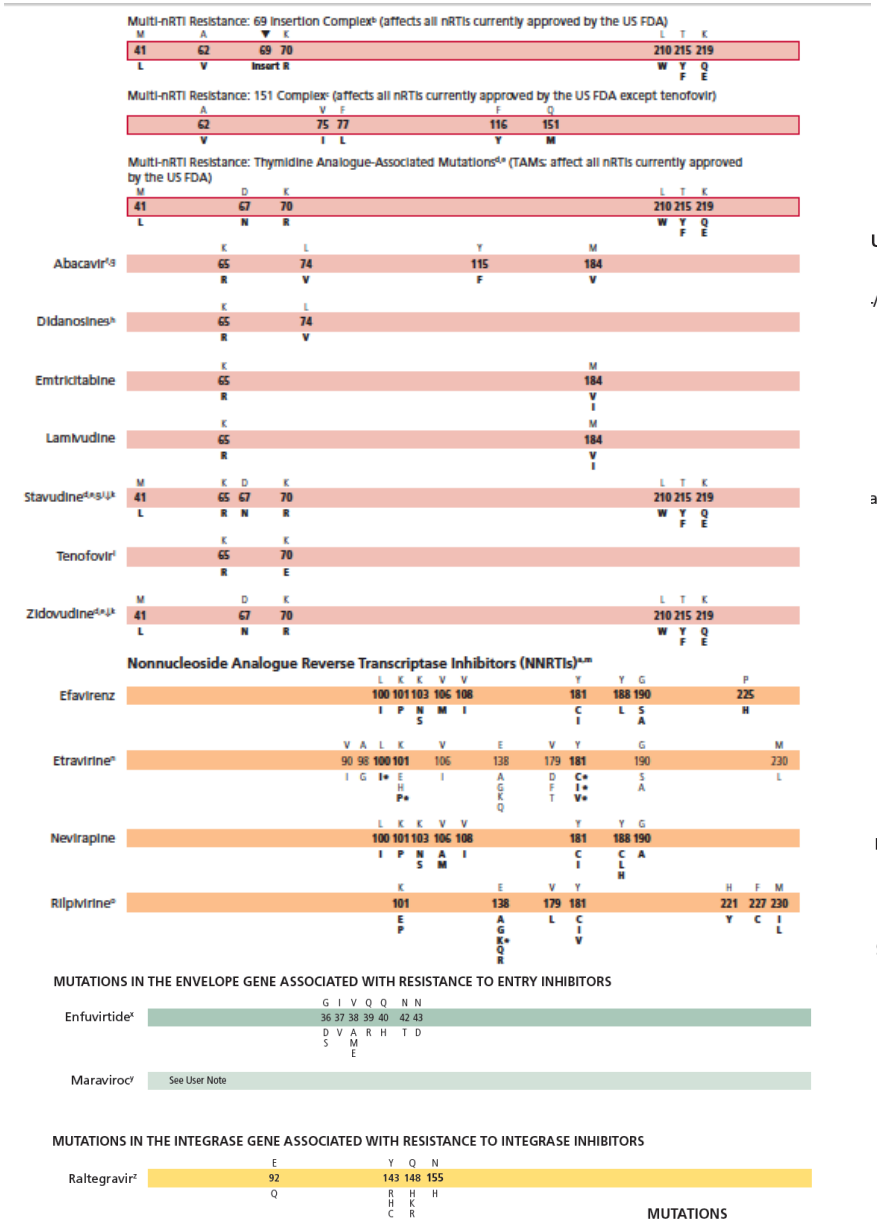
Darwinian Principles in Viral Evolution and Drug Resistance



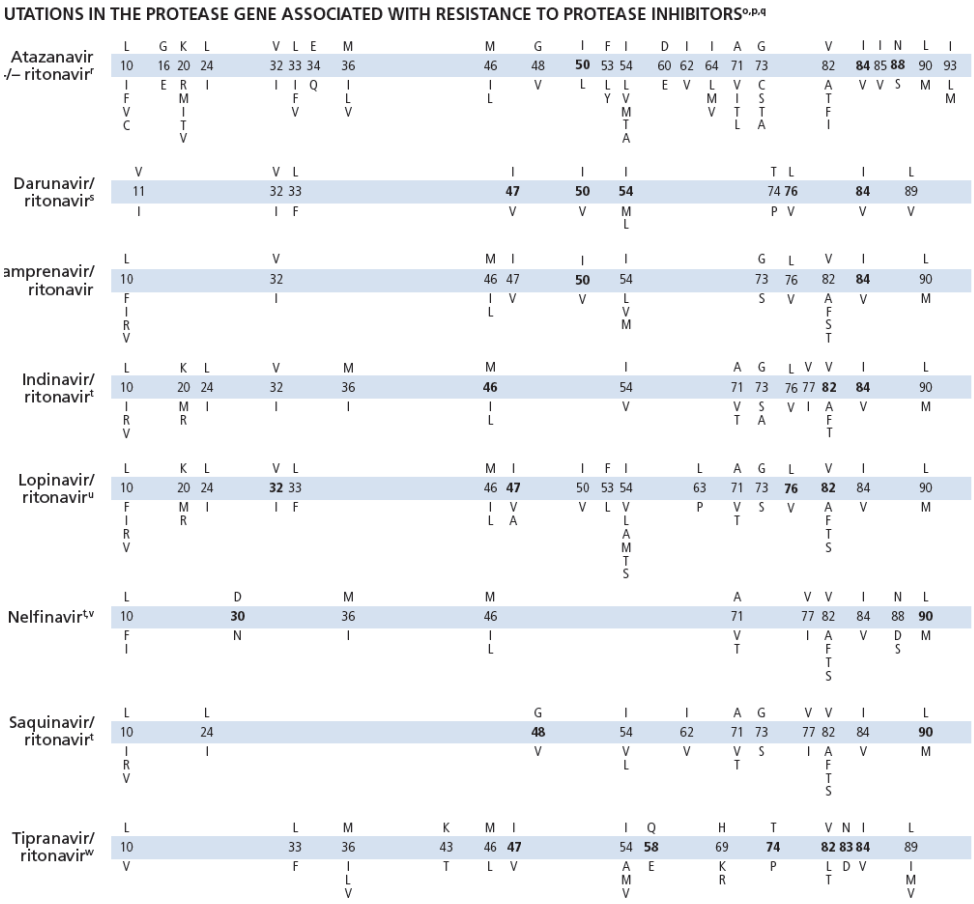
"An antiviral drug is a drug that selects for resistance"

Douglas D. Richman (2000) Hepatology 32:866

For HIV…. more than 100 resistance mutations…



IAS February/March 2013

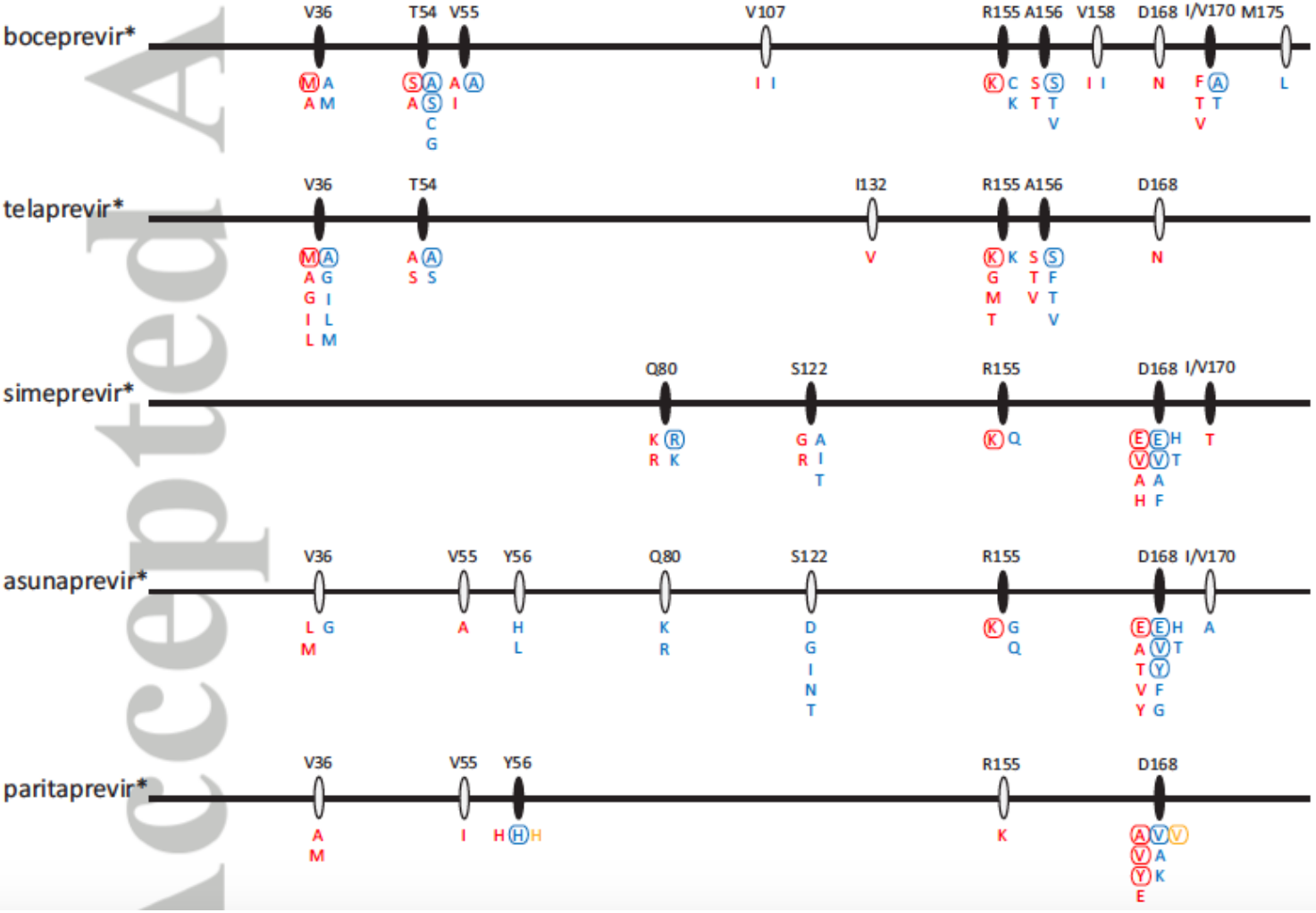


Johnson VA, et al. Top HIV Medicine 2013

Protease Inhibitor Resistance

Major NS3 positions associated to PI resistance across genotypes are: R155 and D168

1a – red
1b – blue
4d – orange

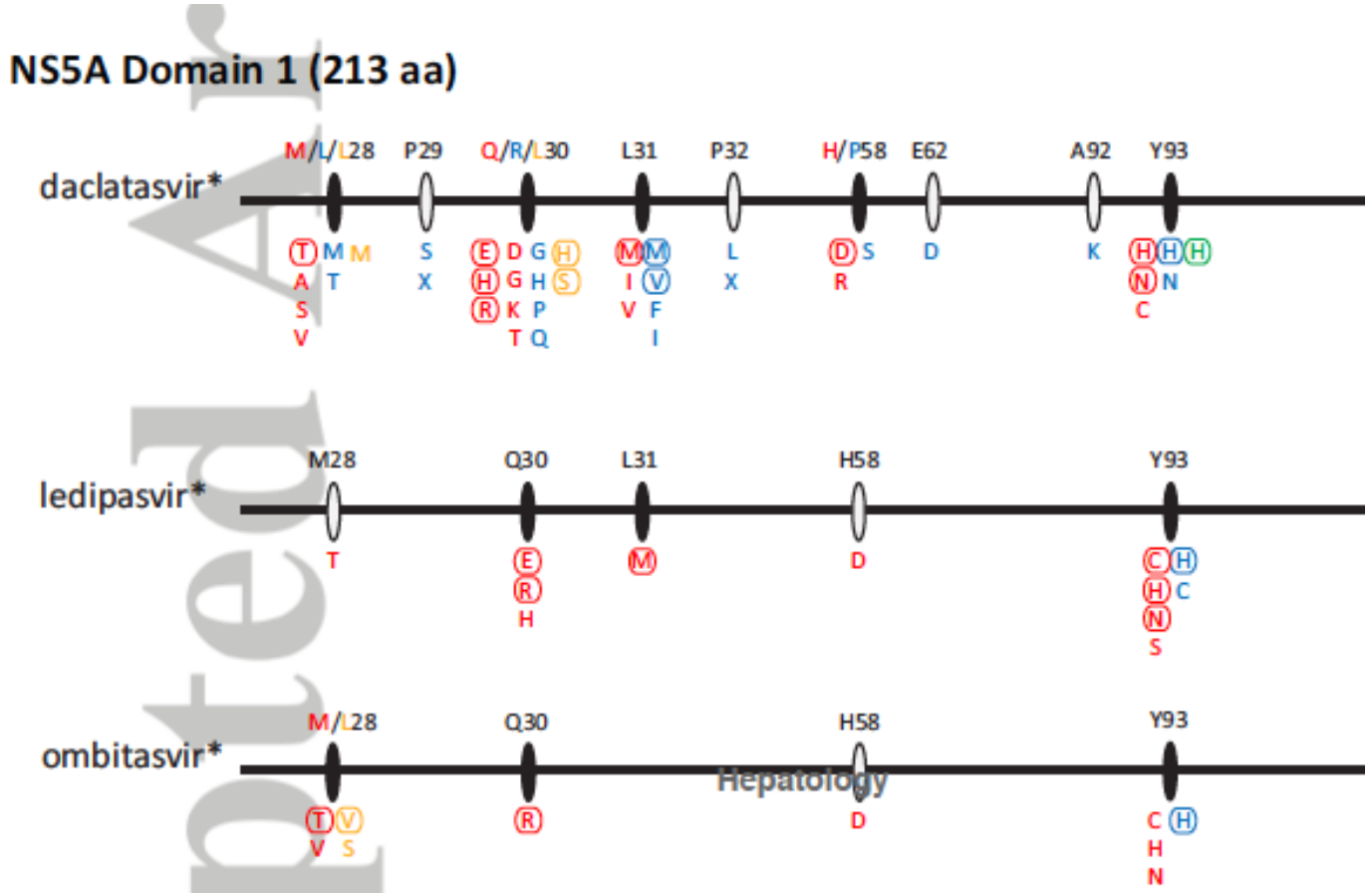


HCV DRAG, Forum for Collaborative HIV Research, Lontok et al Hepatology 2015

NS5A Inhibitor Resistance

Resistance development is qualitatively similar among all first generation NS5A-inhibitors, but variable among HCV-genotypes

1a – red
1b – blue
3a - green
4 – orange



HCV DRAG, Forum for Collaborative HIV Research, Lontok et al Hepatology 2015

Knowledge of HIV-1 resistance is continuously evolving

Knowledge of HIV-1 resistance is continuously evolving

IAS Dec 2010

MUTATIONS IN THE INTEGRASE GENE ASSOCIATED WITH RESISTANCE TO INTEGRASE INHIBITORS

	E	Y	Q	N
Raltegravir[z]	92	143	148	155
	Q	R H C	H K R	H

Note y: **three distinct main genetic pathways** seen in patients failing Raltegravir

1 Pathway: Q148H/K/R +/- L74M+E138A, E138K, **G140S**

2 Pathway: N155H +/- L74M, E92Q, T97A, E92Q+T97A
Y143H, G163K/R, V151I, D232N

3 Pathway: Y143R/H/C less common

Another major mutation, E92Q, has also been described.

Johnson VA, *et* al. Top HIV Medicine 2010

Knowledge of HIV-1 resistance is continuously evolving

MUTATIONS IN THE INTEGRASE GENE ASSOCIATED WITH RESISTANCE TO INTEGRASE STRAND TRANSFER INHIBITORS[aa]

	66	74	92	97	118	121	138	140	143	147	148	155	263
Dolutegravir[bb]		L 74? I		T 97? A	G 118? R	F 121 Y	E 138 A, K	G 140 S, A	Y 143? C		Q 148 H	N 155? H	R 263? K
Elvitegravir[cc]	T 66 I, A, K		E 92 Q, G	T 97 A		F 121 Y	E 138? A, K		Y 143? C	S 147 G	Q 148 R, H, K	N 155 H	
Raltegravir[dd]		L 74 M	E 92 Q	T 97 A	G 118? R	F 121 Y	E 138 A, K	G 140 A, S	Y 143 R, H, C		Q 148 H, K, R	N 155 H	

Top HIV Medicine 2014

Knowledge of HIV-1 resistance is continuously evolving

MUTATIONS IN THE INTEGRASE GENE ASSOCIATED WITH RESISTANCE TO INTEGRASE STRAND TRANSFER INHIBITORS[aa]

Dolutegravir[bb]					L			T	G	F	E	G	Y			Q		N			R				
					74?			97?	118?	121	138	140	143?			148		155?			263?				
					I			A	R	Y	A	S	C			H		H			K				
											K	A													
Elvitegravir[cc]	T			E		T	F		E			Y			S	Q		N						
	66			92		97	121		138?			143?			147	148		155						
	I			Q		A	Y		A			C			G	R		H						
	A			G					K							H								
	K															K								
Raltegravir[dd]			L	E		T	F		E	G	Y			Q		N								
			74	92		97	121		138	140	143			148		155								
			M	Q		A	Y		A	A	R			H		H								
						R			K	S	H			K										
						118?					C			R										

Top HIV Medicine 2014

MUTATIONS IN THE INTEGRASE GENE ASSOCIATED WITH RESISTANCE TO INTEGRASE STRAND TRANSFER INHIBITORS[z]

Dolutegravir[aa]										F	E	G				Q		N			R				
										121	138	140				148		155			263				
										Y	A	A				H		H			K				
											K	S				R									
Elvitegravir[bb]	T			E		T	F								S	Q		N			R				
	66			92		97	121								147	148		155			263				
	I			Q		A	Y								G	H		H			K				
	A			G												K									
	K															R									
Raltegravir[cc]			L	E		T	F		E	G	Y			Q		N			R					
			74	92		97	121		138	140	143			148		155			263					
			M	Q		A	Y		A	A	R			H		H			K					
									K	S	H			K										
											C			R										

Top HIV Medicine 2015

HIV *versus* HCV
Baseline resistance testing

- **HIV treatment failure** expected at <1 to 10% rate, depending on
  - Virus, e.g. viral load
  - Patient, e.g. pretreatment, comorbidity
  - Treatment regimen

- **HCV treatment failure** expected at <1 to 10% rate, depending on
  - Virus, e.g. genotype/subtype, viral load
  - Patient, e.g. pretreatment, cirrhosis
  - Treatment regimen and duration

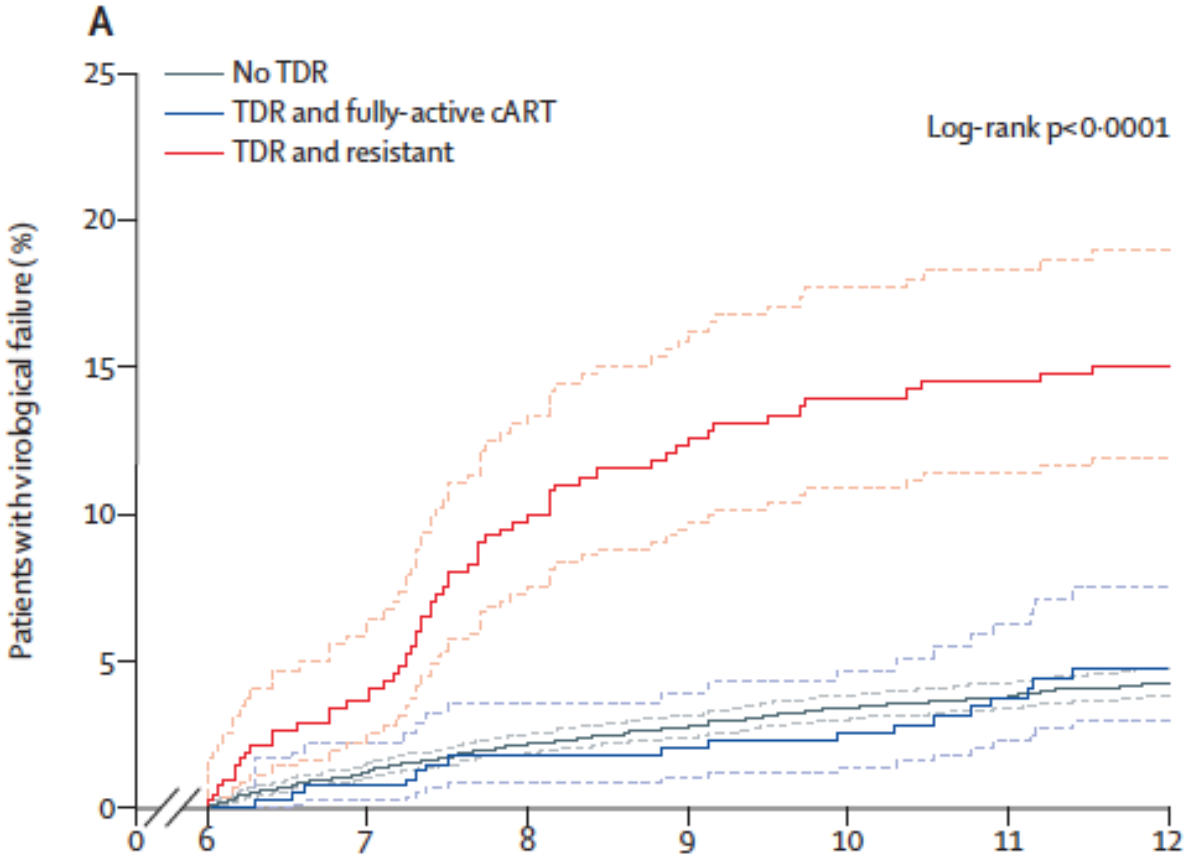
- **HIV transmitted drug resistance** estimated to be <1 to 10-15%, depending on
  - Geographic area
  - Drug class

➢ ***Baseline resistance testing is standard of care***

- **HCV natural resistance** estimated to be <1 to >10-20%, depending on
  - Geographic area
  - Drug class
  - Genotype/subtype

➢ ***Baseline resistance testing is not standard of care***

Transmitted drug resistance is associated with a poorer virological response when patients received cART containing ≥1 drug not fully active

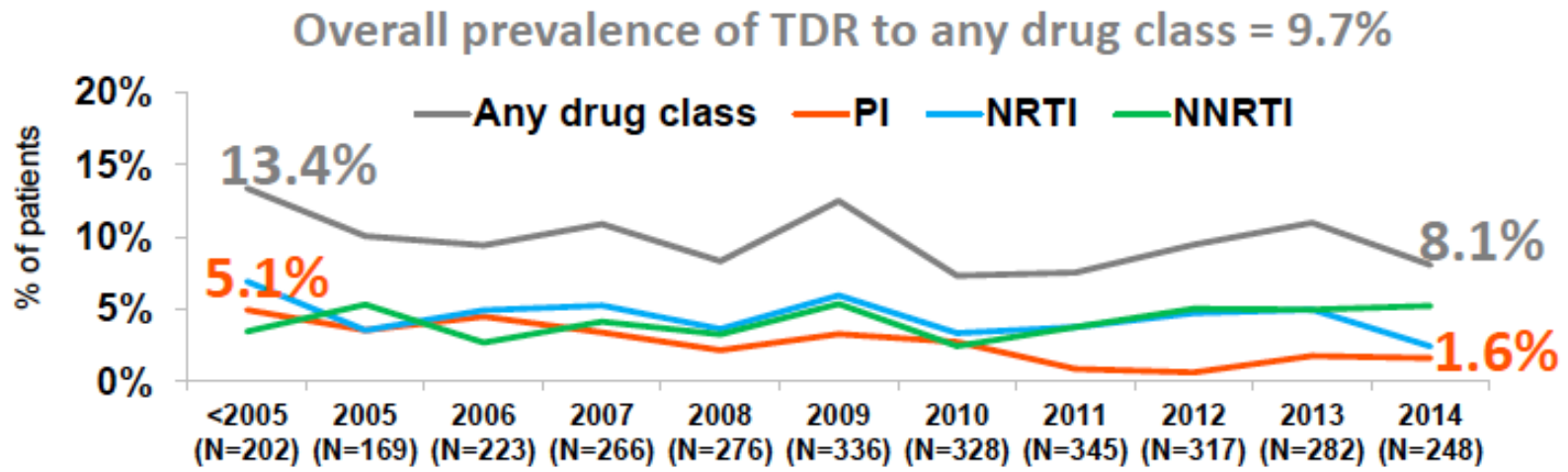


Number at risk							
No TDR	8086	7701	7367	7066	6717	6346	5786
TDR and fully-active cART	427	403	383	361	337	312	284
TDR and resistant	420	394	356	331	310	298	269

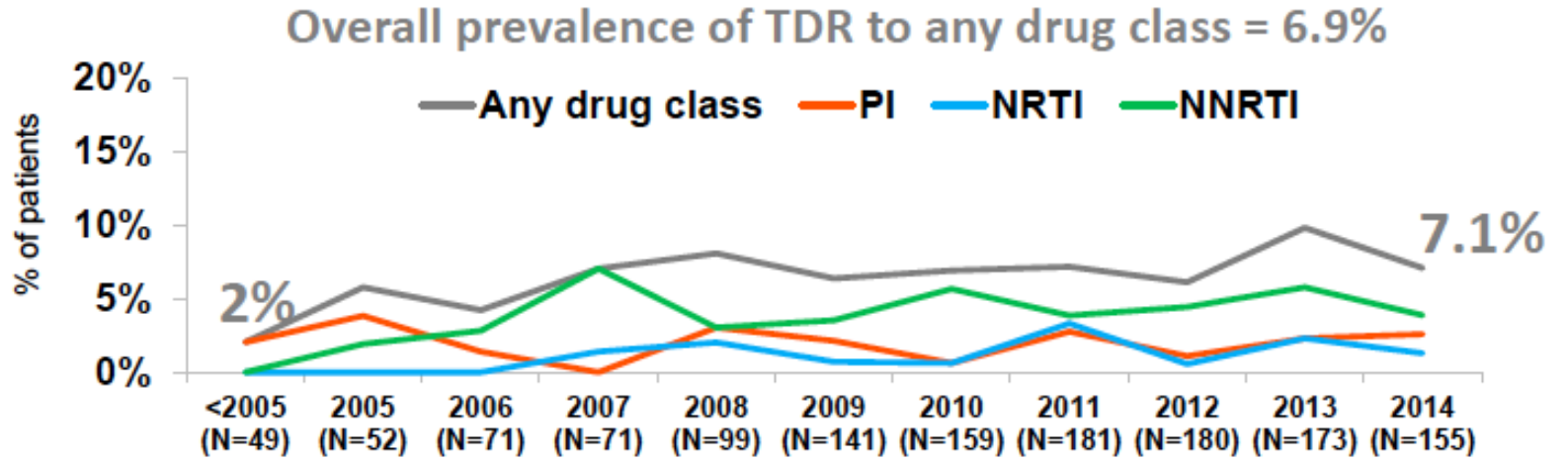
VF rates at M12 were 6.0% (95% confidence interval [CI]: 5.5; 6.5), 6.3% (4.2; 9.3) and 16.2% (13.0; 20.1) for no TDR group, TDR and fully active group and TDR and resistant group, respectively.

Wittkop et al Lancet 2011

Among **2,992 B subtype infected patients analysed, TDR to any drug class decreased over time** with a trend toward significance (<2005-2014: 13.4%-8.1%, p=0.137*). **PI TDR significantly decreased over time** (<2005-2014: 5.0%-1.6%, p<0.001*).



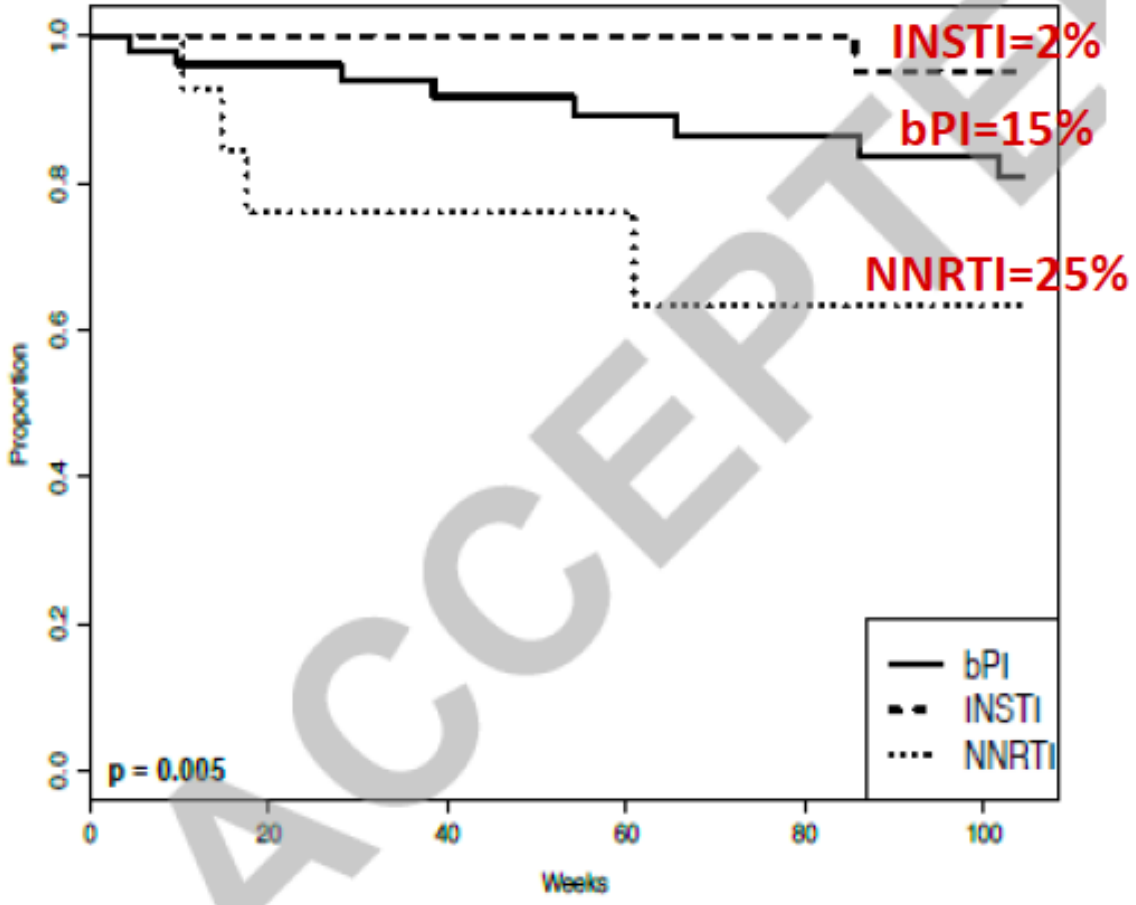
Among **1,331 non-B subtype infected patients analysed, TDR to any drug class increased over time** with a trend toward significance (<2005-2014: 2%-7.1%, p=0.150*).



* By Chi-squared test for trend.

Fabeni L et al. Abstract 74

Patients with isolated NNRTI TDR experienced low VF rates with INSTIs and bPIs. Indeed, in the as-treated analysis, by 100 weeks of treatment, VF occurred in 15% (n=8), 2% (n=1) and 25% (n=4) of patients in the bPI, INSTI and NNRTI groups, respectively.



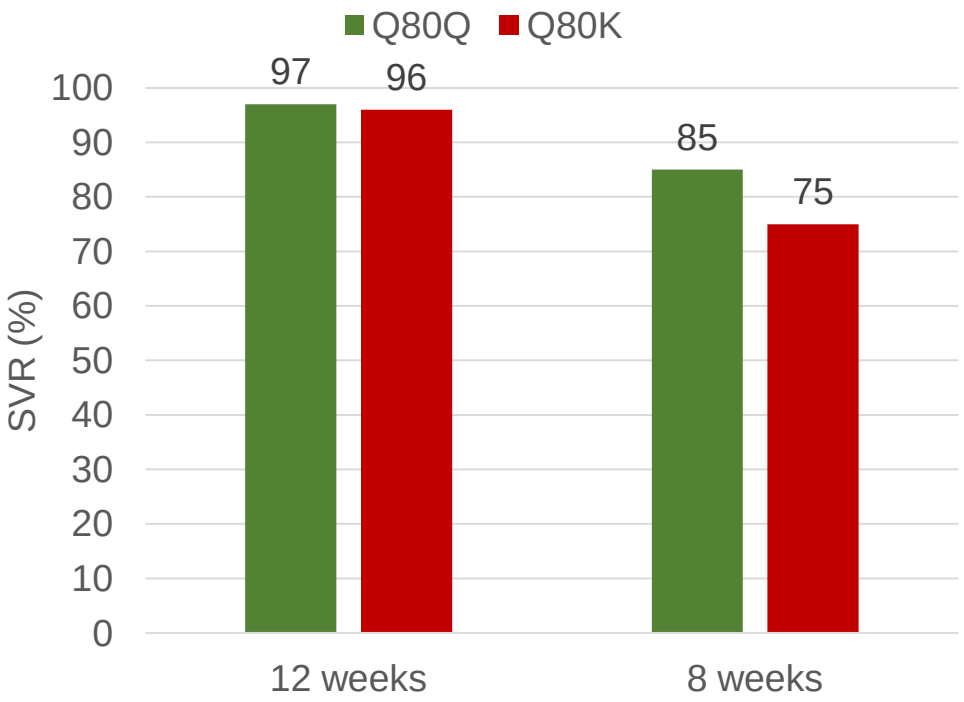
Analysis on 131/3,245 (4.%) patients with isolated TDR.
The inclusion criterion were:
(i) isolated NNRTI resistance defined as an initial genotype containing one or more NNRTI-associated surveillance drug-resistance mutations (SDRMs) without any NRTI or PI-associated SDRMs and
(ii) treatment with a standard regimen defined as a dual NRTI backbone plus a bPI, INSTI or NNRTI received by two or more patients with isolated NNRTI resistance.

Figure 1. Kaplan Meier plot of As -Treated and ITT failure outcomes by base - drug class.

Clutter et al., JAIDS 2016

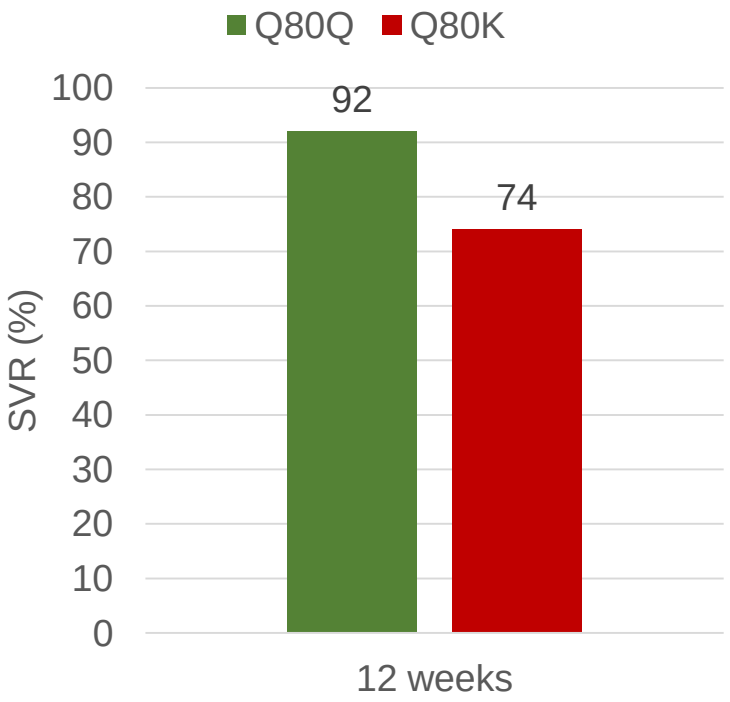
SMV + SOF SVR12 rates according to Q80K presence at baseline in G1a non-cirrhotic and cirrhotic patients

OPTIMIST - 1



Kwo P et al., EASL 2015. AbstractLB14

OPTIMIST - 2



Lawitz E et al., EASL 2015. Poster LP04

Data are lacking with 24 weeks of SOF/SMV therapy in cirrhotic patients

AASLD guidelines recommends Q80K testing in GT-1a patients candidate to a SOF+SMV regimen

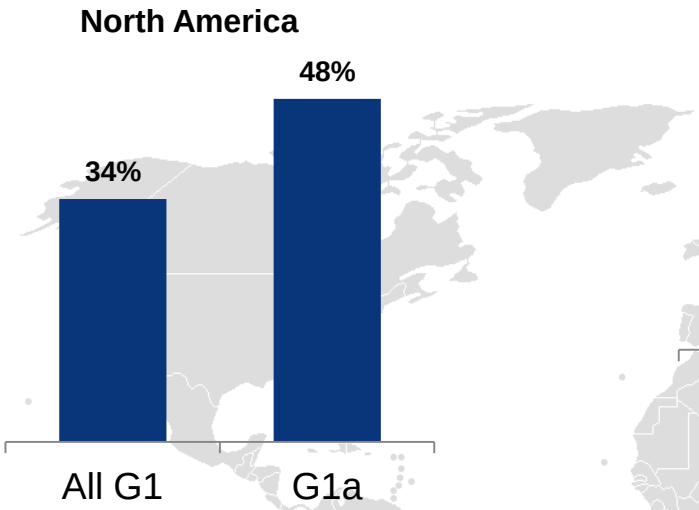
Treatment options for treatment-naive patients with HCV genotype 1a who are initiating therapy

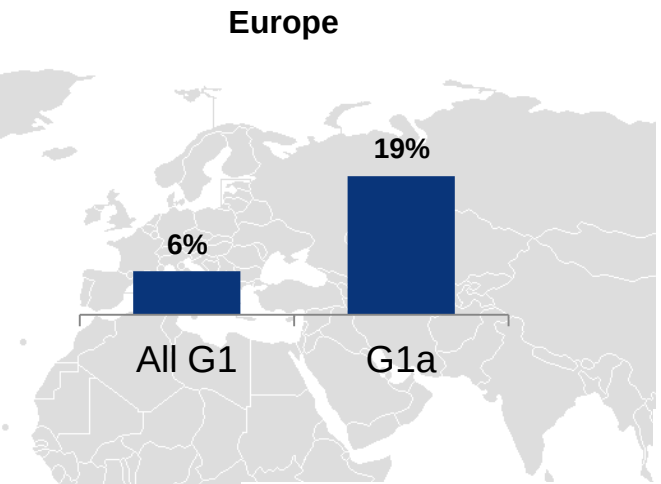
Daily sofosbuvir (400 mg) plus simeprevir (150 mg) with or without weight-based RBV for 12 weeks (no cirrhosis) or 24 weeks (cirrhosis) for patients with a negative test result for the Q80K variant using commercially available resistance assays. *In patients with HCV genotype 1a and cirrhosis who have the Q80K variant, one of the other regimens for cirrhosis detailed above is recommended. (IIa-B)*

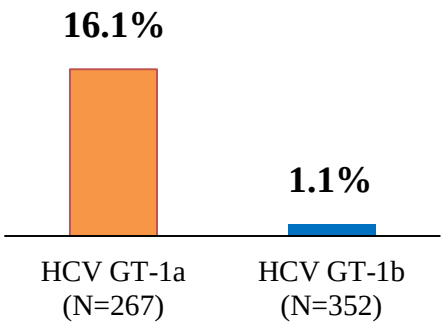
AASLD GUIDELINES HEPATOLOGY UPDATE JUNE 2015

Overall prevalence of Q80K in G1 across different regions

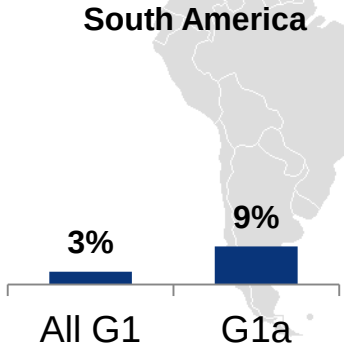
13.7% of patients (274/2007) all HCV G1
29.5% (269/911) of those with HCV GT1a and 0.5% (5/1096) of those with HCV GT1b

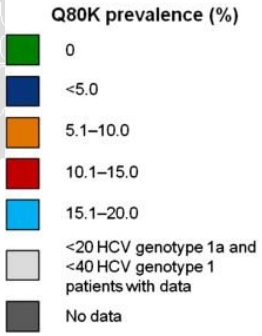


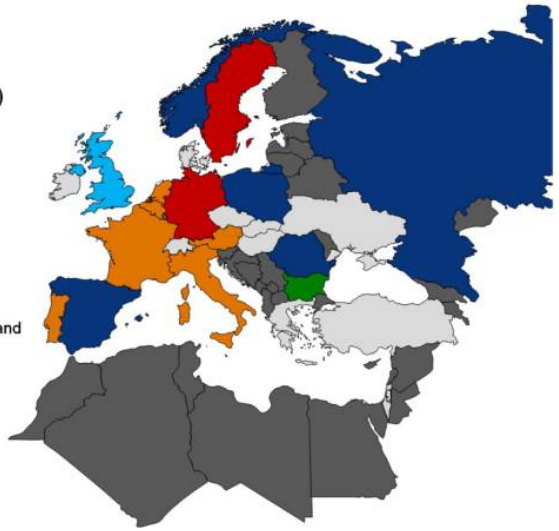




Update from Sorbo MC et al., ICAR 2015







Jacobson et al. Presented at AASLD 2013
Lenz et al. Presented at AASLD 2013

Sarrazin C et al., Antivir Res 2015

The prevalence of pre-treatment NS5A RAVs in GT-1 is different across different countries, ranging from 6% to 25%, and different according to subtype…..

The analysis of >3000 GT-1 NS5A sequences form 14 countries showed a **high prevalence of baseline Y93H mutation** (associated with resistance to **daclatasvir <25 fold and ledipasvir >100 fold) in GT -1 b** infected patients, ranging from **7% to 15%.**

Prevalence of NS5A and NS5B RAVs and TEVs Across Investigated Countries

	Prevalence, % (n)	
	GT 1a Patients With Baseline NS5A RAVs	GT 1b Patients With Baseline NS5A RAVs
Australia	9 (75)	16 (31)
Germany	7 (74)	9 (87)
Spain	6 (33)	14 (22)
France	15 (62)	11 (35)
Italy	17 (41)	13 (67)
Russia	ND	ND
Japan	ND	17 (329)
Korea	ND	17 (85)
Taiwan	ND	13 (68)
USA	10 (2520)	13 (730)
New Zealand	10 (152)	25 (36)

ND, not done due to too few sequences available.

NS5A Resistance Associated Variants Classified by Level of Resistance to LDV*

Genotype	2.5-100 Fold	>100 Fold
GT 1b	**L31M**, P32L, L31I, L31V	P58D, A92K, **Y93H**

*Fold shift in EC_{50} from wild-type replicon

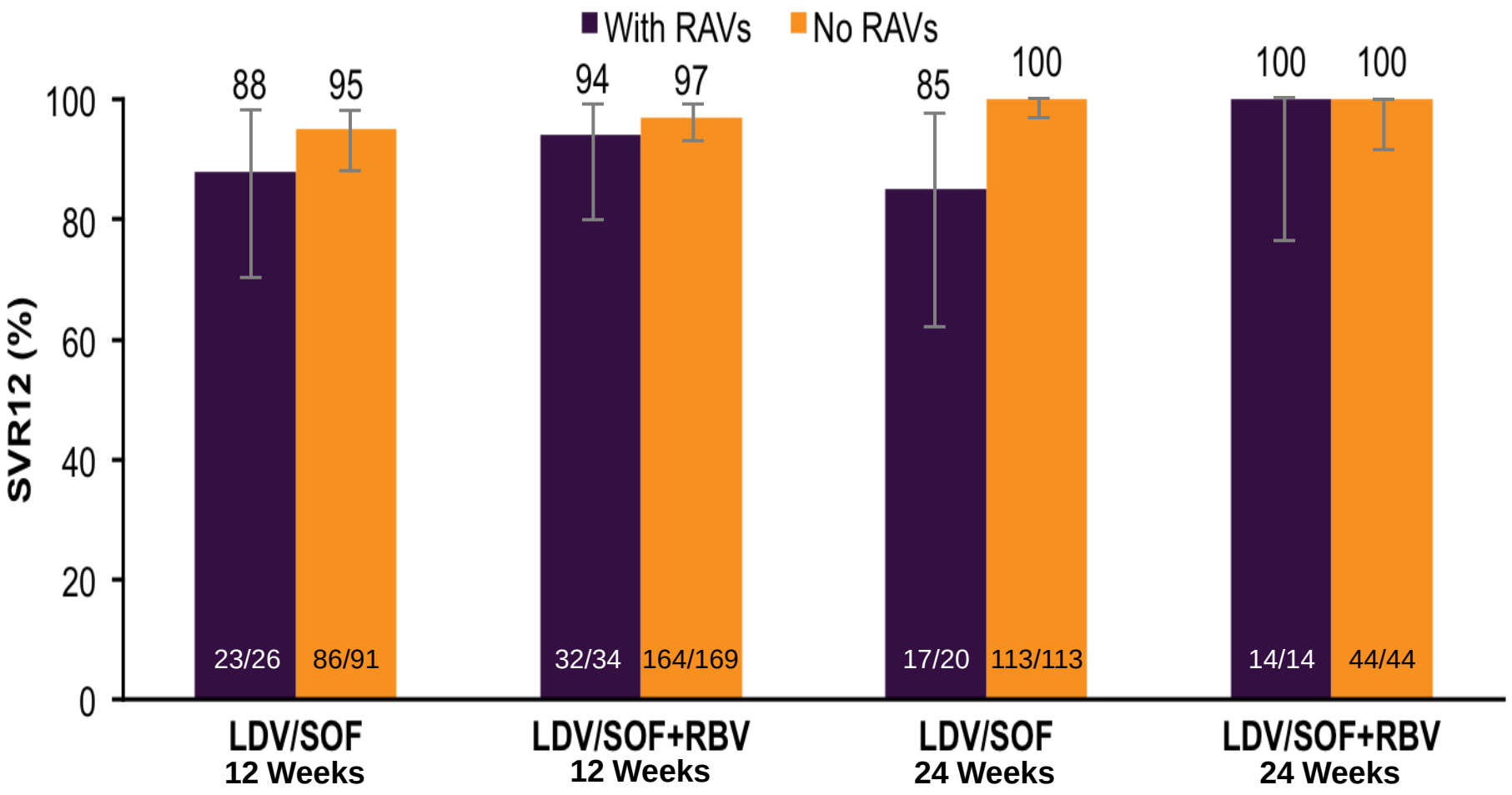
Pretreatment NS5A RAVs in GT 1b Across Investigated Countries

Country, % (n/n)	L31any	A92K	Y93any	Total, %
Australia	3.2 (1/31)	0 (1/31)	12.9 (4/31)	16.1
Germany	2.3 (2/87)	0 (0/87)	6.9 (6/87)	9.2
Spain	0 (0/22)	0 (0/22)	13.6 (2/22)	13.6
France	2.9 (1/35)	0 (0/35)	8.6 (3/35)	11.4
Italy	3.0 (2/67)	0 (0/67)	10.4 (7/67)	13.4
Russia	ND	ND	ND	ND
Japan	3.0 (10/330)	0 (0/330)	13.9 (46/330)	17.0
Korea	1.2 (1/85)	0 (0/85)	15.3 (13/85)	16.5
Taiwan	4.4 (3/68)	0 (0/68)	8.8 (6/68)	13.2
USA	5.9 (43/732)	0 (0/732)	7.4 (54/732)	13.2
New Zealand	13.9 (5/36)	0 (0/36)	11.1 (4/36)	25.0

Svarovskaia E.S., EASL 2015

LDV/SOF ± RBV: SVR12 in GT 1 Treatment-naïve Patients With Cirrhosis ± Baseline NS5A RAVs

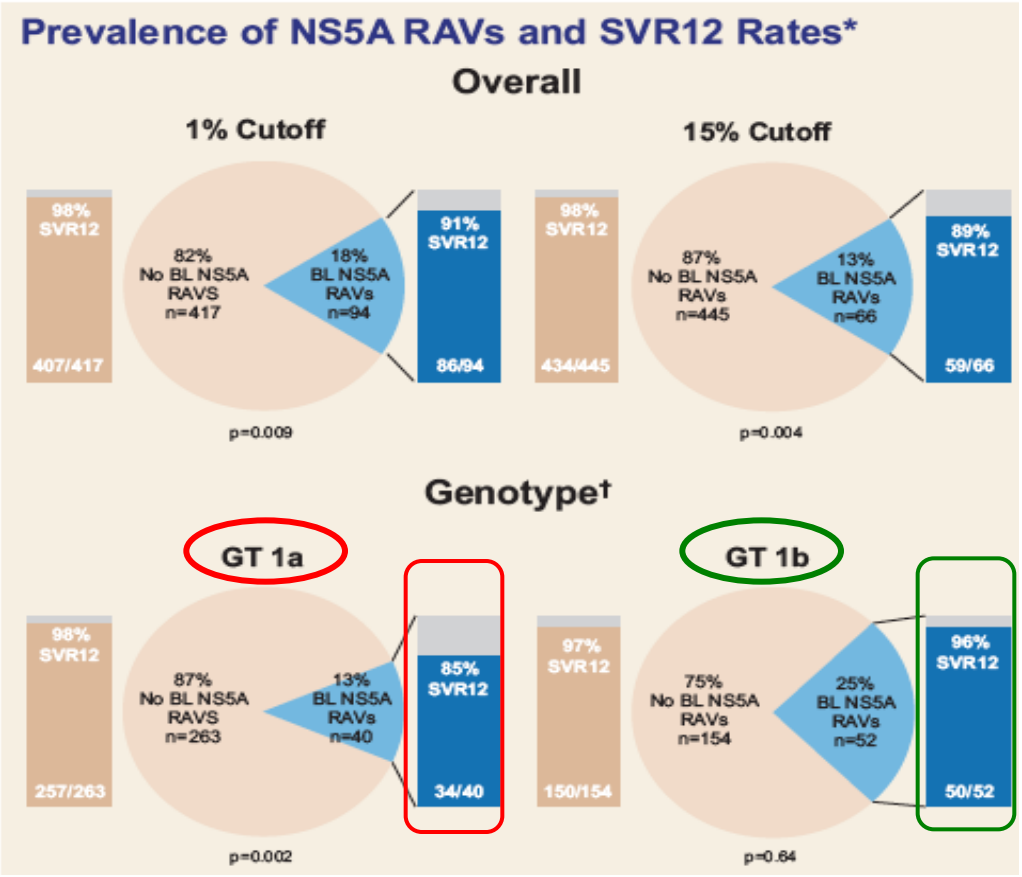
18% (94/511) cirrhotic patients had BL RAVs; Need for RBV?

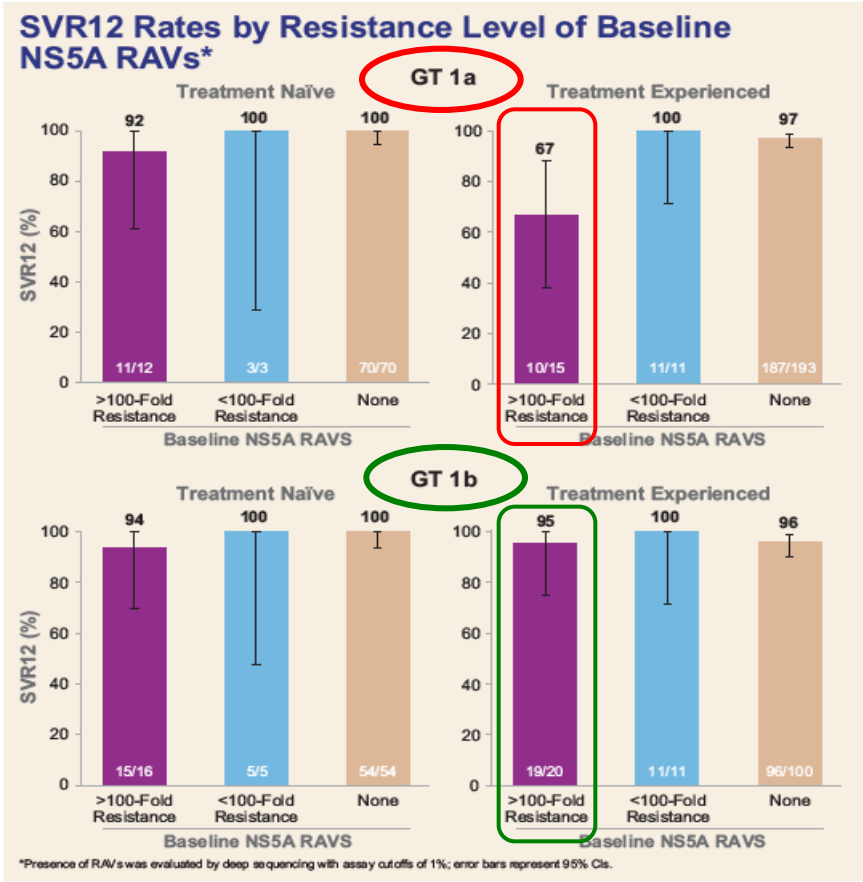


Sarrazin et al., EASL 2015

LDV/SOF ± RBV: SVR12 in GT 1 Treatment-naïve Patients With Cirrhosis ± Baseline NS5A RAVs

Different impact according to HCV-1 subtype……





Sarrazin et al., EASL 2015

Annals of Internal Medicine ORIGINAL RESEARCH

Grazoprevir–Elbasvir Combination Therapy for Treatment-Naive Cirrhotic and Noncirrhotic Patients With Chronic Hepatitis C Virus Genotype 1, 4, or 6 Infection

A Randomized Trial

Zeuzem S et al., Ann Intern Med. 2015

Appendix Table 6. Impact of Baseline HCV RNA Levels and Baseline Selected NS5A RAVs on Efficacy in Noncirrhotic and Cirrhotic GT1-, GT4-, and GT6-Infected Subjects, as Measured by SVR12 (Full Analysis Set)

Treatment	Baseline HCV RNA and selected NS5A RAV	N	HCV RNA < LLOQ	
			n (%)	95% CI†
Immediate-treatment arm: GZR-EBR for 12 weeks	High (>800 000 IU/mL) with selected NS5A RAV	33	22 (66.7)	(48.2, 82.0)
	High (>800 000 IU/mL) without selected NS5A RAV	189	183 (96.8)	(93.2, 98.8)
	Low (≤800 000 IU/mL) with selected NS5A RAV	17	17 (100.0)	(80.5, 100.0)
	Low (≤800 000 IU/mL) without selected NS5A RAV	77	77 (100.0)	(95.3, 100.0)

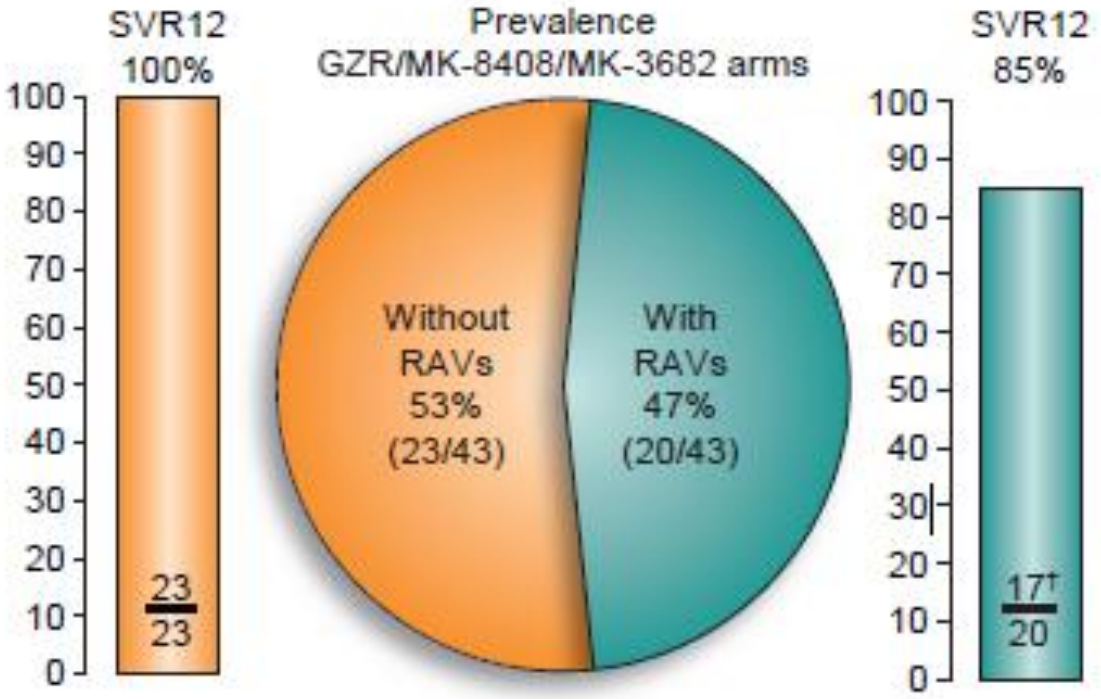
CI=confidence interval; EBR=elbasvir; GZR=grazoprevir; HCV=hepatitis C virus; LLOQ=lower limit of quantification; N=number of subjects included in the analysis; n(%)=number of subjects who achieved the corresponding HCV RNA end point and the percentage calculated as (n/N)*100; RAVs=resistance-associated variants.
† Based on Clopper-Pearson method.
The Roche COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 LLOQ is 15 IU/mL.

Appendix Table 5. Impact of Baseline NS5A RAVs on Efficacy in Noncirrhotic and Cirrhotic GT1-, GT4-, and GT6-Infected Subjects, as Measured by SVR12

Population†	SVR12*			
	Overall efficacy in evaluable patients†	NS5A RAVs not detectable	NS5A RAVs with ≤5-fold shift to EBR	NS5A RAVs with >5-fold shift to EBR
Overall GT1	273/284 (96.1%)	245/247 (99.2%)	10/11 (90.9%)	18/26 (69.2%)
By GT1 Subtypes				
GT1a	144/154 (93.5%)	133/135 (98.5%)	9/10 (90.0%)	2/9 (22.2%)
GT1b	129/130 (99.2%)	112/112 (100%)	1/1 (100%)	16/17 (94.1%)
Overall GT4	18/18 (100%)	9/9 (100%)	9/9 (100%)	
Overall GT6	7/9 (77.8%)	5/6 (83.3%)	2/3 (66.7%)	

n/a = not applicable.
* SVR_{12} = #subjects with the selected RAVs achieving SVR12/#subjects with the selected RAVs in each category.
† Includes all patients in the full analysis population who have relevant sequencing data available and who either achieved SVR12 or met criteria for virologic failure.

SVR rates were reduced in GT-3 patients with natural NS5A RAVs treated with grazoprevir, MK-3682 (NS5B), and MK-8408 (NS5A inhibitor) for 8 weeks

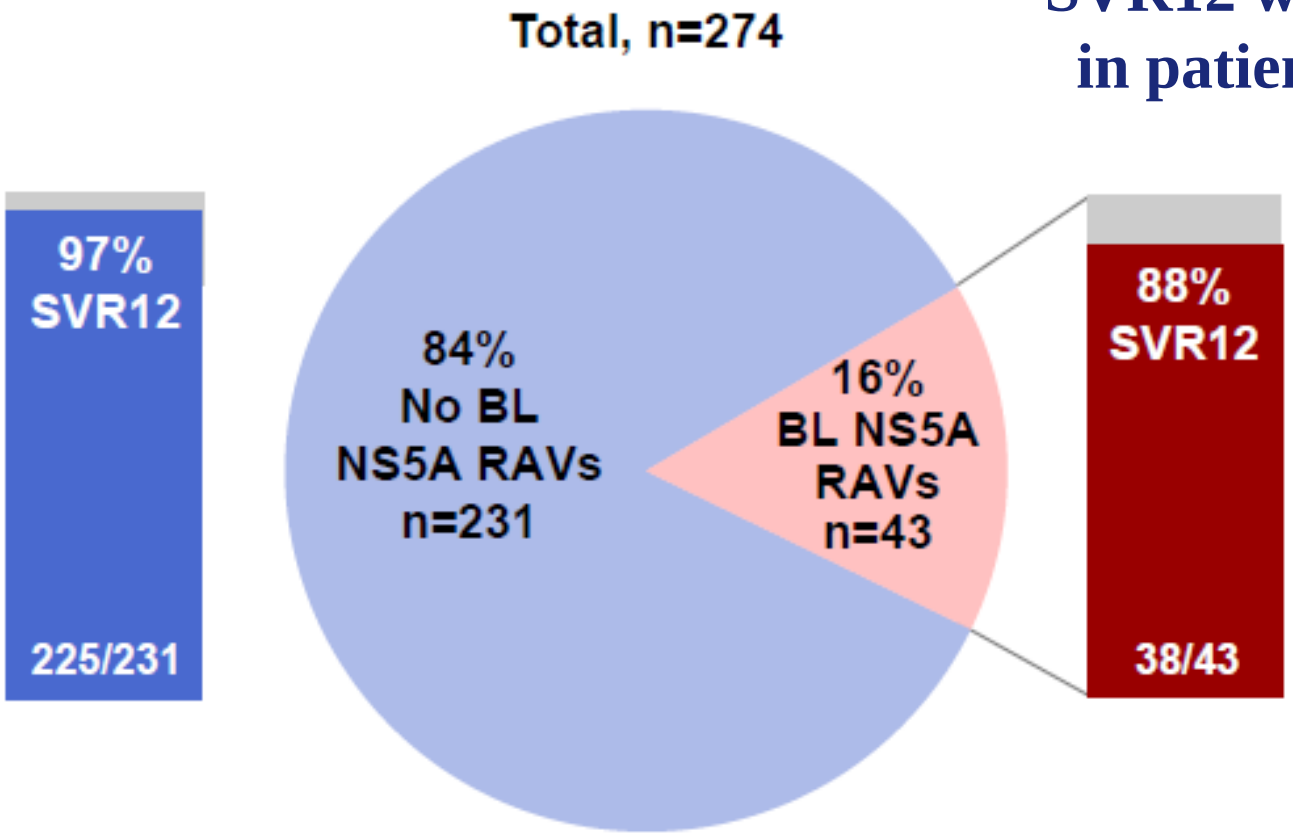


†1 of the 3 relapsers had a treatment-emergent NS5A RAV (Y93H) at the time of failure.

Gane EJ, AASLD 2015, Poster #LB-15

ASTRAL-3: phase 3 study of SOF + VEL for 12 weeks in GT 3 patients

SVR12 was 84% (21/25) in patients with Y93H



Mangia A, AASLD 2015

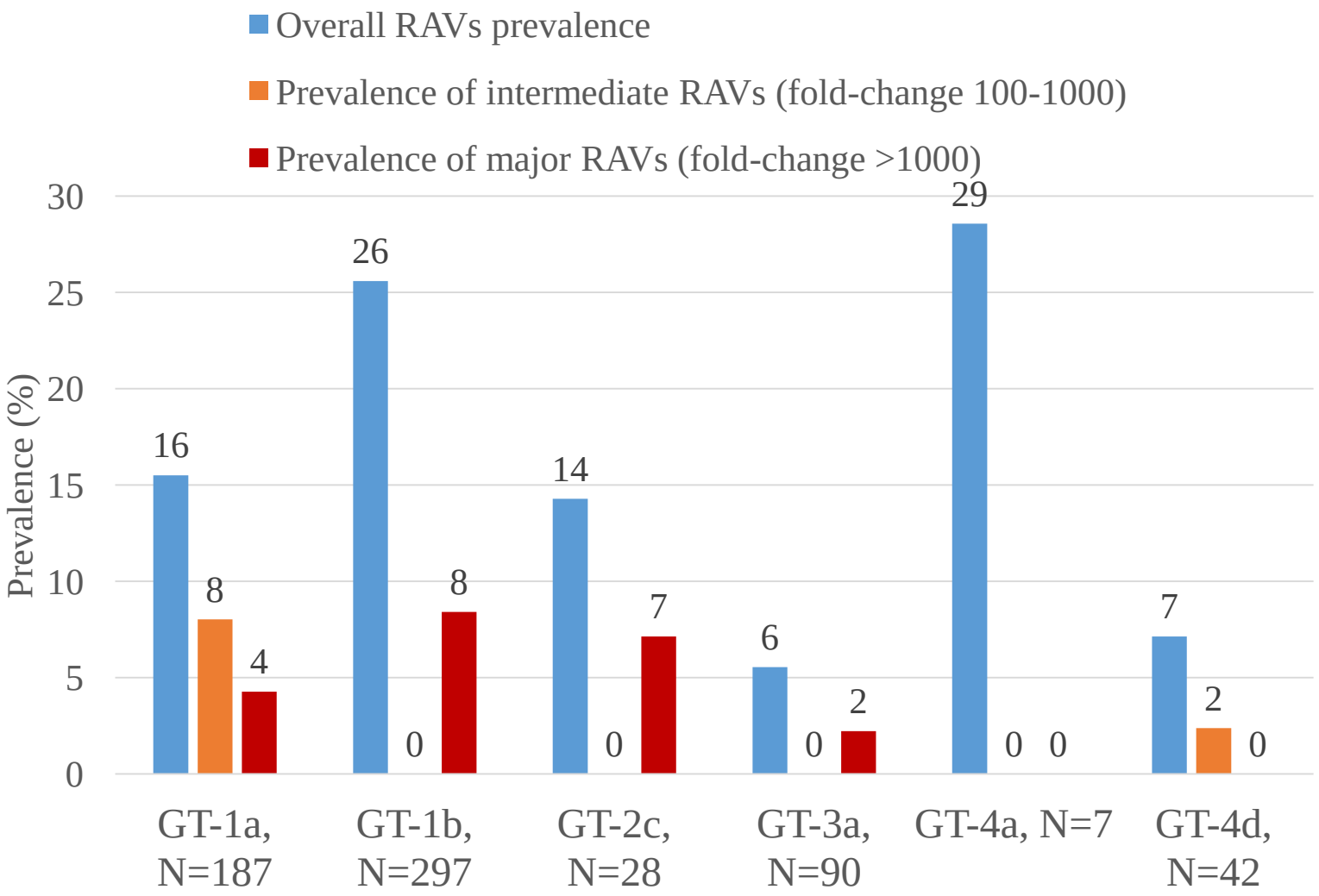
Beware of HCV-genotype for NS5A resistance ...

Table 1 *In vitro* resistance profiles according to hepatitis C virus genotypes[36,40,50-54,64]

EC_{50}	< 10 pmol/L	< 100 pmol/L	< 1 nmol/L	< 10 nmol/L	< 100 nmol/L	< 1 μmol/L	> 1 μmol/L
DCV HCV GT							
1b	Wild (2.6 pmol/L) L28M L31M R30Q	R30E, H L31F, V P32L Y93H, N 37L or 54H/93H 23F/31F	23F/93H 30Q/31F 31V/58S 30H/31M		31F, M, V/93H 30Q/31M/93H		Δ30/32L
1a	Wild (6 pmol/L)			M28T Q30H, R L31M P32L H58D	L31V Y93C, H	Q30E, K Y93N (> 500 nmol/L) 28T/30H 30H/93H 30R/93C 30R/62D	31V/93H
2-6		GT2a (JFH1) GT4a, 5a, 6a	GT3a	GT2a (L31M) GT2a (C92R)	GT2a (Y93H) GT2b (31M) GT3a (A30K) GT3a (L31F) GT4a (R30G) GT4a (L30H)	GT2a (F28S) GT3a (Y93H) GT4a (L30I/Y93R)	
ACH-3102 HCV GT							
1b	Wild (7 pmol/L) L31V	Y93H 31V/93H	P58S/Y93H P58S/T64A/Y93H				
1a		wild (20 pmol/L) Q30H L31M, V	Q30R, E, K M28T P32L H58D	Y93C	Y93H, N[1] 28T/30H/93C[1]		
2-6			GT2a (JFH1) GT2a (L31M) GT2b (31M) GT3a, 4a, 5a, 6a				

Nakamoto S., WJG 2014

The Italian experience: the prevalence of patients with at least 1 natural NS5A RAV is different according to genotype and subtype



Cento V, unpublished data

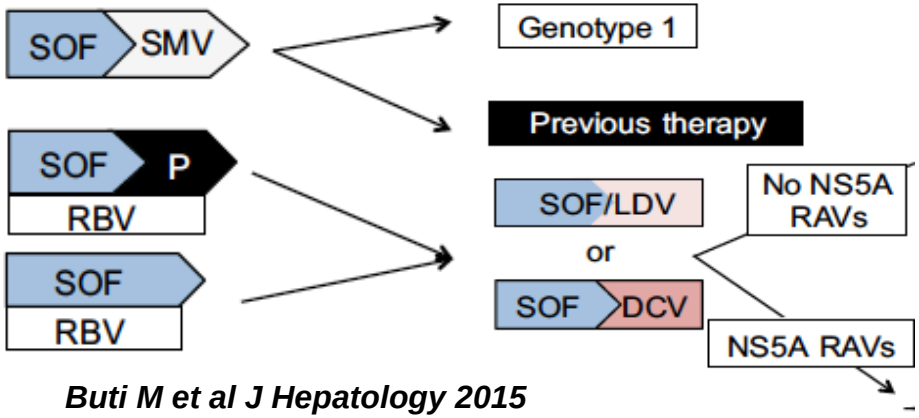
Natural NS5A RAVs in different HCV genotypes in Italy

	Natural NS5A RAV prevalence, N (%)						
	GT-1a, N=187	GT-1b, N=297	GT-2c, N=28	GT-3a, N=90	GT-4a, N=7	GT-4d, N=42	Overall, N=651
Major NS5A RAVs (fold-change ≥100)							
M28V	7 (3.7)	-	-	-	-	-	7 (1.1)
Q30H	1 (0.5)	-	-	-	-	-	1 (0.2)
Q30R	4 (2.1)	-	-	-	-	-	4 (0.6)
R30S	-	-	-	-	-	1 (2.4)	1 (0.2)
L31M	8 (4.3)	-	1 (3.4)	-	-	-	9 (1.4)
Y93C	1 (0.5)	-	-	-	-	-	1 (0.2)
Y93H	1 (0.5)	25 (8.4)	1 (3.4)	2 (2.2)	-	-	29 (4.4)
Y93N	1 (0.5)	-	-	-	-	-	1 (0.2)
Minor NS5A RAVs (fold-change <100)							
K24R	1 (0.5)	-	-	-	-	2 (4.8)	3 (0.5)
L28M	-	8 (2.7)	-	-	-	-	8 (1.2)
A30K	-	-	-	2 (2.2)	-	-	2 (0.3)
L30R	-	-	-	-	2 (28.6)	-	2 (0.3)
R30H	-	1 (0.3)	-	-	-	-	1 (0.2)
R30Q	-	17 (5.7)	-	-	-	-	17 (2.6)
L31F	-	-	1 (3.4)	-	-	-	1 (0.2)
L31M	-	10 (3.4)	-	-	-	-	10 (1.5)
L31P	-	-	-	1 (1.1)	-	-	1 (0.2)
P58L	-	2 (0.7)	-	-	-	-	2 (0.3)
P58S	-	11 (3.7)	1 (3.4)	-	-	-	12 (1.8)
E62D	7 (3.7)	-	-	-	-	-	7 (1.1)
A92T	-	13 (4.4)	-	-	-	-	13 (2)

NS5A RAVs are reported according to genotype-specific wild-type amino acid.

Cento V, unpublished data

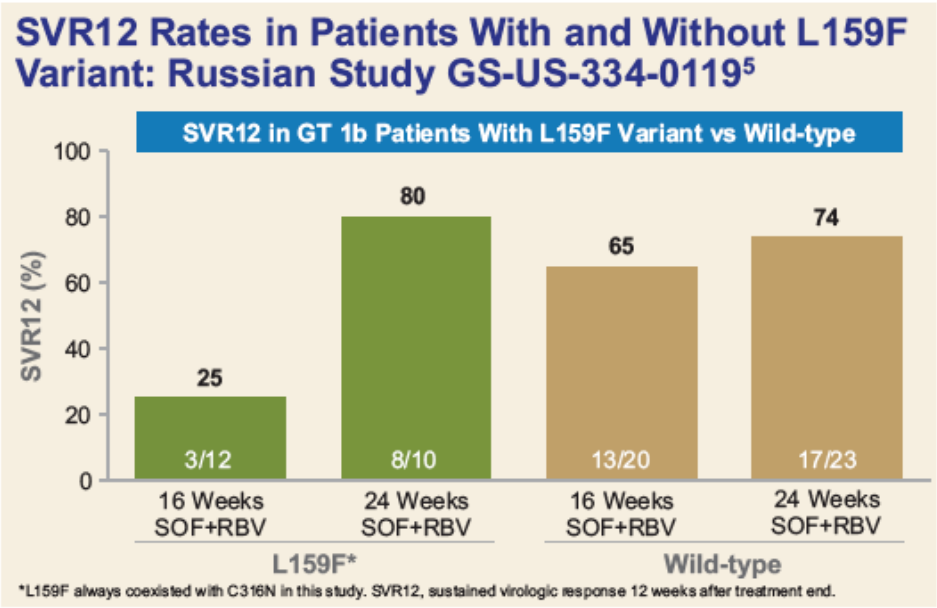
The role of NS5B resistance test for patients who failed a Sofosbuvir containing regimen is not yet defined

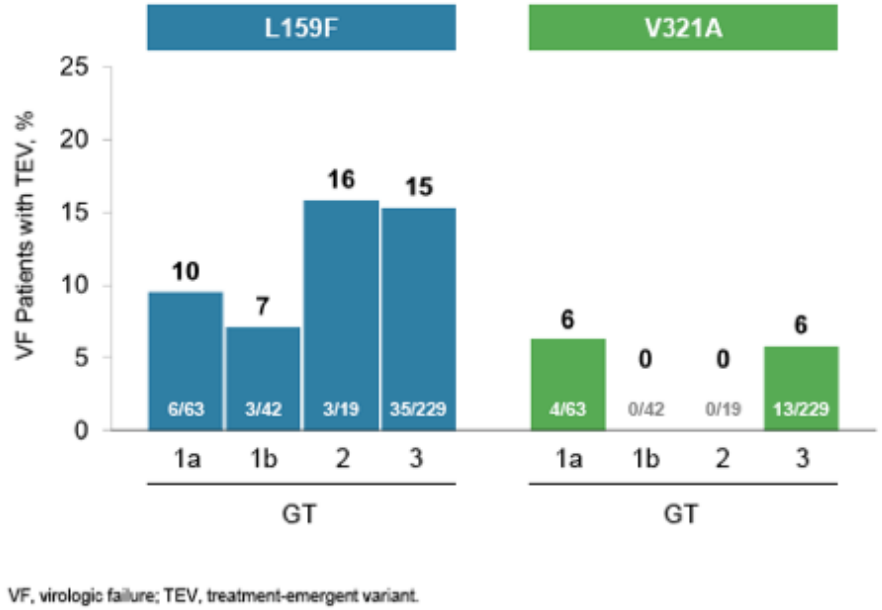


Buti M et al J Hepatology 2015

n =12,012 in SOF or LDV/SOF clinical studies
n=1025 with virologic failure
n=901* with deep sequencing
⇨ **1% 282T SOF virologic failures**

Gane et al AASLD 2015

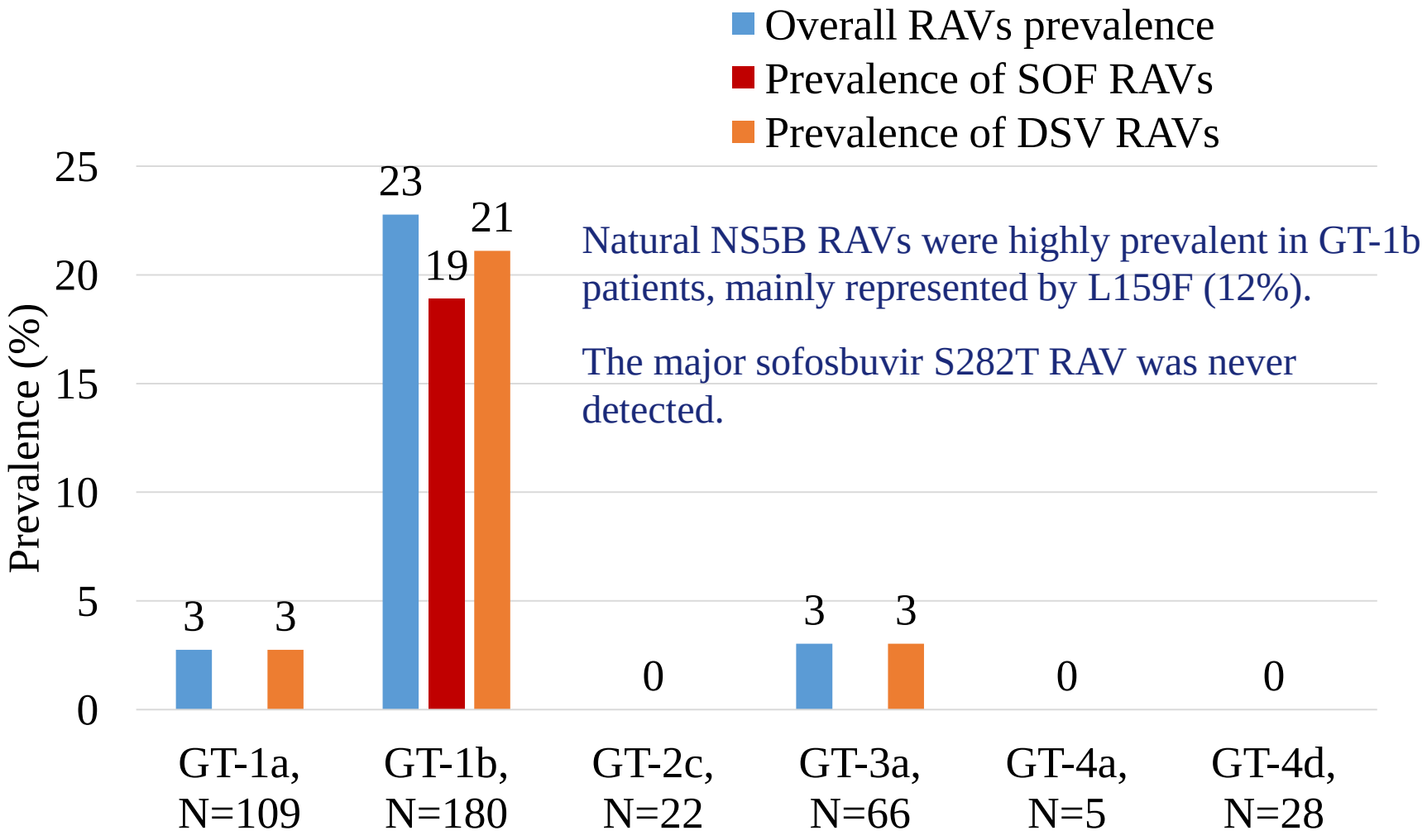




Svarovskaia et al, JID 2015

Zhdanov K., APASL 2015

The Italian experience: the prevalence of patients with at least 1 natural NS5B RAV is 3% in GT1a, 23% in GT1b, 3% in GT-3, 0% in GT2 & GT4

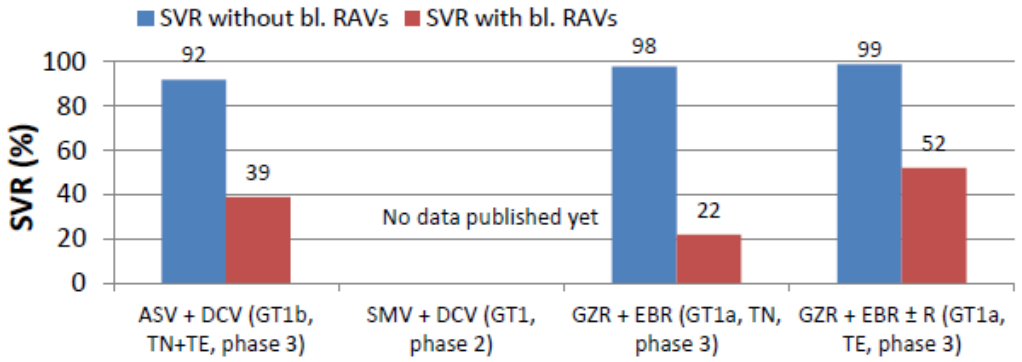


Natural NS5B RAVs were highly prevalent in GT-1b patients, mainly represented by L159F (12%).

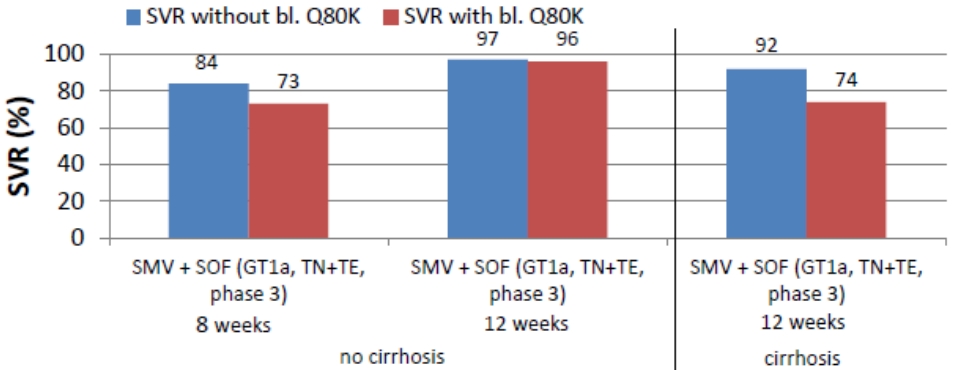
The major sofosbuvir S282T RAV was never detected.

Cento V, unpublished data

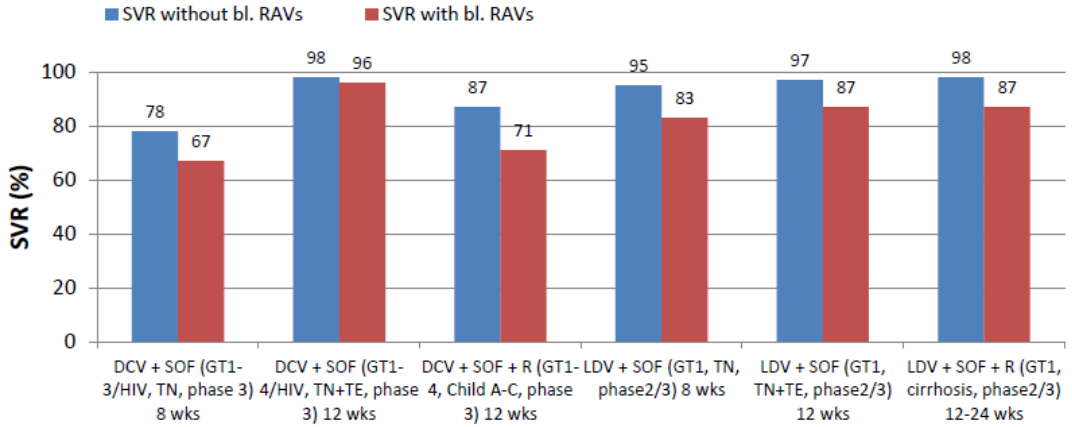
Should we be worried about baseline RAVs?



SVR rates to **NS3** protease inhibitor plus **NS5A** inhibitor combination regimens in HCV genotype 1 infected patients according to the presence of baseline RAVs.



SVR rates for **NS3** protease inhibitor plus nucleos(t)ide **NS5B** inhibitor combination regimens in HCV genotype 1 infected patients according to the presence of baseline RAVs.

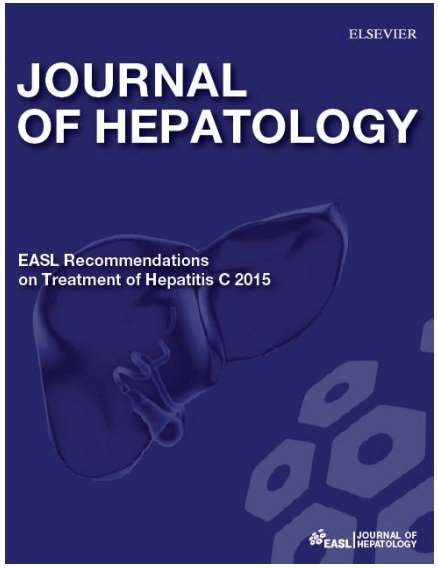


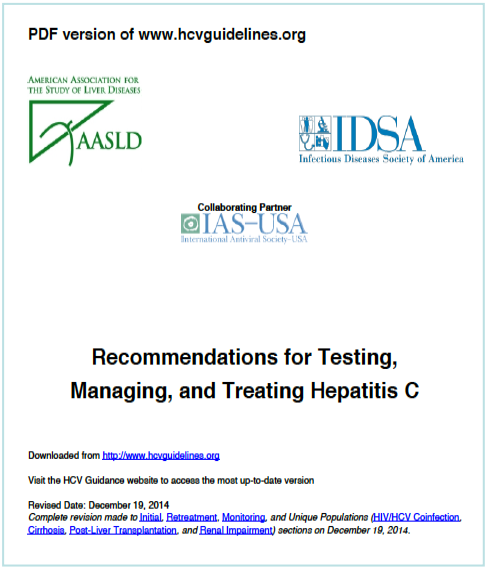
SVR rates **NS5A** inhibitor plus nucleos(t)ide **NS5B** inhibitor DAA combination regimens in HCV genotype 1 infected patients according to the presence of baseline RAVs.

Sarrazin C et al., J Hepatol 2015

Virological issues in the DAAs Era

After treatment failure: useful / recommended the resistance test?




PDF version of www.hcvguidelines.org

AMERICAN ASSOCIATION FOR THE STUDY OF LIVER DISEASES
AASLD

IDSA
Infectious Diseases Society of America

Collaborating Partner
IAS–USA
International Antiviral Society–USA

Recommendations for Testing, Managing, and Treating Hepatitis C

Downloaded from http://www.hcvguidelines.org

Visit the HCV Guidance website to access the most up-to-date version

Revised Date: December 19, 2014
Complete revision made to Initial, Retreatment, Monitoring, *and Unique Populations (*HIV/HCV Coinfection, Cirrhosis, Post-Liver Transplantation, *and* Renal Impairment*) sections on December 19, 2014.*

[…] Currently, there is no data to firmly support retreatment recommendations, which must be based on indirect evidence (HCV genotype, known resistance profiles of the administered drugs, number of drugs used, use of ribavirin, treatment duration). Whether assessing the sequence of the target HCV genes (HCV resistance testing) prior to retreatment is helpful to make a decision remains unknown, as well as which therapeutic decision should be made based on this result.

- ***Routine monitoring for HCV drug RAVs during or after therapy is not recommended except prior to treatment of (1) persons with HCV genotype 1a infection who are being considered for treatment with simeprevir with PEG-IFN and RBV, simeprevir, or sofosbuvir (cirrhosis) or (2) persons with HCV genotype 1 infection who were previously treated with an NS5A inhibitor and are being considered for retreatment. (III-C)***

Baseline characteristics of 72 HCV failures to DAAs with resistance test available at failure

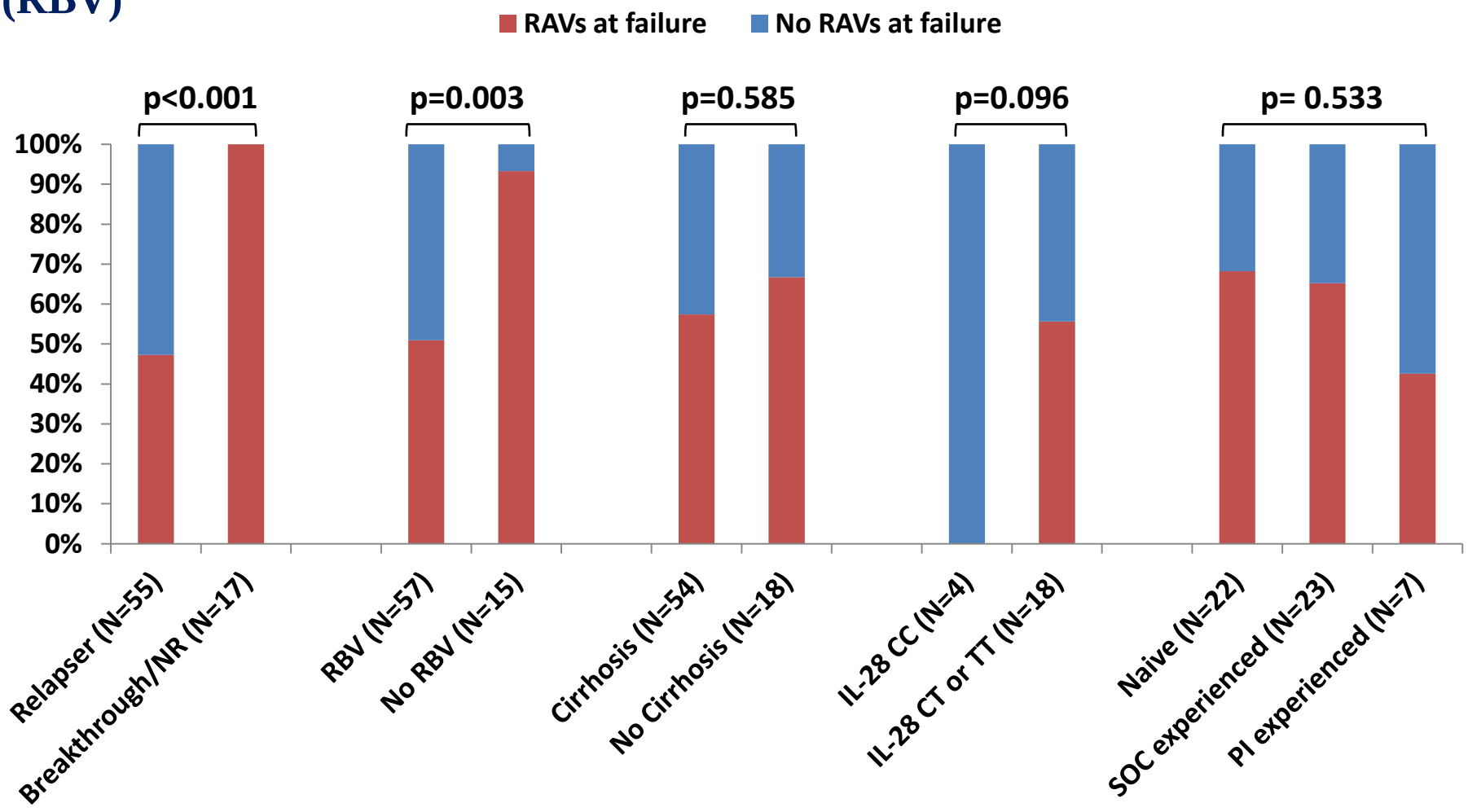
Patients, N		72
Males, N(%)		48 (66.7)
Age (years), Median (IQR)		57 (52-66)
Liver Transplant, N (%)		11 (15.0)
Liver HCC, N (%)		7 (9.9)
Cirrhotic, N (%)		54 (75.0)
Stiffness at baseline (Kpa), Median (IQR) *		22 (14-33)
Naïve patients, N (%)		22 (30.6)
Treatment experienced, N(%)	**Breakthrough**	4 (5.6)
	Non-responder	20 (27.8)
	Relapse	7 (9.7)
	Unknown/other	4 (5.6)
PI experienced		7 (12.3)
Unknown previous treatment		15 (20.8)
Baseline HCV-RNA (logIU/ml), Median (IQR)		6.1 (5.5-6.5)
Baseline ALT (IU/ml), Median (IQR)*		57 (38-88)
HCV geno/subtype	**1a**	16 (22.2)
	1b	27 (37.5)
	2c	2 (2.8)
	3a	14 (19.4)
	4 (a-d-n-r)	13 (18.1)

IQR, interquartile range, * Information not available for all patients

Di Maio et al., AISF 2016

Overall, 43/72 patients (59.7%) showed at least one RAV at failure

RAVs prevalence was significantly higher in breakthrough/ non responders (N=17) than in relapsers (N=55) and in patients who did not receive ribavirin (RBV)



Di Maio et al., AISF 2016

All NS5A failing-patients showed NS5A RAVs at failure

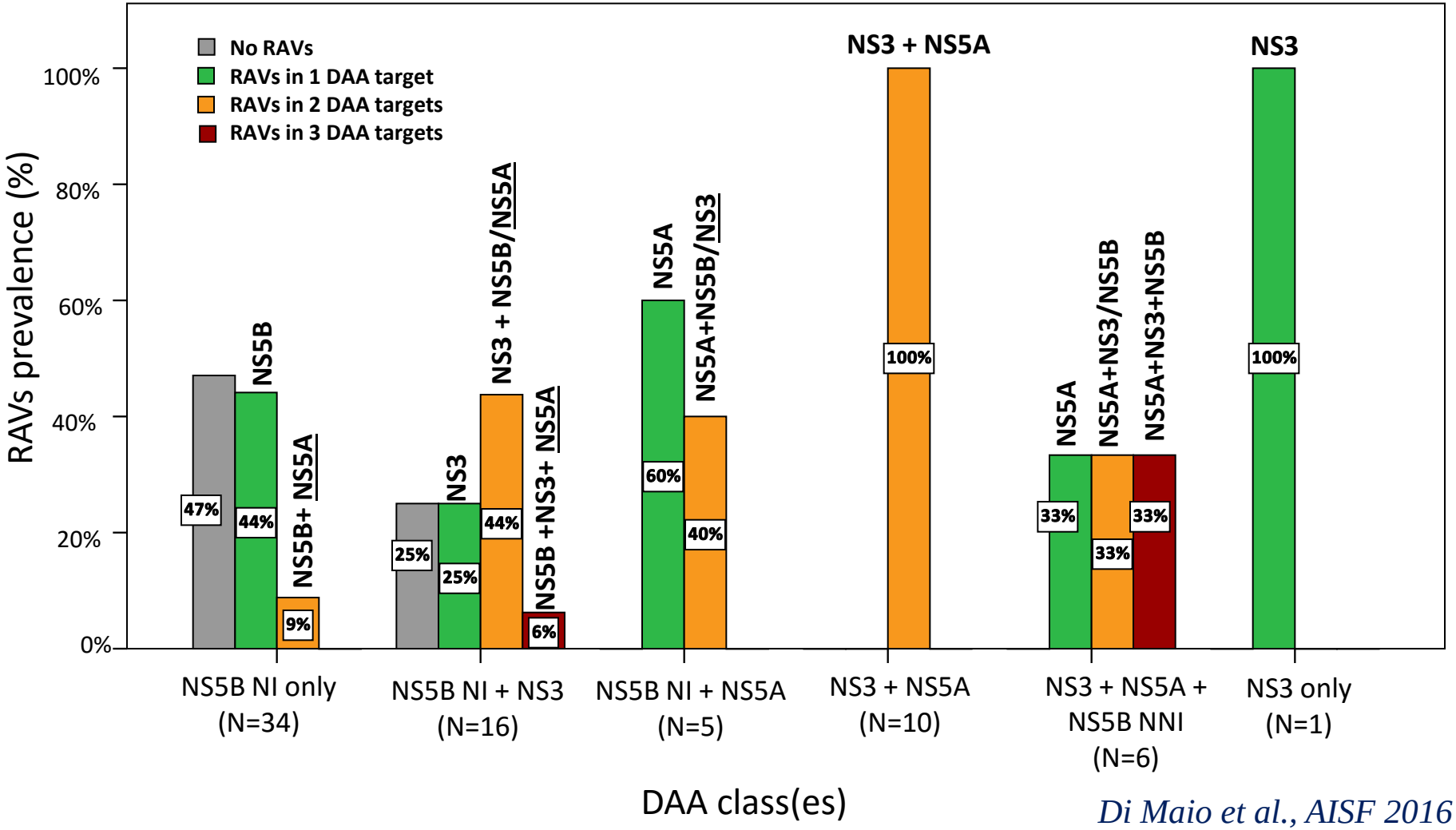
NS5A containing regimen	Failing patients	Patients with NS5A RAVs
PAR/r+OMB+DAS+RBV	N=6	100%
ASU+DCV+/-RBV	N=3	100%
SMV+DCV+/-RBV	N=7	100%
DCV+SOF+/-RBV	N=3	100%
LDV+SOF+/-RBV	N=2	100%

RAVs, Resistance Associated Variants

Di Maio et al., AISF 2016

26/72 (31.9%) patients showed RAVs on ≥2 DAA-targets

….If we consider only the 37 patients treated with 2 or more classes, the rate of 2 or more RAV classes is 62.1%



Di Maio et al., AISF 2016

17/22 (77.2%) HCV-1b relapsers showed at least one RAV related to the DAA-regimen at virological failure

DAA-Target	Regimen	Failure Ravs		
		NS3	NS5A	NS5B
NS3+NS5A+NS5B	PAR/r+OMB+DAS	S122T+D168V	Y93H	S556G
NS3+NS5A	SMV+DCV+RBV	D168D/V	L31M+Y93H	
NS3+NS5B	SMV+SOF	D168D/V	L31M+Y93H	L159F+C316N
	SMV+SOF	Q80Q/R		L159F+C316N+S556G
	SMV+SOF	V36I		A421V
	SMV+SOF+RBV	D168V		C316H
	SMV+SOF+RBV	D168V		C316N
	SMV+SOF+RBV	D168D/V		L159F+C316N
NS3	SMV+PegINF+RBV	D168V		
NS5A+NS5B	DCV+SOF+RBV		L28M+Y93H	
NS5B	SOF+RBV			L159F+C316N
	SOF+RBV			L159F+C316N
	SOF+RBV		R30H+Y93H	L159F+C316N+S556G
	SOF+RBV			L159F
	SOF+PegINF+RBV			L159F+C316N+S556S/G
	SOF+PegINF+RBV			L159F+C316N+S556G
	SOF+PegINF+RBV			L159F+C316N

DAA, Direct Acting Antivirals, RAVs, Resistance Associated Variants

Di Maio et al., AISF 2016

The Sofosbuvir resistance mutation L159F was found in 11/51 (21.6%) relapsers treated with a Sofosbuvir containing regimen, including 10/19 (52.6%) HCV-1b relapsers

DAA-Target	Regimen	HCV geno/ subtype	Failure Ravs		
			NS3	NS5A	NS5B
NS3+NS5B	SMV+SOF	1b	D168D/V	L31M+Y93H	**L159F+C316N**
	SMV+SOF	1b	Q80Q/R		**L159F+C316N**+S556G
	SMV+SOF	1b	V36I		A421V
	SMV+SOF+RBV	1b	D168V		C316H
	SMV+SOF+RBV	1b	D168V		C316N
	SMV+SOF+RBV	1b	D168D/V		**L159F+C316N**
NS5A+NS5B	DCV+SOF+RBV	1b		L28M+Y93H	
NS5B	SOF+RBV	1b			**L159F+C316N***
	SOF+RBV	1b			**L159F+C316N***
	SOF+RBV	1b		R30H+Y93H	**L159F+C316N**+S556G
	SOF+RBV	1b			**L159F**
	SOF+PegINF+RBV	1b			**L159F+C316N**+S556S/G
	SOF+PegINF+RBV	1b			**L159F+C316N**+S556G
	SOF+PegINF+RBV	1b			**L159F+C316N**
	SOF+RBV	3a			**L159F**

DAA, Direct Acting Antivirals, RAVs, Resistance Associated Variants

* RAVs Present already at Baseline

Di Maio et al., AISF 2016

All 13 (100%) breakthrough-, and 4 non responder patients showed at least one RAV at failure in at least one DAA-target

DAA-Target	Regimen	HCV geno/ subtype	Baseline RAVs			Failure RAVs		
			NS3	NS5A	NS5B	NS3	NS5A	NS5B
NS3+NS5A +NS5B	PAR/r+OMB+DAS+RBV	1a	V55A			D168A+V36M/V+V55A	Q30R	
	PAR/r+OMB+DAS+RBV	1a				D168A+Q80K+Y56H	Q30R	A553T
	PAR/r+OMB+DAS+RBV	1a				Y56H+D168A	Q30R	
	PAR/r+OMB+DAS+RBV	3a					Y93H	
	PAR/r+OMB+DAS+RBV	3a					Y93H	
NS3+NS5A	ASU+DCV	1b	D168V	Y93H		D168V+Y56H/Y	L31M+Y93H	
	ASU+DCV	1b				D168V	L28G+L31I+Y93H/I	
	ASU+DCV+RBV	4d				D168V	L28V+M31M/V+Y93Y/H	
	SMV+DCV	1b				D168V	Y93H	
	SMV+DCV	4d	D168E	R30S		A156G+D168E	L28V+R30S	
	SMV+DCV+RBV	1b				D168V	L31F/I+Y93H	
	SMV+DCV+RBV	4d				D168V	T58A+Y93H	
	SMV+DCV+RBV	4r				D168V	P58S	
NS3+NS5B	SMV+SOF	4a				D168E+Q80R		S282T
NS5A+NS5B	LDV+SOF	4a					L30H	S282T
	LDV+SOF+RBV	1b					L31M+Y93H	
NS5B	SOF	3a						S282T

RAVs, Resistance Associated Variants

Baseline resistance test not available

Di Maio et al., AISF 2016

All 13 (100%) breakthrough-, and 4 non responder patients showed at least one RAV at failure in at least one DAA-target

3/4 non responder patients showed a different HCV genotype at failure

DAA-Target	Regimen	HCV geno/ subtype	Baseline RAVs			Failure RAVs		
			NS3	NS5A	NS5B	NS3	NS5A	NS5B
NS3+NS5A +NS5B	PAR/r+OMB+DAS+RBV	1a	V55A			D168A+V36M/V+V55A	Q30R	
	PAR/r+OMB+DAS+RBV	1a				D168A+Q80K+Y56H	Q30R	A553T
	PAR/r+OMB+DAS+RBV	1a				Y56H+D168A	Q30R	
	PAR/r+OMB+DAS+RBV	1a->3a*					Y93H	
	PAR/r+OMB+DAS+RBV	1b->3a*					Y93H	
NS3+NS5A	ASU+DCV	1b	D168V	Y93H		D168V+Y56H/Y	L31M+Y93H	
	ASU+DCV	1b				D168V	L28G+L31I+Y93H/I	
	ASU+DCV+RBV	1b->4d*				D168V	L28V+M31M/V+Y93Y/H	
	SMV+DCV	1b				D168V	Y93H	
	SMV+DCV	4d	D168E	R30S		A156G+D168E	L28V+R30S	
	SMV+DCV+RBV	1b				D168V	L31F/I+Y93H	
	SMV+DCV+RBV	4d				D168V	T58A+Y93H	
	SMV+DCV+RBV	4r				D168V	P58S	
NS3+NS5B	SMV+SOF	4a				D168E+Q80R		S282T
NS5A+NS5B	LDV+SOF	4a					L30H	S282T
	LDV+SOF+RBV	1b					L31M+Y93H	
NS5B	SOF	3a						S282T

RAVs, Resistance Associated Variants

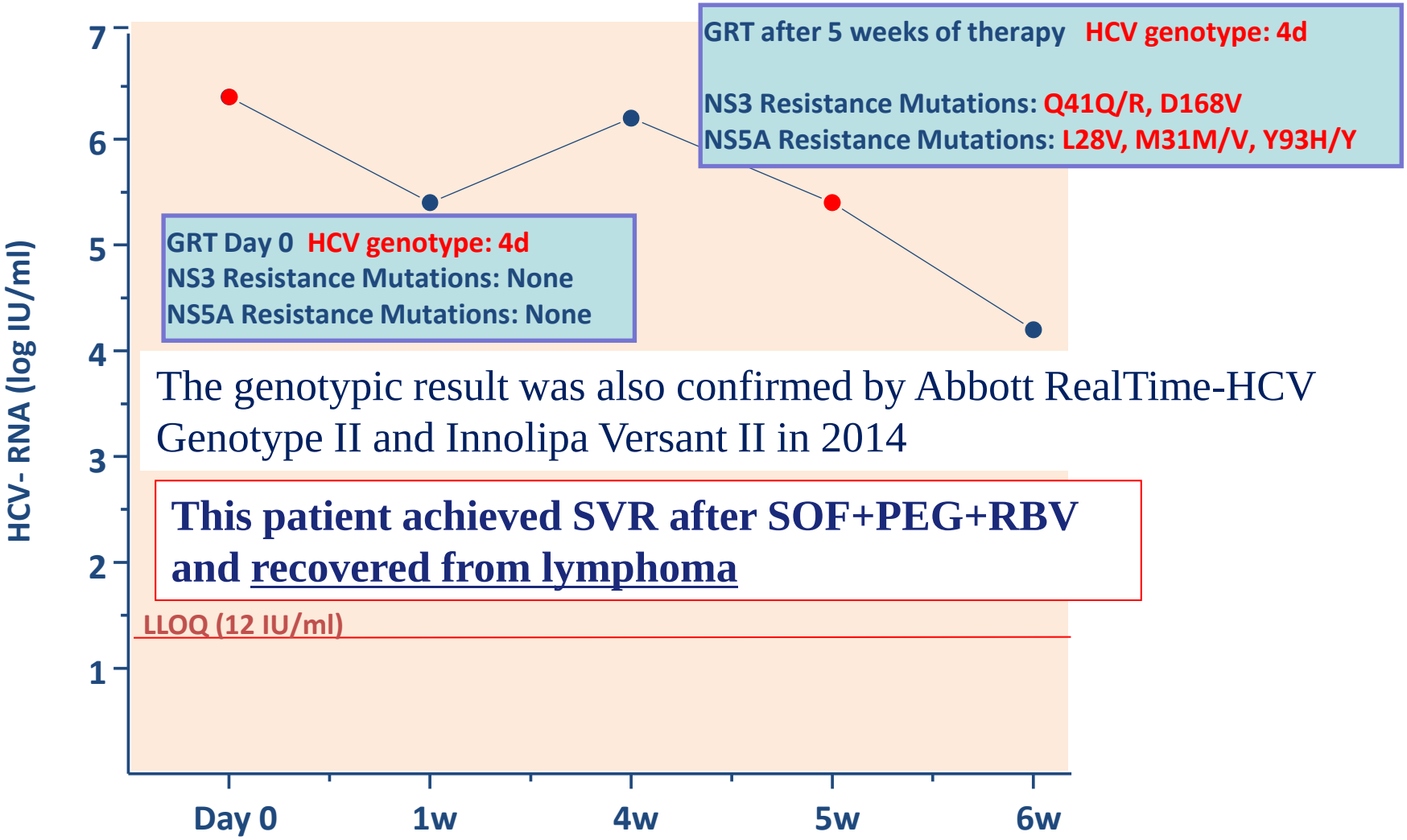
Baseline resistance test not available

Di Maio et al., AISF 2016

A clinical case of a HCV-4d infected patient previously classified as HCV-1b who failed Asunaprevir+ Daclatasvir+RBV treatment

HCV genotype: 1b (performed in 1998)	Sex: M	Non Responder to SOC	Follicular Lymphoma

Asunaprevir+ Daclatasvir+RBV



The genotypic result was also confirmed by Abbott RealTime-HCV Genotype II and Innolipa Versant II in 2014

This patient achieved SVR after SOF+PEG+RBV and recovered from lymphoma

3 HCV-infected patients who experienced a breakthrough to a Sofosbuvir containing regimen showed the major NI RAV S282T at virological failure

DAA-Target	Regimen	HCV geno/ subtype	Failure RAVs		
			NS3	NS5A	NS5B
NS3+NS5A +NS5B	PAR/r+OMB+DAS+RBV	1a	D168A+V36M/V+V55A	Q30R	
	PAR/r+OMB+DAS+RBV	1a	D168A+Q80K+Y56H	Q30R	A553T
	PAR/r+OMB+DAS+RBV	1a	Y56H+D168A	Q30R	
	PAR/r+OMB+DAS+RBV	3a		Y93H	
	PAR/r+OMB+DAS+RBV	3a		Y93H	
NS3+NS5A	ASU+DCV	1b	D168V+Y56H/Y	L31M+Y93H	
	ASU+DCV	1b	D168V	L28G+L31I+Y93H/I	
	ASU+DCV+RBV	4d	D168V	L28V+M31M/V+Y93Y/H	
	SMV+DCV	1b	D168V	Y93H	
	SMV+DCV	4d	A156G+D168E	L28V+R30S	
	SMV+DCV+RBV	1b	D168V	L31F/I+Y93H	
	SMV+DCV+RBV	4d	D168V	T58A+Y93H	
	SMV+DCV+RBV	4r	D168V	P58S	
NS3+NS5B	SMV+**SOF**	**4a**	D168E+Q80R		**S282T**
NS5A+ NS5B	LDV+**SOF**	**4a**		L30H	**S282T**
	LDV+SOF+RBV	1b		L31M+Y93H	
NS5B	**SOF**	**3a**			**S282T**

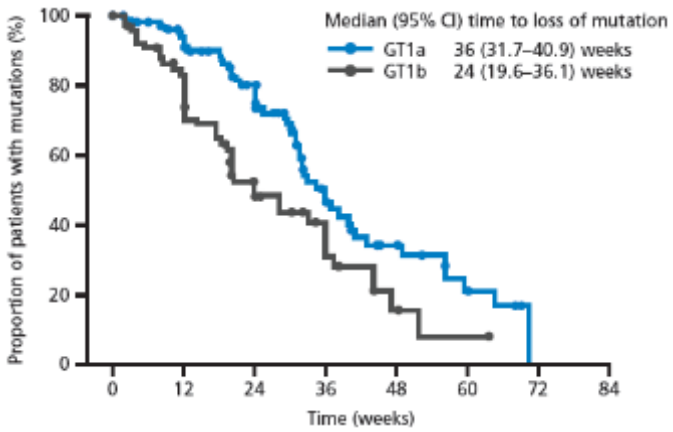
DAA, Direct Acting Antivirals, RAVs, Resistance Associated Variants

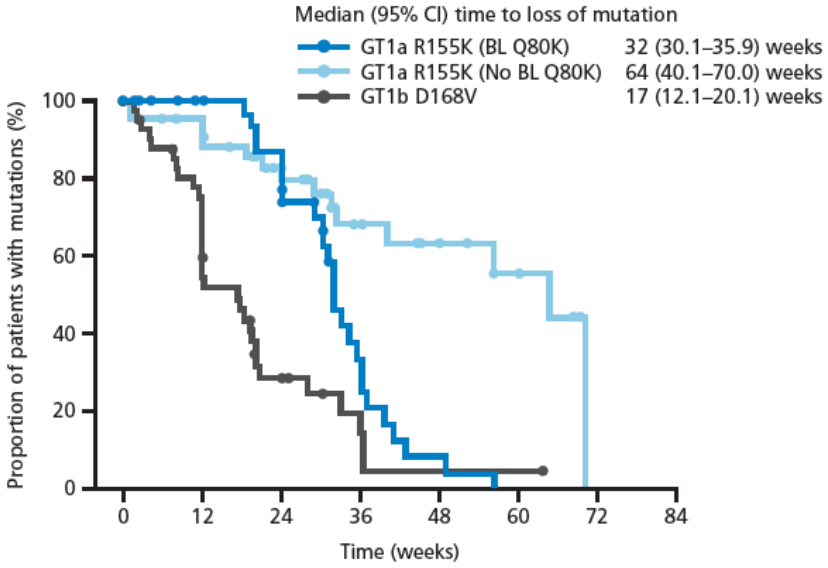
Di Maio et al., AISF 2016

Do DAA resistance mutations “disappear” following discontinuation of therapy?

In the majority of patients PR RAVs disappear….

Simeprevir





Lentz O, et al. EASL 2014

Paritaprevir/r

	Non-Cirrhotics n/N (%)				Cirrhotics n/N (%)		All PTV/r-Containing Regimens n/N (%)	
	2D ± RBV		3D ± RBV		3D + RBV			
Time window	PTW24	PTW48	PTW24	PTW48	PTW24	PTW48	PTW24	PTW48
TEVs (any)	6/19 (32)	1/17 (6)	19/37 (51)	3/32 (9)	8/11 (73)	1/8 (13)	31/67 (46)	5/57 (9)
D168 (any)	4/16 (25)	0/16 (0)	11/30 (37)	1/30 (3)	6/9 (67)	1/7 (14)	21/55 (38)	2/53 (4)
R155K	2/5 (40)	1/5 (20)	6/6 (100)	1/2 (50)	2/2 (100)	LTFU	10/13 (77)	2/7 (29)

2D = PTV/r + (DSV or OBV); PTW = post-treatment Week; LTFU = lost to follow-up

Krishnan P et al. EASL 2015

Persistence of NS5a Resistance Associated Variants Following Ombitasvir/Paritaprevir/r + Dasabuvir Treatment

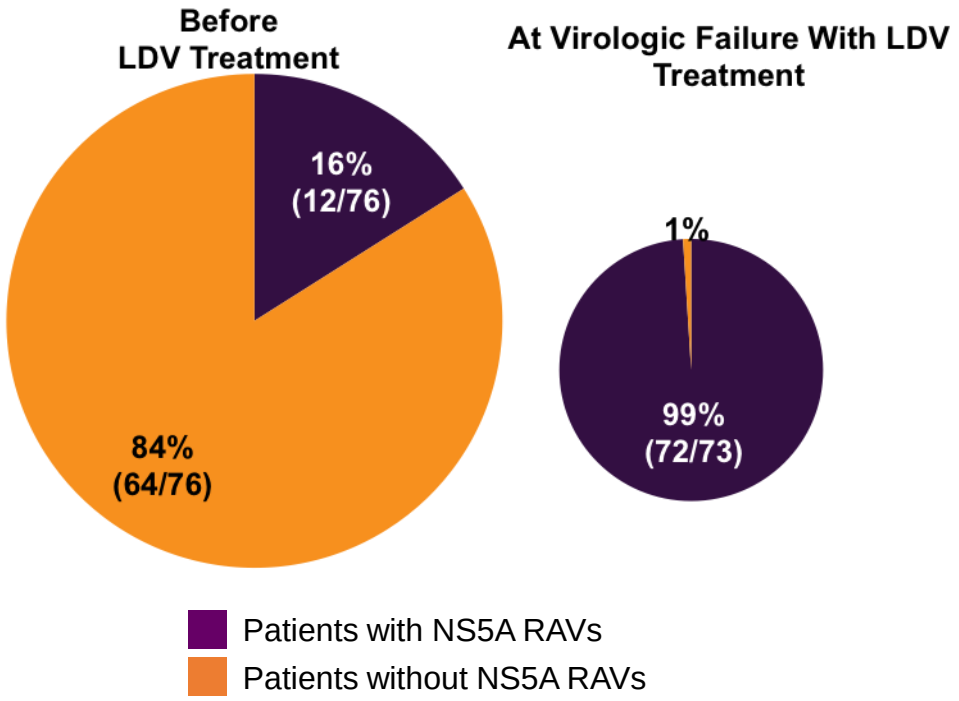
- Pooled patients with virologic failure from all clinical trials (n=2510)
  - 67 patients with HCV genotype 1a
  - 7 patients with HCV genotype 1b (no long-term follow-up reported)

	Post-treatment 24 Weeks	Post-treatment 48 Weeks
NS3/4A (any)	31/67 (46%)	5/57 (9%)
NS5A (any)	68/70 (97%)	**49/51 (96%)**
NS5B (non-nuc)	33/44 (75%)	20/35 (57%)

Krishnan et al., Abstract #O057, EASL 2015

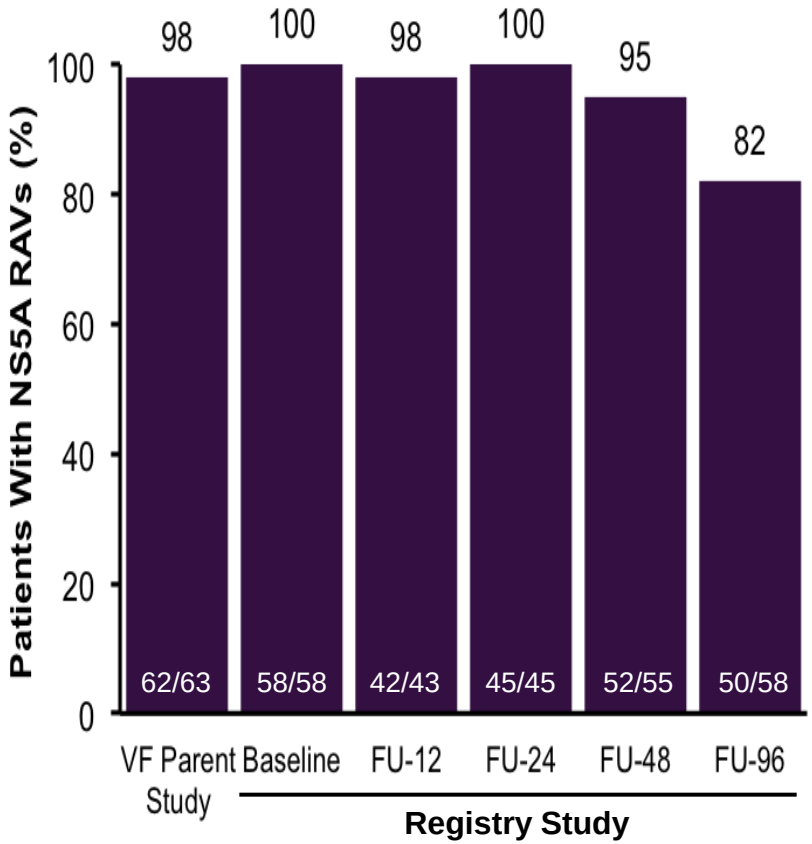
Long-Term Persistence of HCV NS5A Variants After Treatment With LDV

- NS5A RAVs in patients who failed HCV treatment with ledipasvir (LDV) in the absence SOF
  - Positions 24, 28, 30, 31, 32, 58, 93 that confer >2.5-fold reduced susceptibility to LDV *in vitro* were included



Almost all patients developed NS5A RAVs at treatment failure

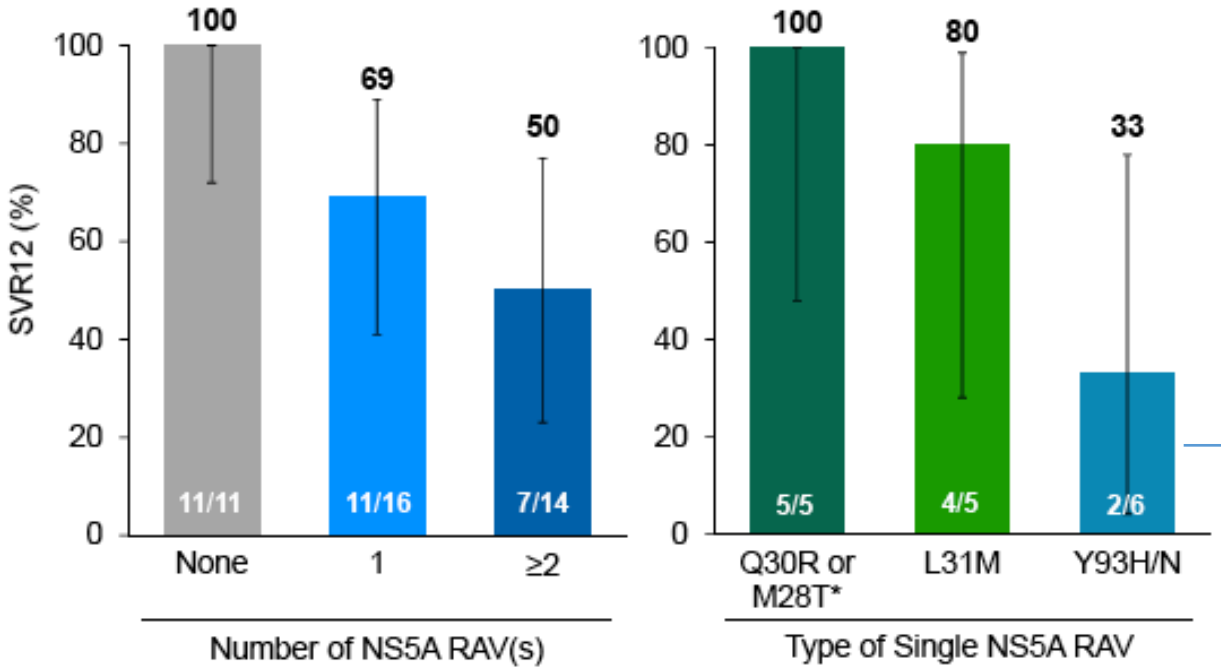
Majority of RAVs Detected After 96 Weeks (> 1% of Population)



Wyles et al. Abstract O059, EASL 2015

Retreatment of Patients Who Failed 8 or 12 Weeks of Ledipasvir/Sofosbuvir-Based Regimens With Ledipasvir/Sofosbuvir for 24 Weeks

SVR12 by baseline RAVs shows that the presence of baseline NS5A RAV(s) is associated with virological failure.

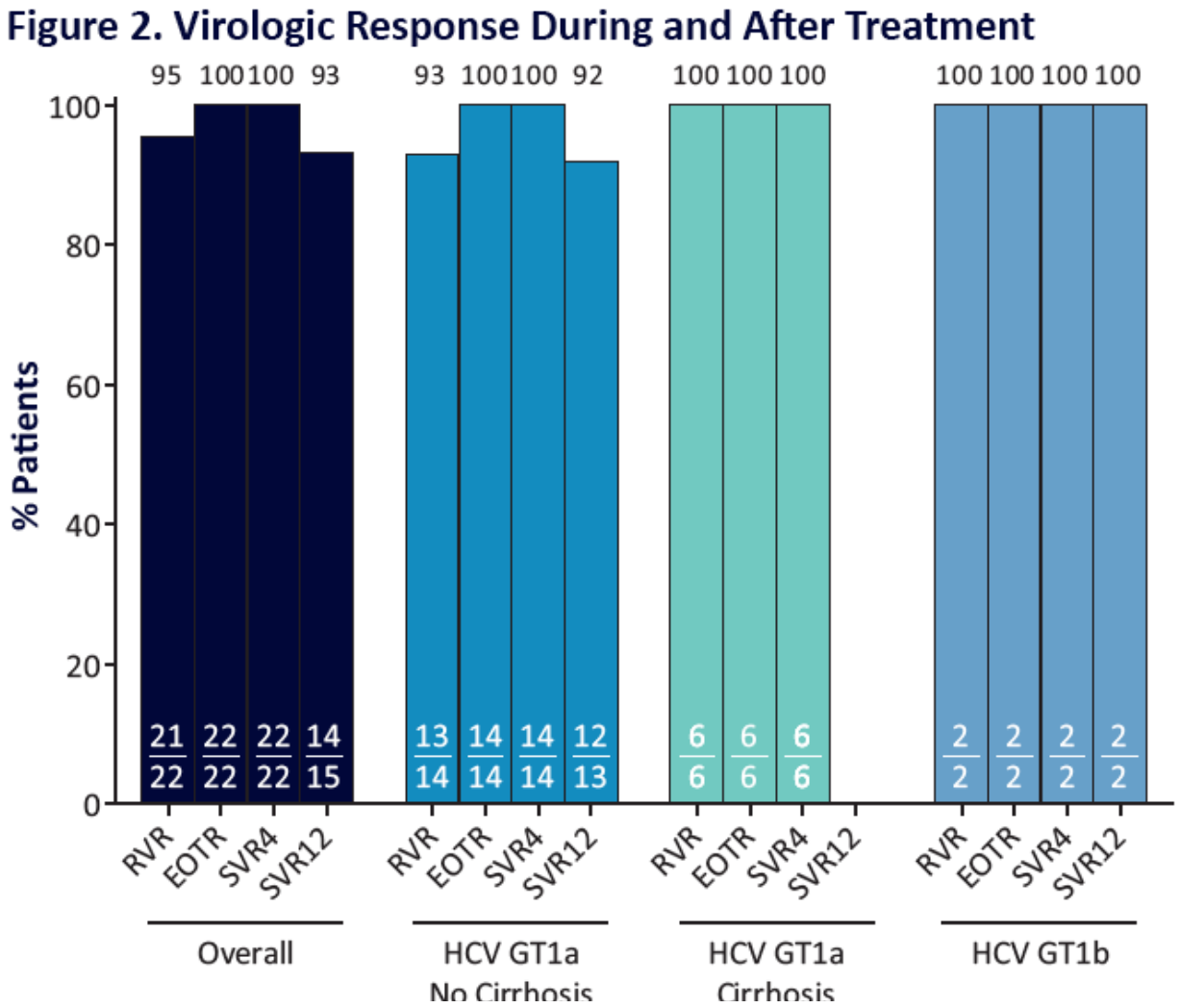


*M28T (n=1).

Prior to re-treat no NS5B RAVs (S282T, L159F, V321A)
At second virologic failure
4 of 12 (33%) patients had NS5B RAVs:
S282T (n=2)
L159F (n=1)
S282T + L159F (n=1)

Lawitz E. et al., EASL 2015
Hadas Dvory-Sobol IWDR Berlin, June 2014

QUARTZ-I: Retreatment of HCV Genotype 1 DAA-failures with Ombitasvir/Paritaprevir/r, Dasabuvir, and Sofosbuvir

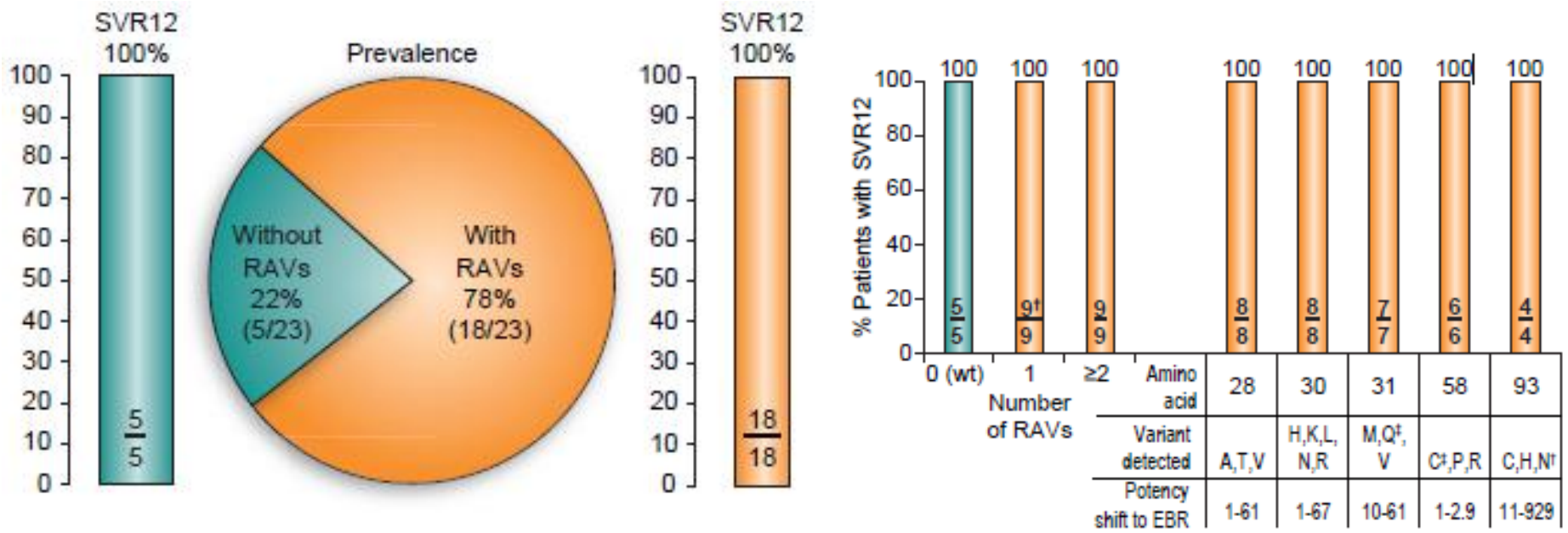


At BL, 17/22 patients had at least 1 RAV in 1 of the 3 DAA targets, with the remaining 5 had the Q80K in NS3. 7 patients had RAVs (other than NS3 Q80K) in 2 targets; 2 patients had RAVs in all 3 targets

Poordad F et al., AASLD 2015

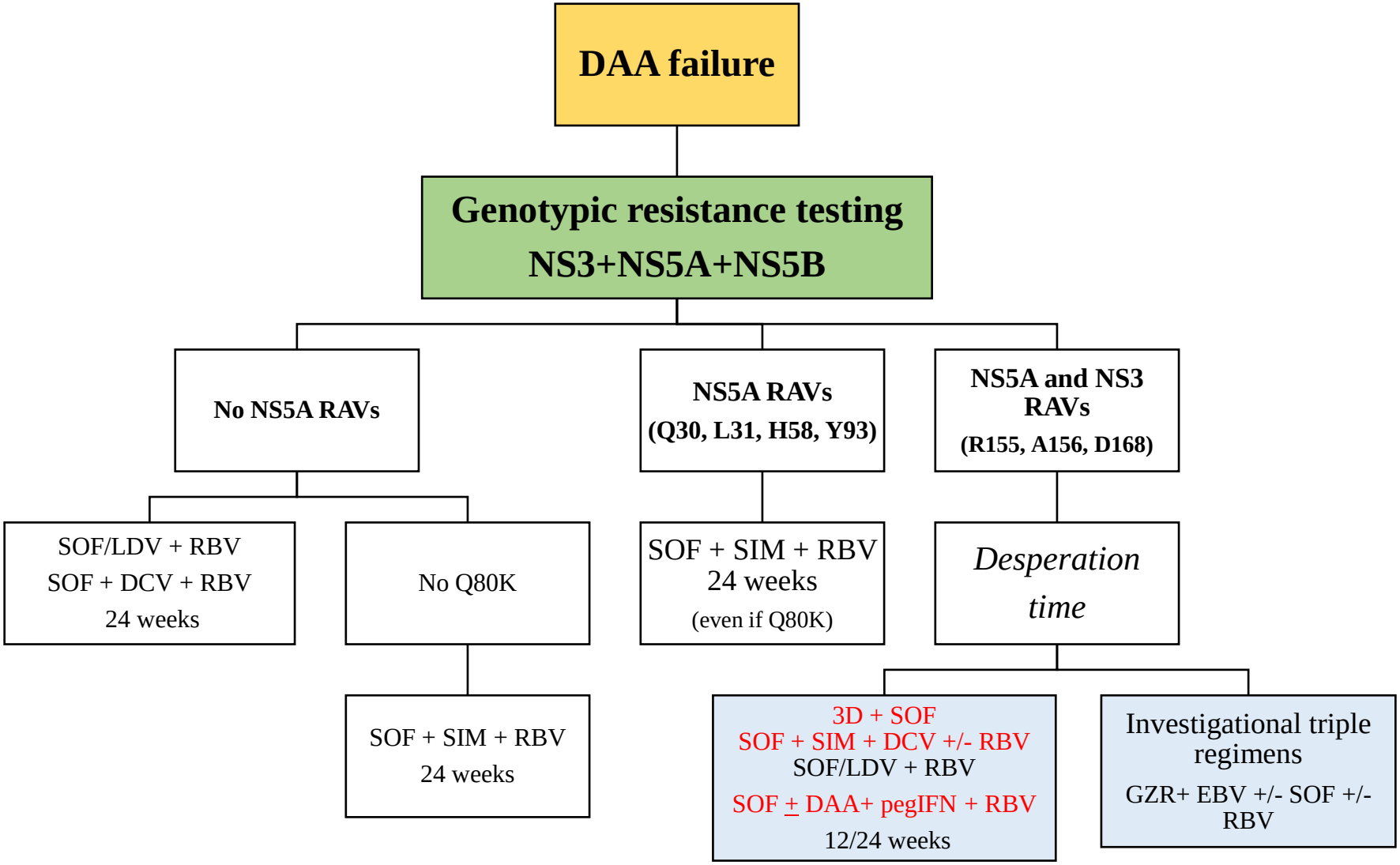
Retreatment may require «unconventional» approaches with multiple DAAs
C-SWIFT retreatment Part B

HCV GT1-infected patients who failed 4, 6, or 8 weeks of EBR/GZR + SOF in Part A were offered retreatment with EBR/GZR + SOF + RBV for 12 weeks



Lawitz E., AASLD 2015, Poster #LB-12

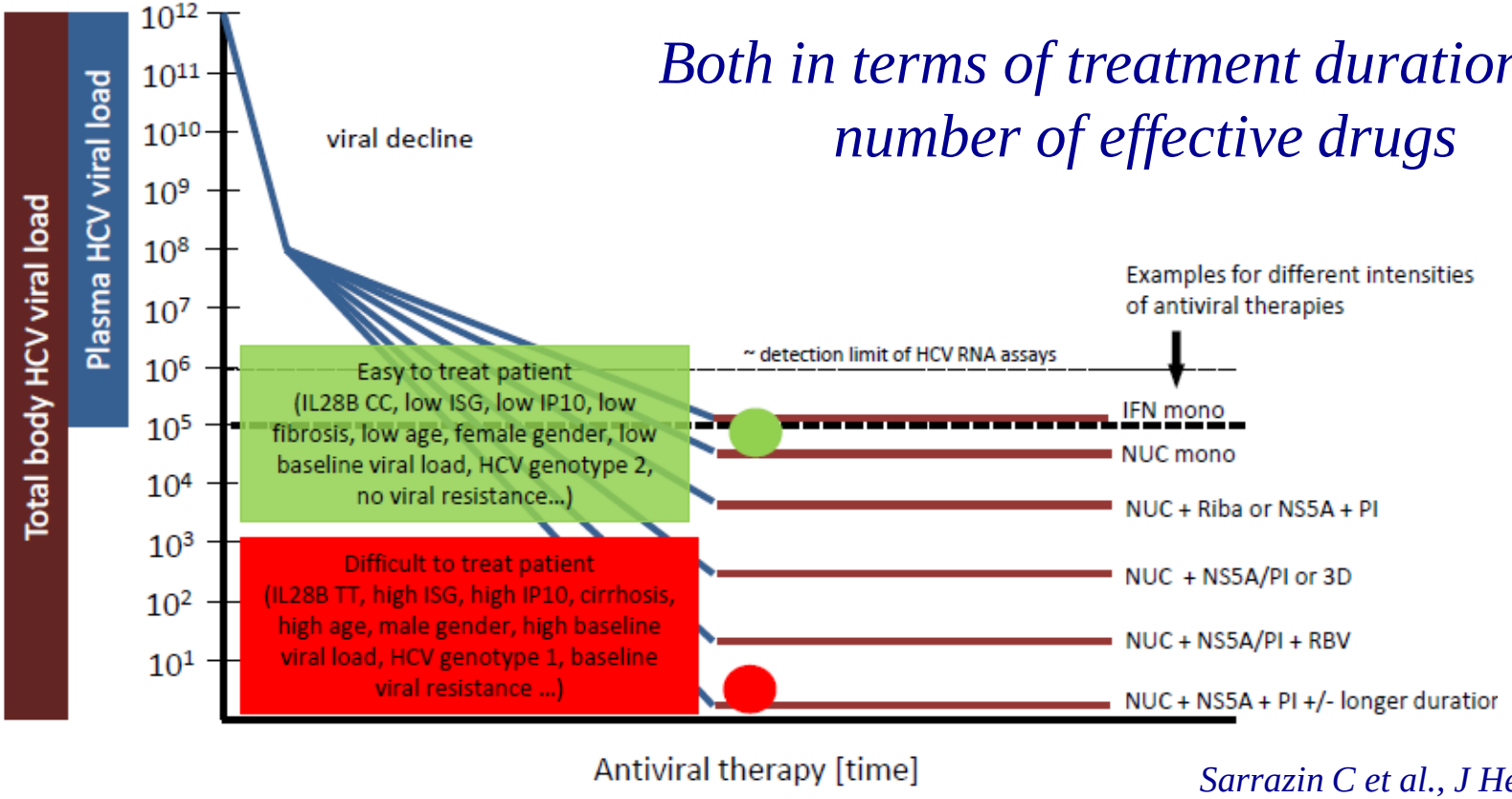
Retreatment may require «unconventional» approaches with multiple DAAs



Modified by Wyles D, AASLD 2015

Treatment should be individualized

Both in terms of treatment duration and number of effective drugs



Sarrazin C et al., J Hepatol 2015

Summary

HIV-1 is incurable to date because effective antiviral therapies target only replicating viruses and do not eradicate latently integrated viral genomes.

We can cure HCV. SVR is a validated surrogate of clinical efficacy because it predicts long-term clinical benefit.

Resistance testing is not routinely performed in HCV clinical practice, in contrast to HIV where it is recommended both prior to start of treatment and during follow-up, in order to prevent therapy failure.

Many lessons learnt from HIV can be helpful for designing adequate treatment strategies against viral hepatitis.

Strategies to avoid sequential weak or "functional" monotherapies and the emergence of viral resistance are therefore very important.

Conclusions

In the era of (expensive) new treatment options anti HCV, **the first choice is very important.**

Prior to treatment: it is mandatory to assess (repeat) HCV-genotype and GT-1 subtype with a "second generation assay".

According to the status of patient (cirrhotic, experienced to SOC/DAA, no CC, GT1a/GT3, high viral load) **baseline HCV sequencing** can provide **two important virological information:** 1) **a correct genotype/subtype assignment based on sequence analysis** often incomplete, or even wrong, with old/other diagnostic methods; 2) **detection of variants that are potential non responders to therapy,** by natural resistance or previous failure resistance.

Although GRT at baseline is not yet recommended (exception: NS3-Q80K, soon NS5A-test for elbasvir), it is indeed currently considered (helpful to store a sample).

At failure: the resistance test should be performed in all 3 genes NS3 + NS5A + NS5B. Patients that fail are few, but critical in term of cost and therapeutic outcome. Resistance test at failure is becoming more and more mandatory for re-treatment strategies.

Thanks for your attention