10TH RESIDENTIAL COURSE ON CLINICAL PHARMACOLOGY OF ANTIRETROVIRALS

HCV/HBV:
SURFING THE WAVE

Virological variables of anti-HCV/HBV therapies

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Università degli Studi di Roma "Tor Vergata"

Cattedra di Virologia

Torino 21-23 Gennaio 2015

Many lessons learnt from HIV can be helpful for designing adequate treatment strategies against viral hepatitis?……such as HCV and HBV….?

HCV discovery: one of the most significant biomedical breakthroughs in the last 25 years

SCIENCE, VOL. 244 21 APRIL 1989

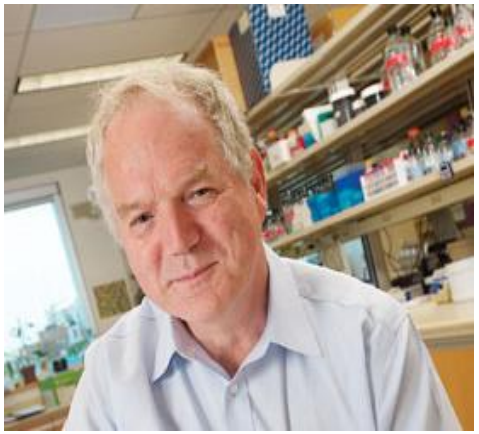
Isolation of a cDNA Clone Derived from a Blood-Borne Non-A, Non-B Viral Hepatitis Genome

QUI-LIM CHOO, GEORGE KUO, AMY J. WEINER, LACY R. OVERBY, DANIEL W. BRADLEY, MICHAEL HOUGHTON

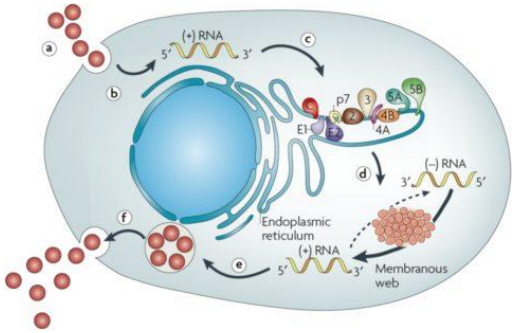
21 APRIL 1989 SCIENCE, VOL. 244

An Assay for Circulating Antibodies to a Major Etiologic Virus of Human Non-A, Non-B Hepatitis

G. KUO, Q.-L. CHOO, H. J. ALTER, G. L. GITNICK, A. G. REDEKER, R. H. PURCELL, T. MIYAMURA, J. L. DIENSTAG, M. J. ALTER, C. E. STEVENS, G. E. TEGTMEIER, F. BONINO, M. COLOMBO, W.-S. LEE, C. KUO, K. BERGER, J. R. SHUSTER, L. R. OVERBY, D. W. BRADLEY, M. HOUGHTON



Michael Houghton



Replication of Subgenomic Hepatitis C Virus RNAs in a Hepatoma Cell Line

V. Lohmann,[1] F. Körner,[1] J.-O. Koch,[1] U. Herian,[1] L. Theilmann,[2] R. Bartenschlager[1]*

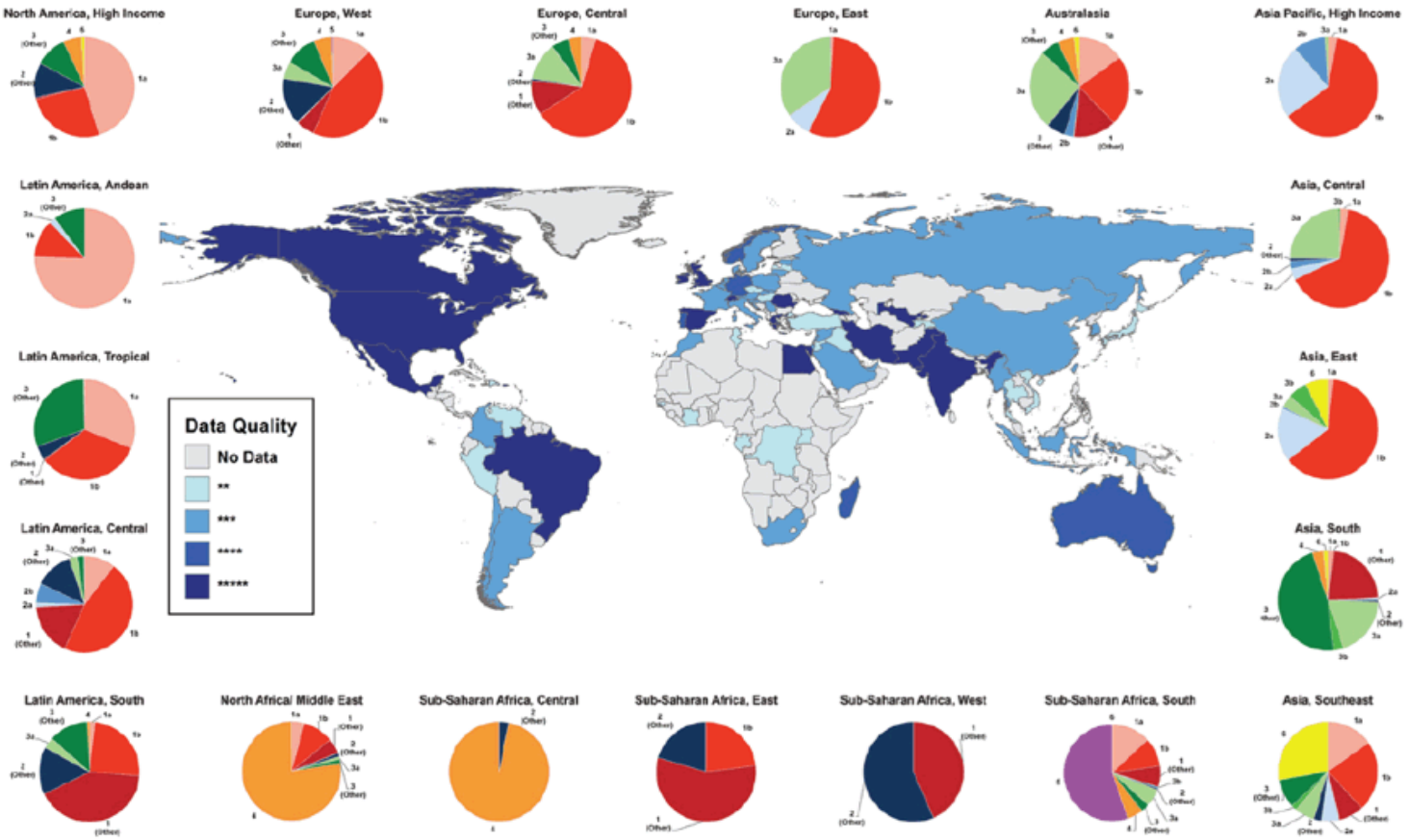
Science, July 1999;285:110-113

This discovery has facilitated the development of effective diagnostics, blood screening tests and the elucidation of promising drug and vaccine targets to control this global pathogen and save the lives of millions of people around the world….

Hepatitis C is one of the most pressing health emergencies worldwide

The global prevalence of HCV infection has been estimated at 2-3%, which equates to 130-170 million people

>350,000 mortality cases each year for HCV chronic disease related

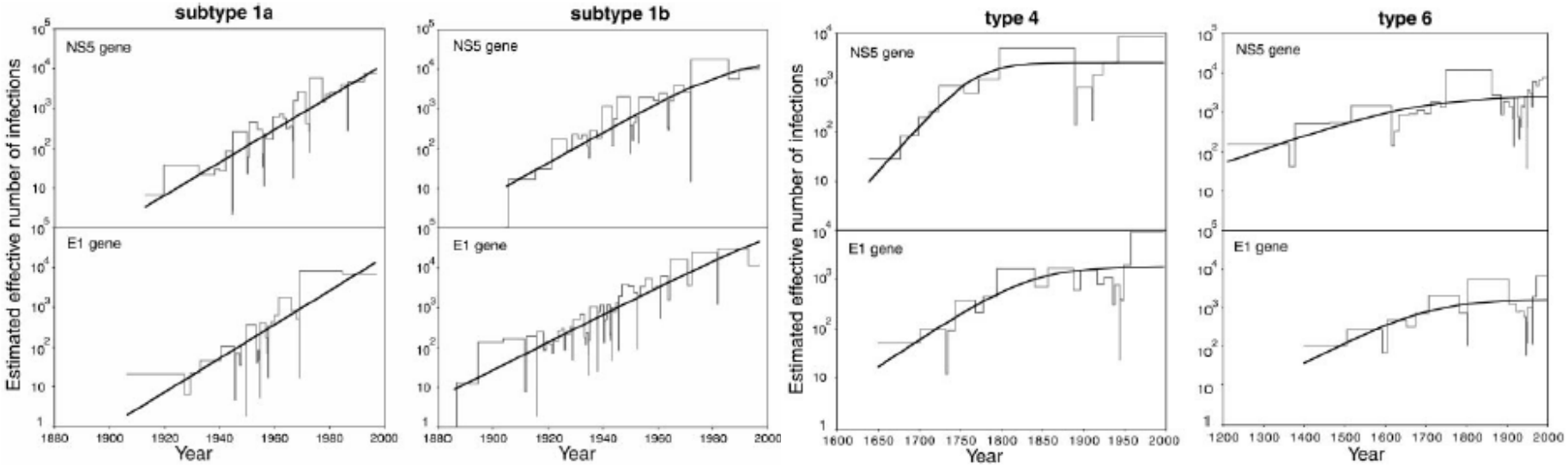


Messina JP, Hepatology 2014

The origin of the primate Flaviviridae could be as ancient as the differentiation of primate species some 35 million years ago

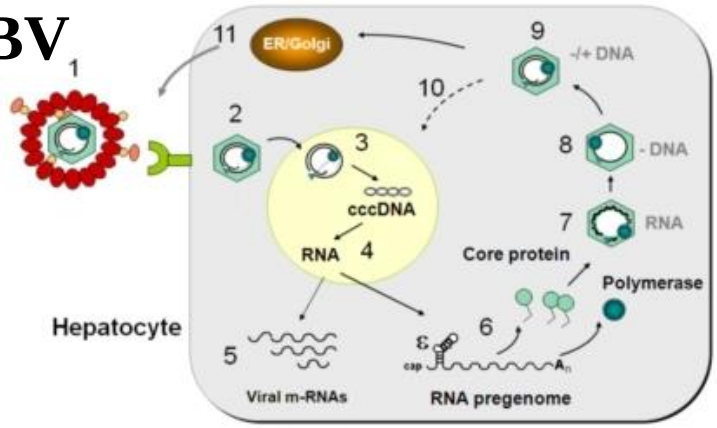
HCV could have been coevolving with human populations during their migration out of Africa within the past 100,000 to 150,000 years, **but the current HCV genotypes appeared much more recently**.

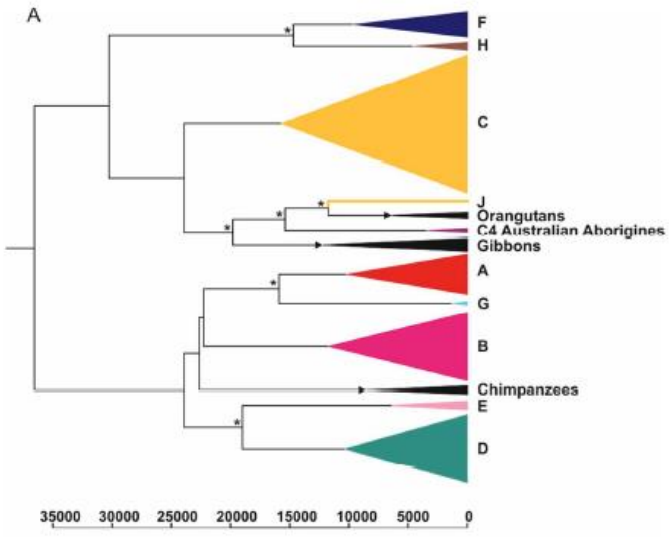
A study suggested that types 6 and 4 could have originated 700 years and 350 years ago, respectively, whereas **subtypes 1a and 1b could have arisen less than 100 years ago.**



Pybus et al Science 2001

HBV





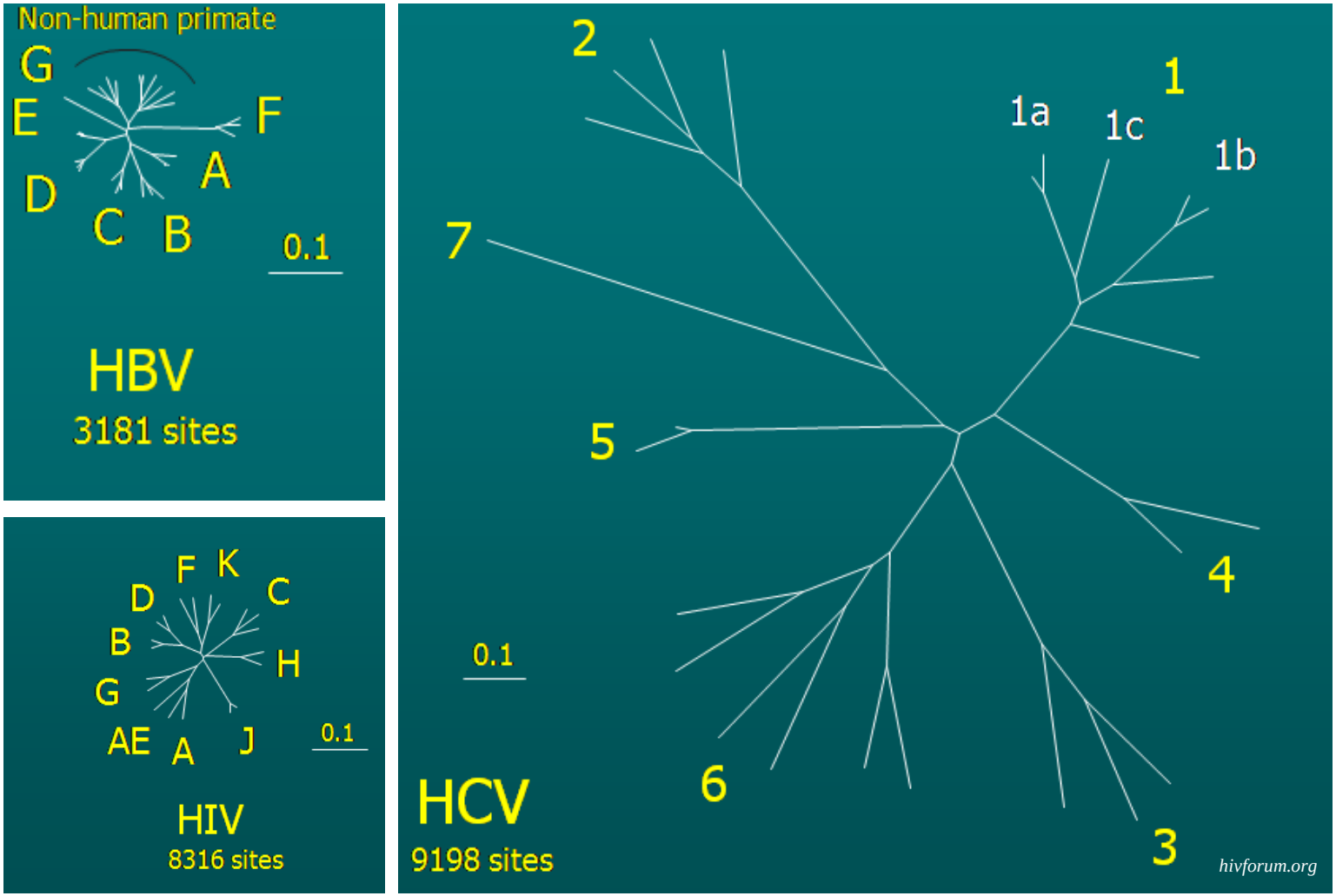
Hepatitis B is also a major global public health concern with approximately 2 billion individuals infected with HBV and with more than 350 million chronic carriers.

Calibrating the HBV molecular clock using the divergence times of different indigenous human populations based on archaeological and genetic evidence, HBV jumped into humans around 33,600 years ago. This coincides with the origin of modern non-African humans.

Crucially, the most pronounced increase in the HBV pandemic correlates with the global population increase over the last 5,000 years.

Paraskevis D et al Hepatology. 2012

Consequences of HCV variability at population level: HCV genotypes



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

HBV and HCV share several biological similarities

	HIV	HBV	HCV
Virus			
daily production of virions per day	10^{10}	10^{12}–10^{13}	10^{12}
half-life of free virions (h)	1	3–24	2–3
half-life of intracellular virions	days (dependent on infected cells $t_{1/2}$)	months (dependent on infected cells $t_{1/2}$)	hours (not dependent on infected cells $t_{1/2}$)
mutation rate	very high	high	very high
constraints due to ORFs in targeted viral enzymes	moderate	high	none
immune-mediated escape mutants	frequent	infrequent	frequent
Target cells			
half-life of infected cells	days	months	weeks
size of susceptible cells compartment	large	small	probably large
intracellular viral reservoir	yes (integrated cDNA)	yes (cccDNA)	no

ORFs, open reading frames; cDNA, complementary DNA; cccDNA, covalently closed circular DNA.

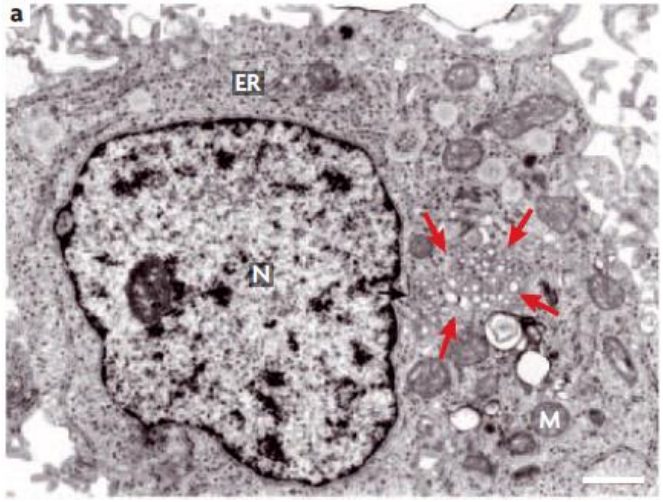
Soriano et al., JAC 2008

HBV and HCV share several biological similarities, but …

	HIV	HBV	HCV

Differently from HIV and HBV:

- HCV replication occurs only in cytoplasm
- Viral genome is not archived into the genome of infected cells



Moradpour D et al., Nature 2007

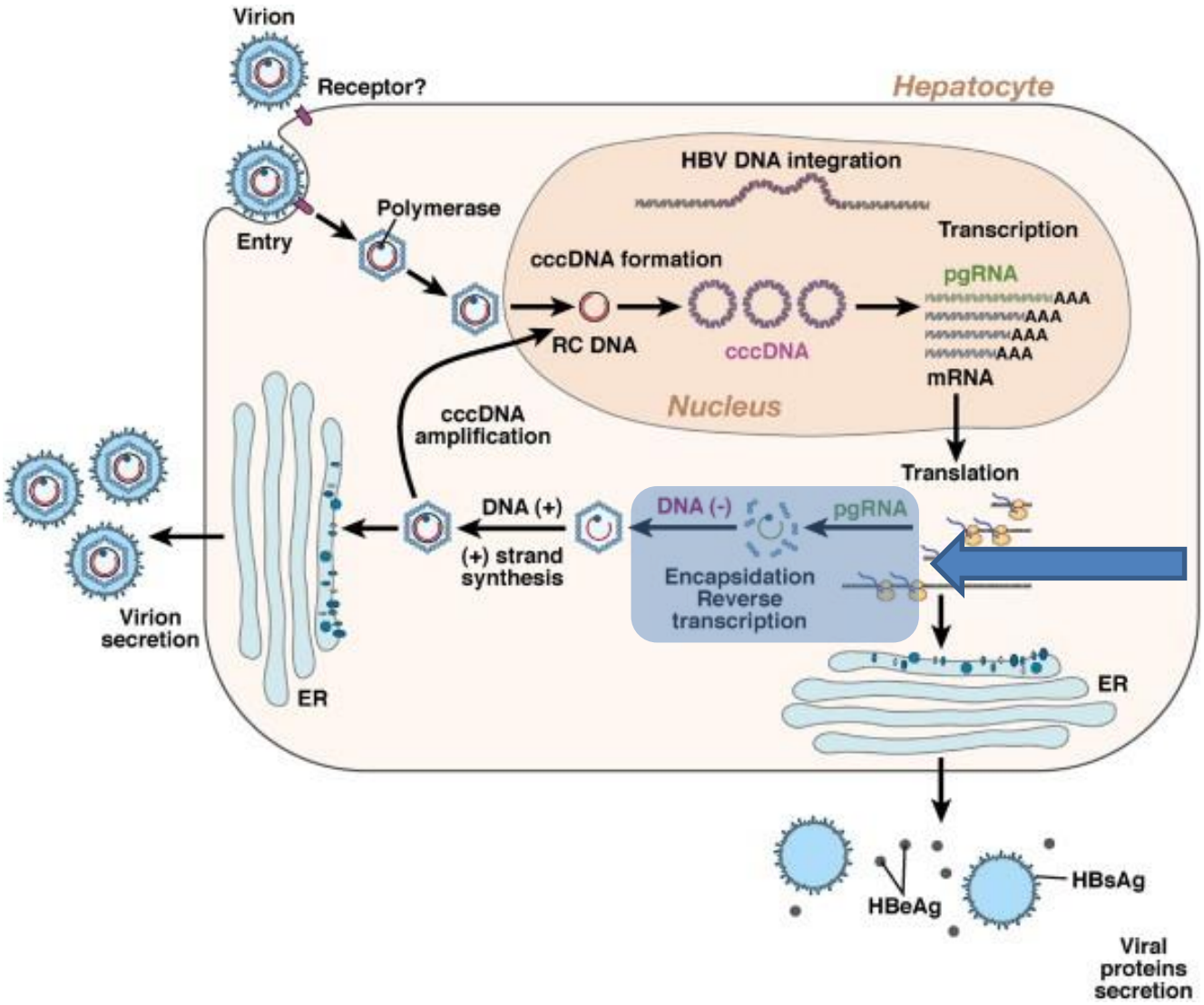
… This makes HCV curable!!!!

• Thus, the goal of the current HCV treatment is not the suppression of HCV replication and viremia but the eradication of infection.

• This is so far possible to a larger number of people thanks to the next introduction of new anti-HCV drugs.

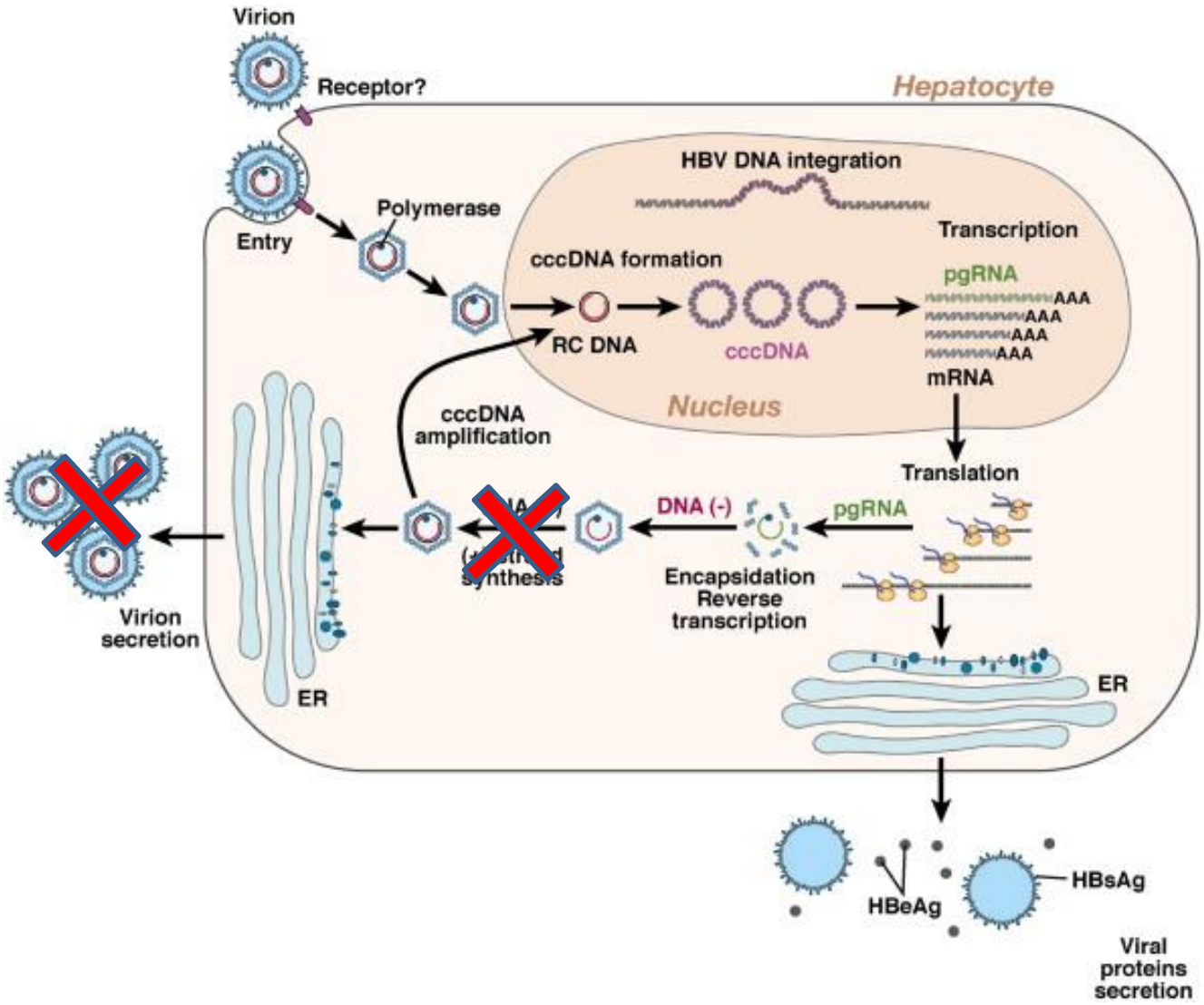
HIV-1 and HBV have been incurable to date because effective antiviral therapies target only replicating viruses and do not eradicate latently integrated or non replicating episomal viral genomes….

HBV Reverse Transcriptase acts during the late stages of HBV life cycle, downstream cccDNA production



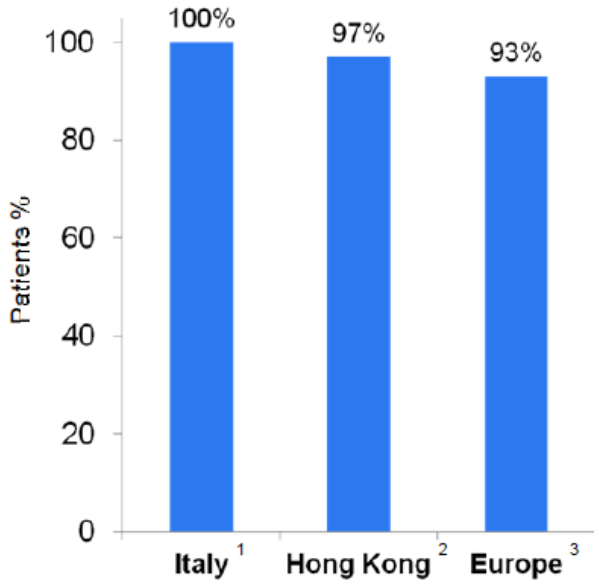
Reverse Transcription occurs during late stages of HBV life cycle

The current NUCs can efficiently inhibit HBV Reverse Transcriptase activity thus suppressing the production of viral particles



Current antiviral drugs for the treatment of chronic hepatitis B are highly potent

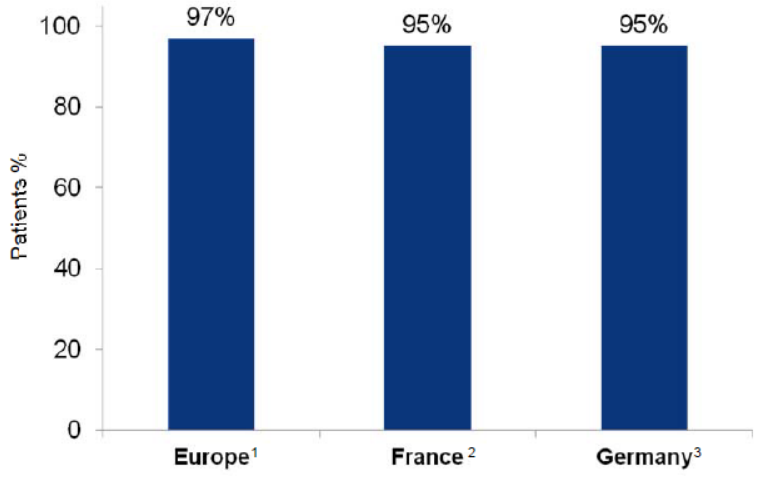
Virological response to Entecavir



Follow up (yrs)	5	5	3
N° of patients	418	222	333

1. Lampertico, et al. EASL 2013; 2. Seto, et al. J Gastroenterol Hepatol 2014; 3 Zoutendijk, et al. Hepatology 2011

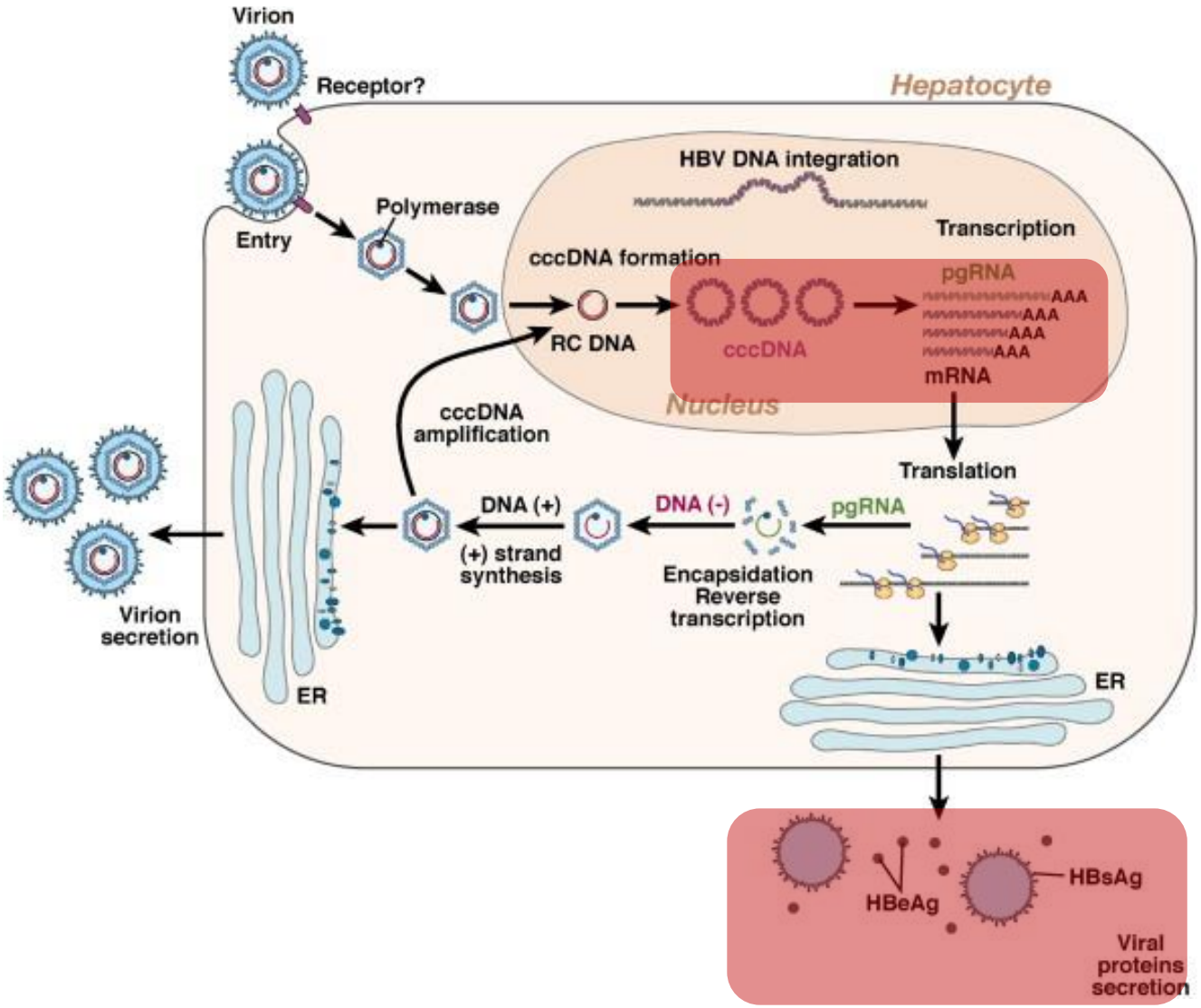
Virological response to Tenofovir



	4	3	3
	374	440	400

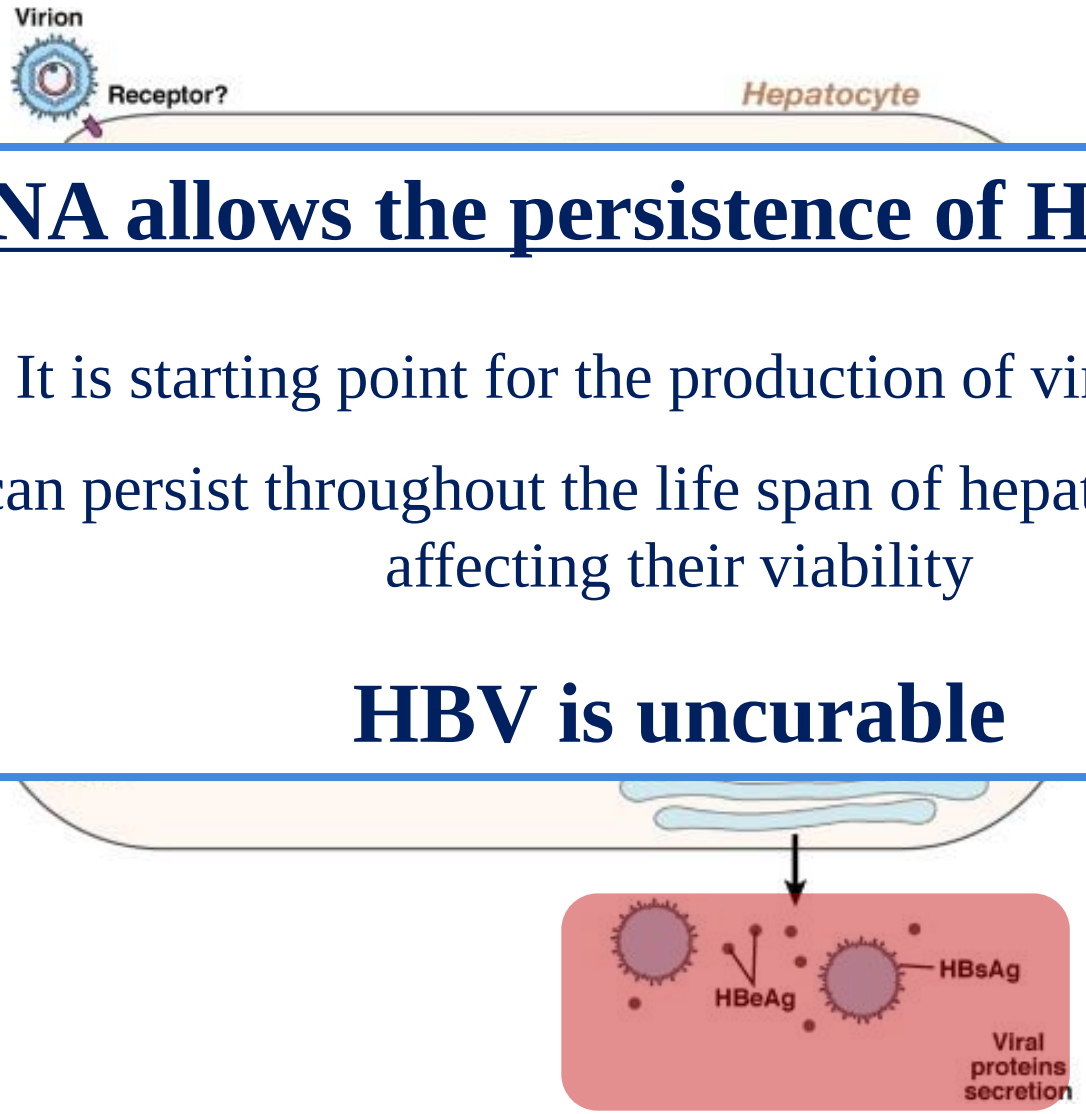
1.Lampertico, et al. AISF 2014; 2.Pageaux, et al EASL 2014; 3.Petersen, et al EASL 2014

The currently available anti-HBV drugs cannot efficiently affect the burden of cccDNA and its metabolic activity



Production of viral proteins despite virological suppression

The currently available anti-HBV drugs cannot efficiently affect the burden of cccDNA and its metabolic activity



cccDNA allows the persistence of HBV infection

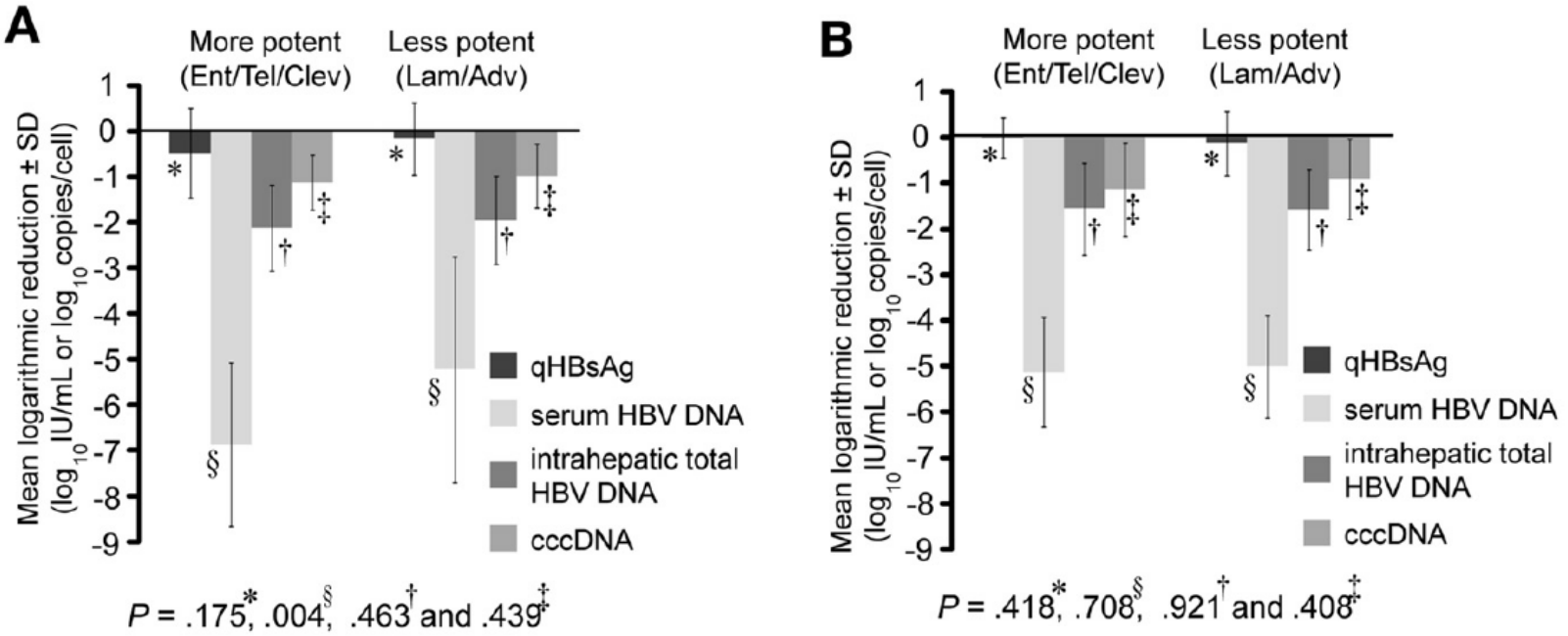
- It is starting point for the production of viral particles
- It can persist throughout the life span of hepatocytes without affecting their viability

HBV is uncurable

Reduction of Hepatitis B Surface Antigen and Covalently Closed Circular DNA by Nucleos(t)ide Analogues of Different Potency

CLINICAL GASTROENTEROLOGY AND HEPATOLOGY 2013;11:1004–1010

DANNY KA-HO WONG,*,‡ WAI-KAY SETO,* JAMES FUNG,*,‡ PHILIP IP,§ FUNG-YU HUANG,* CHING-LUNG LAI,*,‡ and MAN-FUNG YUEN*,‡



HBeAg positive **HBeAg positive**

- After **1 year of NA therapy**, there was a **4–7 log_{10} IU/mL reduction in serum HBV DNA**, whereas the **overall reduction of qHBsAg and cccDNA was relatively small** (implying a time period for cccDNA clearance of at least 14 years).
- Serum HBV DNA was undetectable in 75% (88/117) of patients, HBsAg and cccDNA was undetectable in 0% and 4.3% of patients, respectively.

The persistence of cccDNA and its metabolic activity have **potential implications in hepatocarcinogenesis….**

The persistence of cccDNA creates conditions for **HBV reactivation** during immunosuppression also in anti-HBs positive, anti-HBc positive patients, and thus **in patients with resolved (but not cured!!) infection.**

There are 2 types of HBV cure:

Biological Cure

- It implies the complete eradication of each cccDNA molecules by inducing the killing of each HBV infected cell

Functional Cure

- Functional cure
  - off-therapy persistent HBV suppression in the absence of liver damage

Which is the best surrogate marker of functional HBV cure?

Achieving the state of inactive carrier

HBsAg positivity
Serum HBV DNA <2000IU/ml
Persistently normal transaminases

Achieving the state of latent (occult) HBV infection

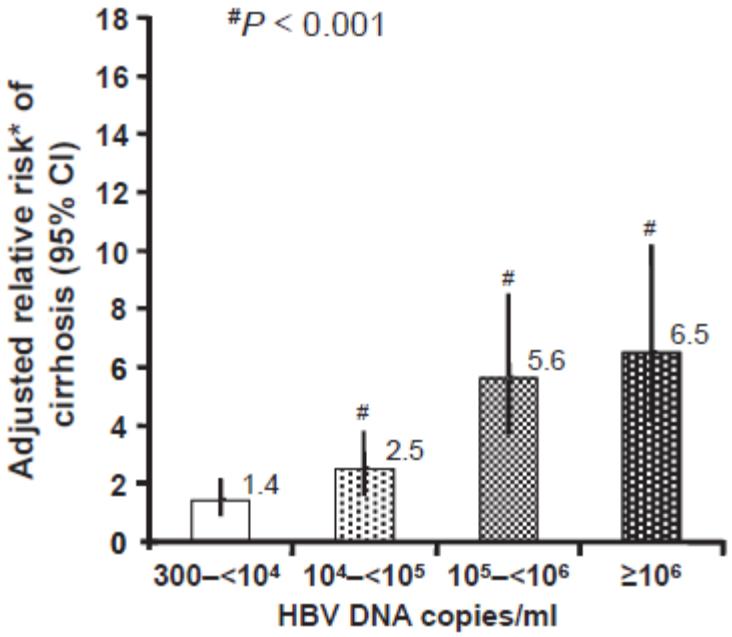
HBsAg negativity (as a marker of dormant cccDNA)
Undetectable serum HBV DNA
Persistently normal transaminases

The functional HBV cure implies the silencing of cccDNA but not the silencing of integrated HBV genome……

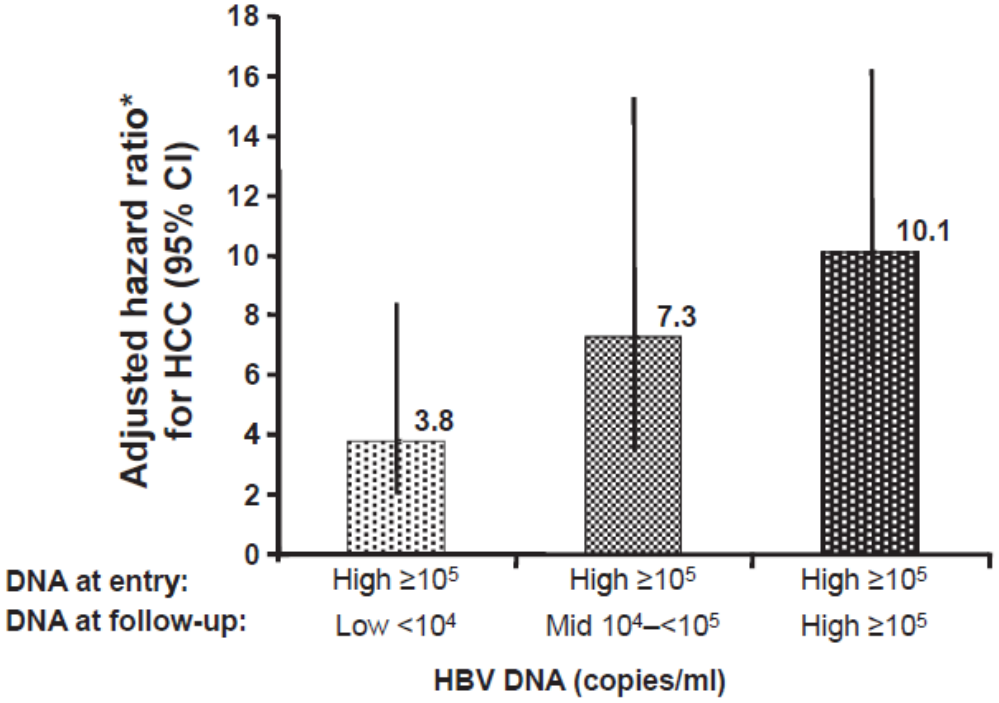
…. This implies continuing HCC monitoring

Chronic elevation of viral replication correlates with a higher risk of cirrhosis and HCC

Risk of cirrhosis
according to HBV DNA level

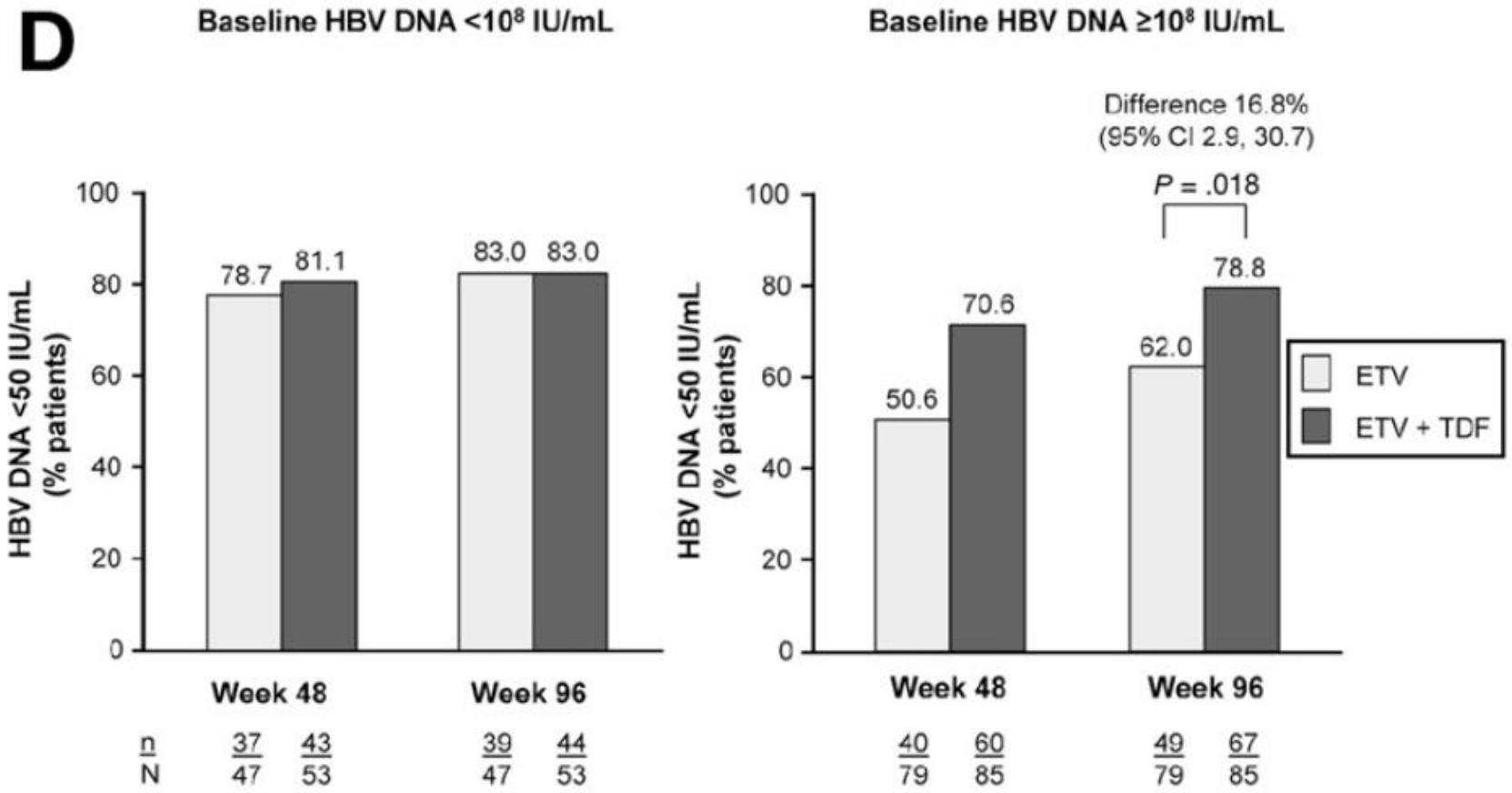


Risk of HCC
according to HBV DNA level



From Iloeje et al., Liver International 2012

In the setting of high serum HBV DNA at baseline (>10^8IU/ml), combination therapy correlates with a high rate of virological success (HBV DNA < 50 IU/ml) at week 48 and 96 of treatment

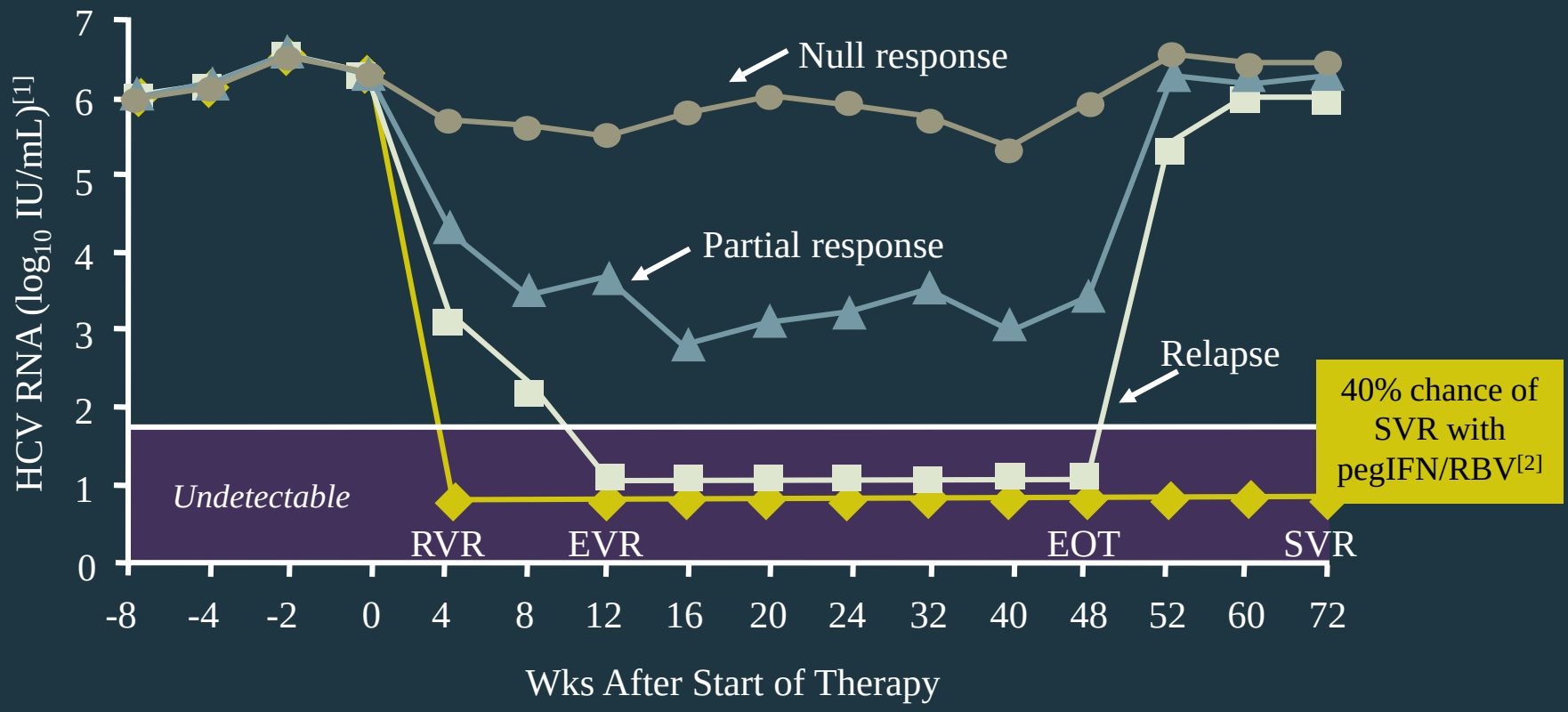


Lok et al., Gastroenterology 2012

The importance of viral load….

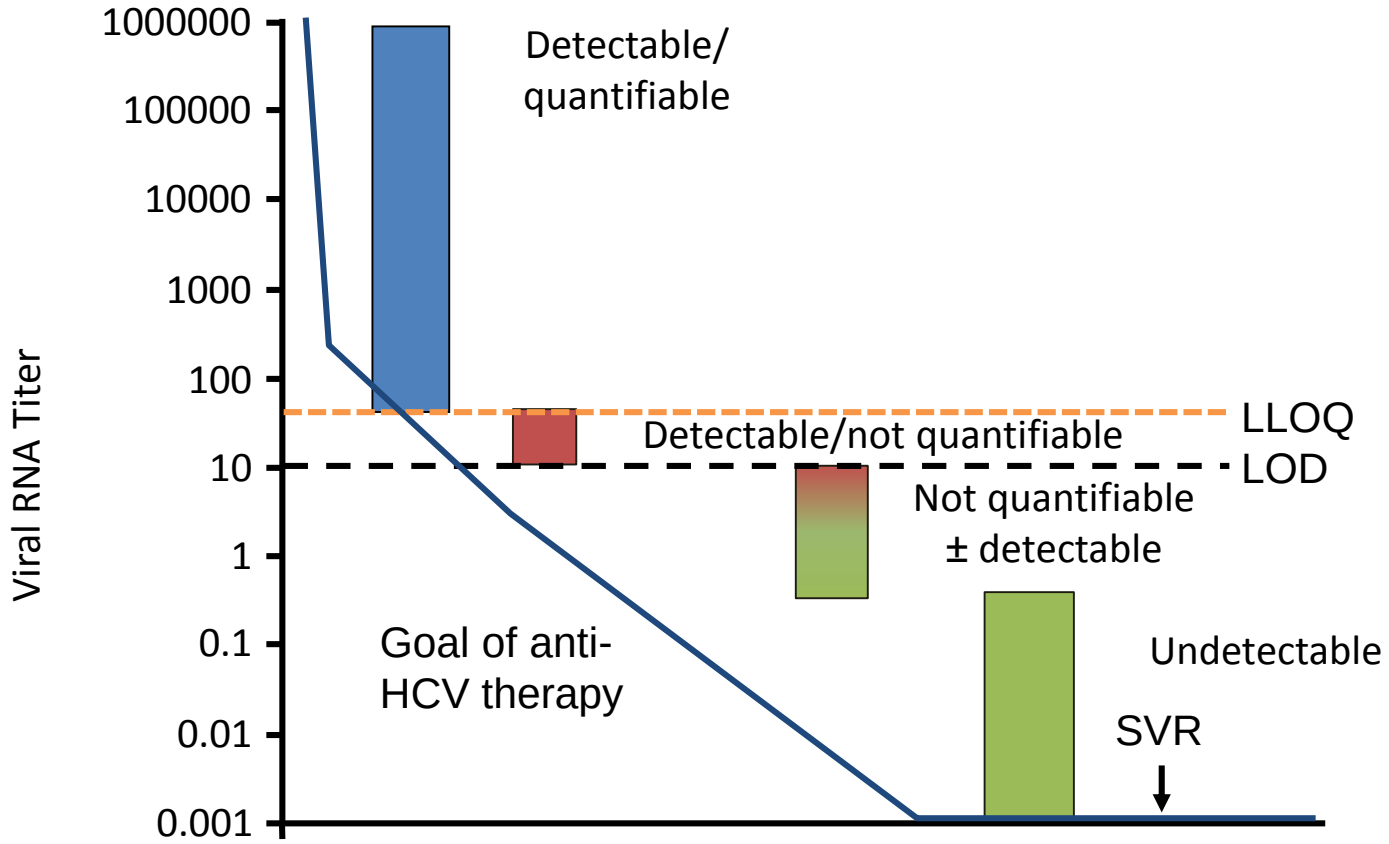
The primary goal of the current HCV treatment is not the suppression of HCV replication and viremia, but the eradication of infection

PATTERNS OF VIROLOGIC RESPONSE



1. Ghany MG, et al. Hepatology. 2009;49:1335-1374.
2. McHutchison JG, et al. N Engl J Med. 2009;361:580-593.

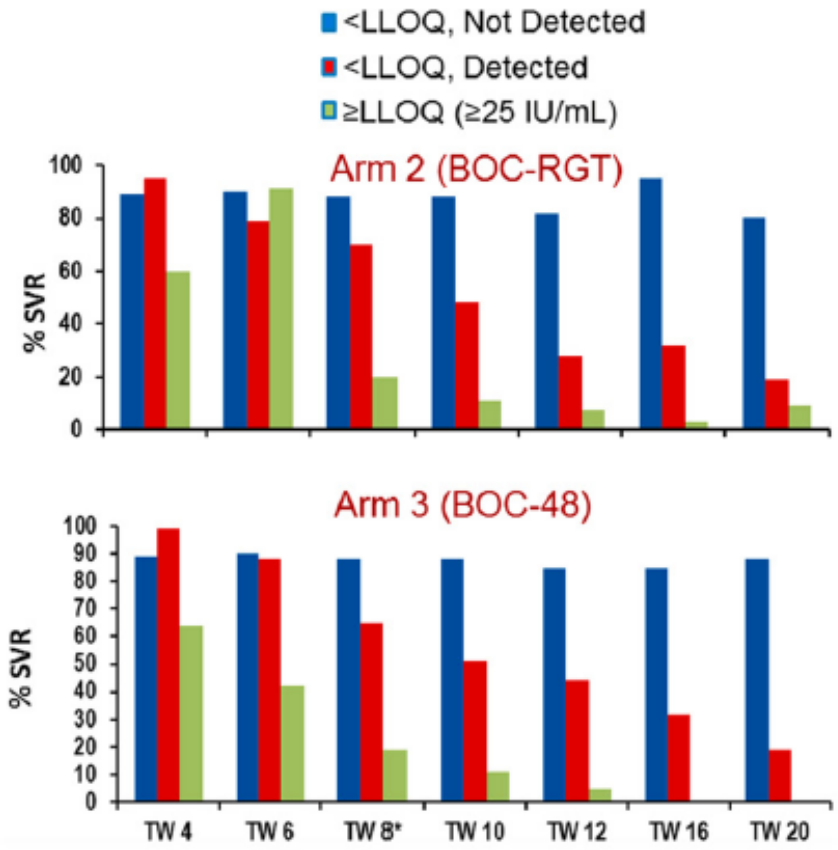
It is strongly recommended, during treatment, the use of highly sensitive assays for HCV-RNA quantification which will allow to distinguish between HCV-RNA values under the limit of detection (undetectable viremia) or under the limit on quantification

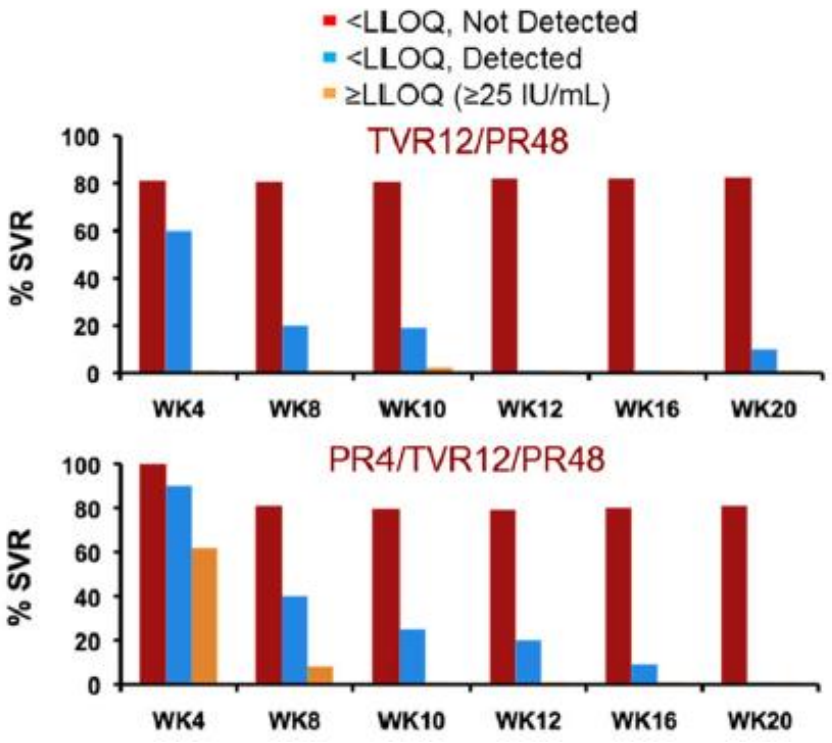


Vierling J.M, Clin Liv Dis 2013

Differences in test interpretation are clinically relevant

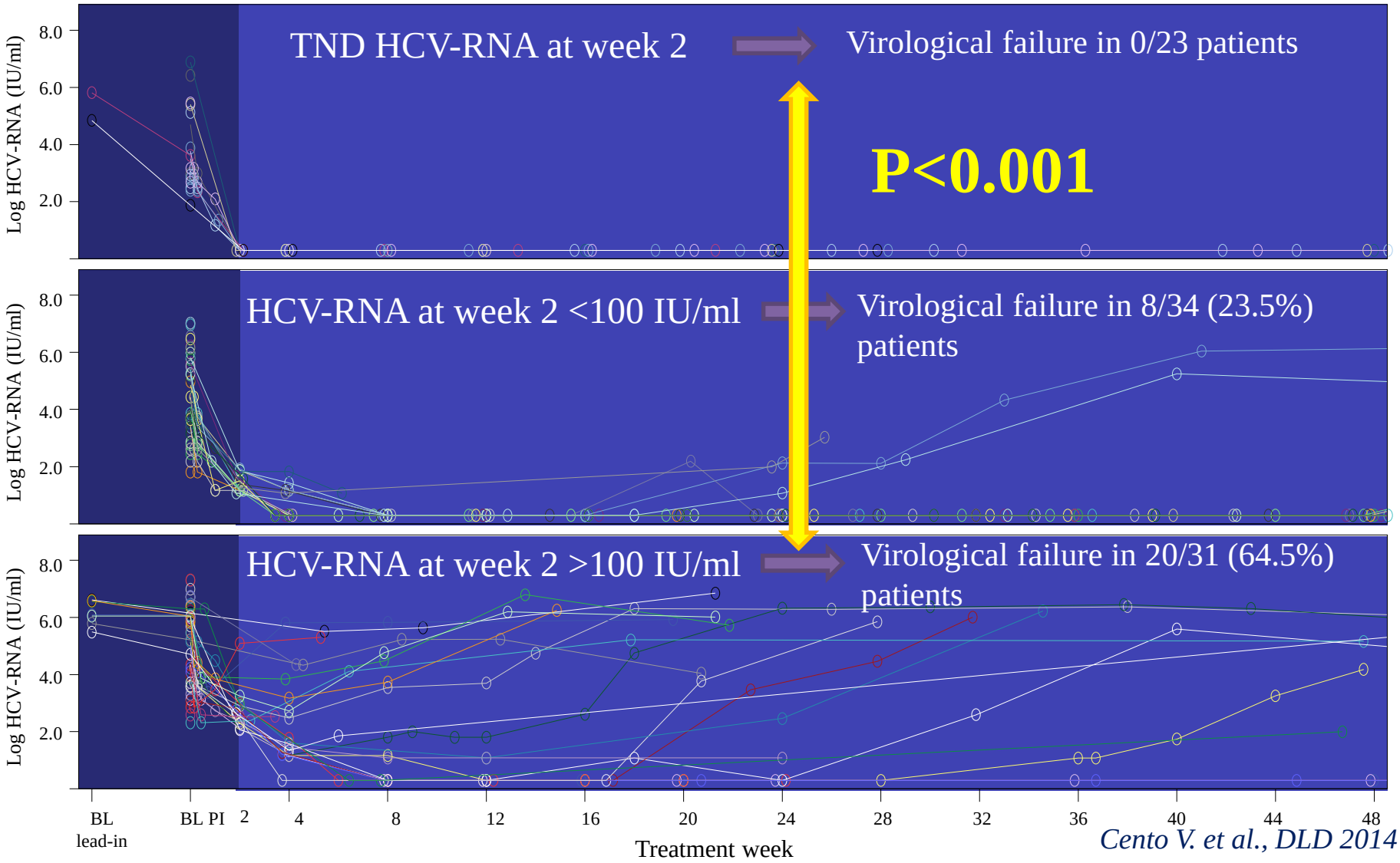
Patients with HCV RNA detected (not quantifiable) on-treatment had a reduced SVR rate vs patients with undetectable HCV RNA, at the same time points





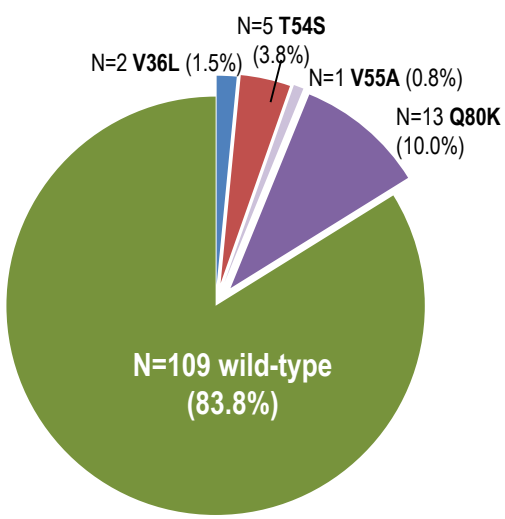
Vierling J.M, Clin Liv Dis 2013

2 weeks HCV-RNA values >100 IU/ml during triple therapy (BOC/TVR+Peg-IFN+Ribavirin) are associated with virological failure



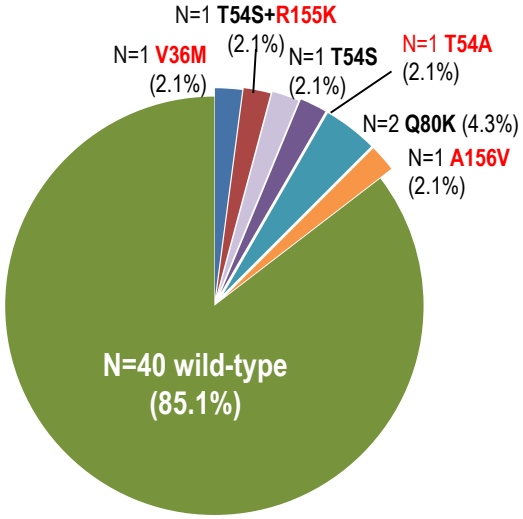
Cento V. et al., DLD 2014

Slow HCV-RNA decay and early resistance predict the risk of failure to TVR/BOC treatment



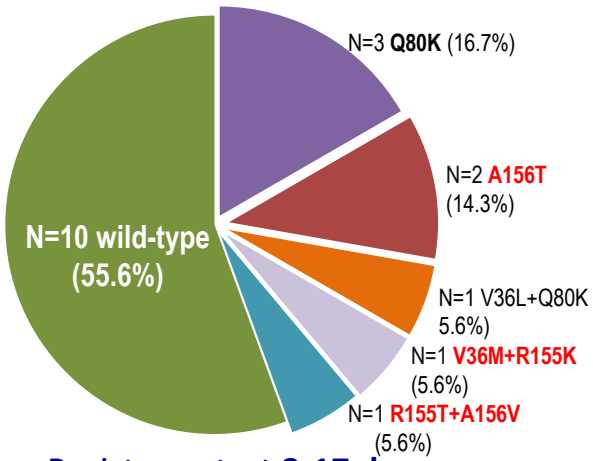
Baseline resistance test
(N=130, TVR & BOC)

Baseline RAVs in 21 patients (16.2%)



Resistance test **48h**
(N=47, TVR & BOC)

48h new RAVs in 4 patients (8.5%)



Resistance test **3-17 days**
(N=18, TVR & BOC)

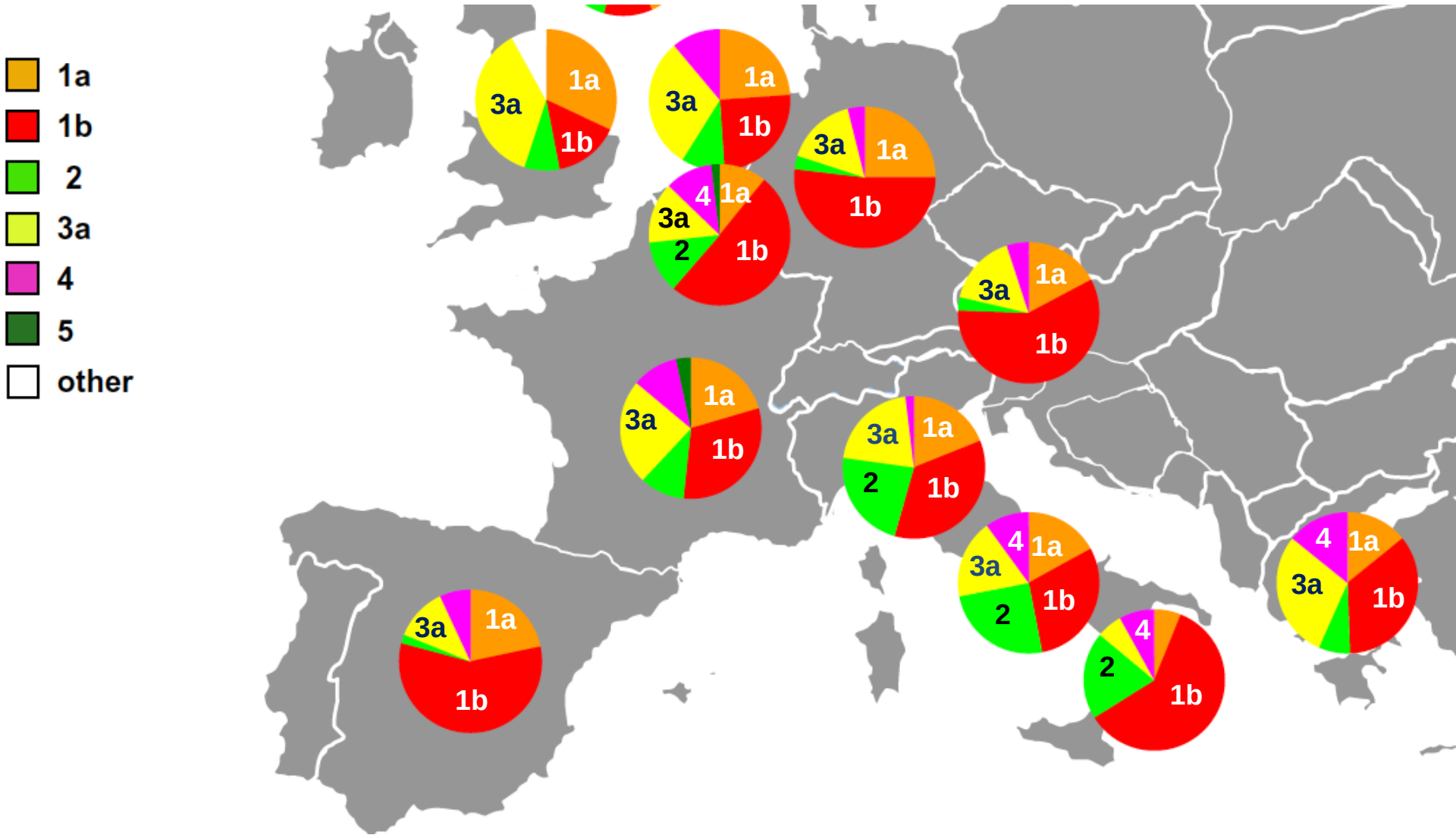
3-17 days new RAVs in 4 patients (22.2%)

Characteristics	Crude OR	95% C.I.		P-value	Adjusted OR	95% C.I.		P-value
		Lower	Upper			Lower	Upper	
HCV-RNA >100 IU/ml at week 2	**12.667**	**3.308**	**48.504**	**<0.001**	**27.955**	**1.885**	**414.500**	**0.015**
At least one baseline/early resistance mutation	3.300	1.070	10.179	0.038	2.460	0.335	18.068	0.376
HCV genotype	0.541	0.220	1.329	0.180	0.820	0.130	5.166	0.832
Cirrhosis	1.616	0.670	3.895	0.285	0.383	0.052	2.820	0.346
Unfavourable IL-28b genotype (TT or CT)	6.097	0.705	52.754	0.101	3.532	0.175	71.470	0.411
Previous null responder to SOC	1.895	0.685	5.239	0.218	0.299	0.019	4.705	0.390

OR, odds ratio; CI, confidence interval; SOC, standard of care

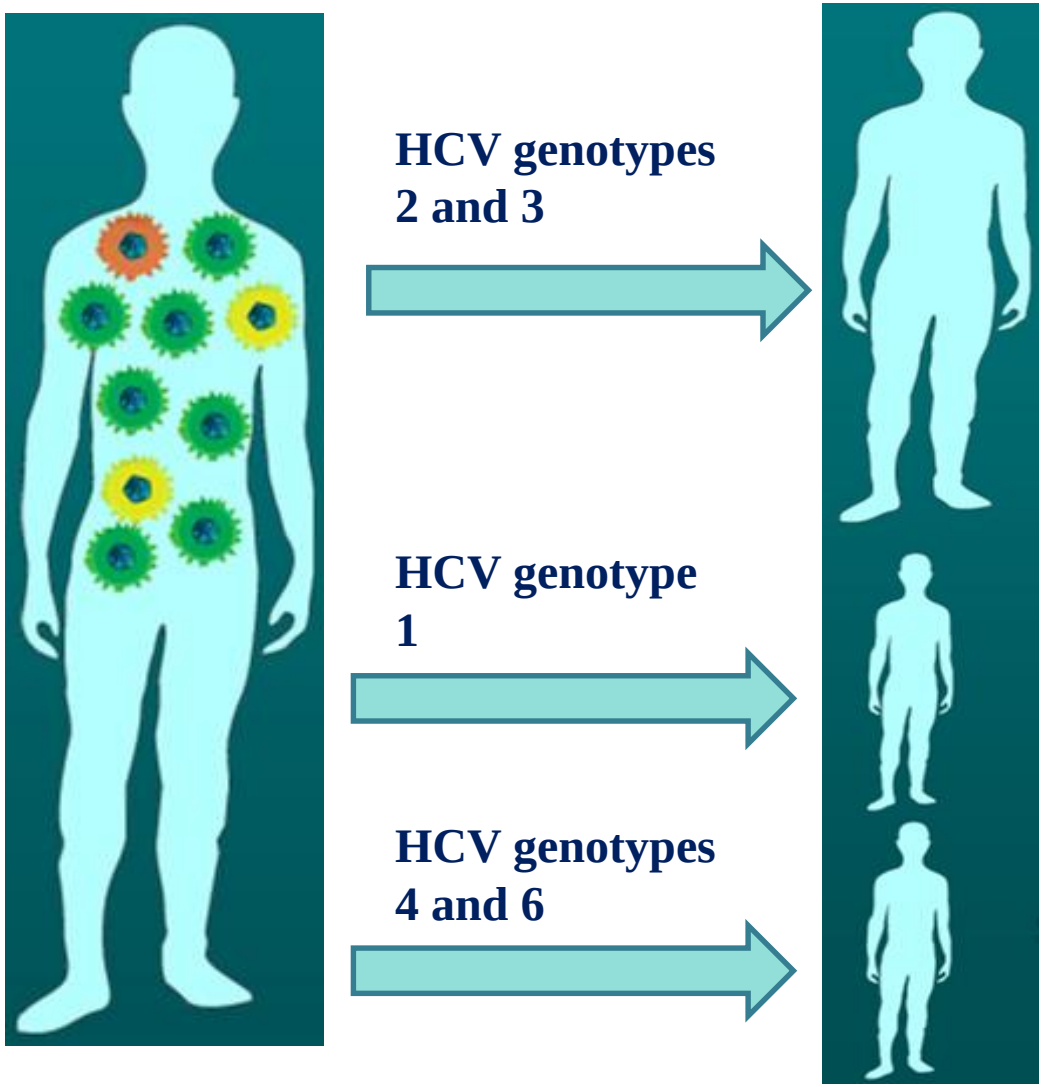
Cento V. et al., EASL 2014 Ceccherini-Silberstein et al CROI 2014

Genotype 1 is by far the most frequent genotype in chronically infected patients worldwide as well as in Europe



Esteban JI et al J Hepatol 2008;48:148-162

HCV genotype was the most important baseline predictor for response to Peg-IFN + Ribavirin Combination Therapy



HCV genotypes 2 and 3

SVR = 78-86 %

HCV-2= 80-95%

HCV-3 Low viremia = 75-80%

HCV-3 High viremia = 60-70%

HCV genotype 1

SVR = 35-65 %

HCV-1 Low viremia = 50%

HCV-1 High viremia = 30-35%

HCV genotypes 4 and 6

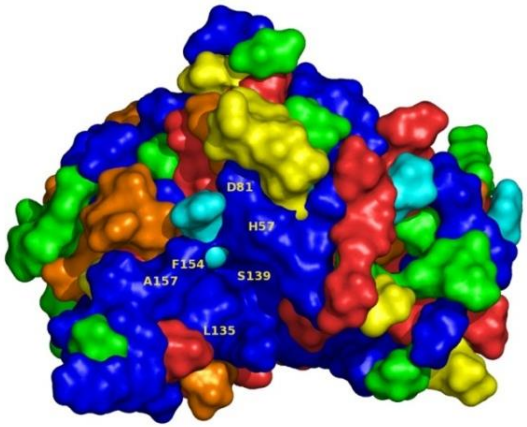
SVR = 42-52 %

What about today or tomorrow in the era of new DAAs?

Still genotype important predictor for response?

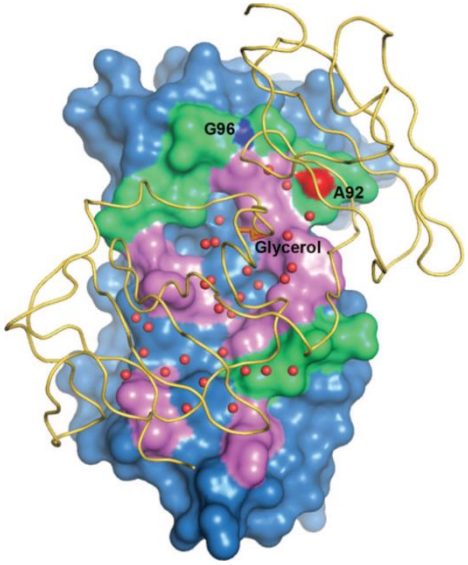
Today only few DAAs are pangenotypic…….

(Grazoprevir)



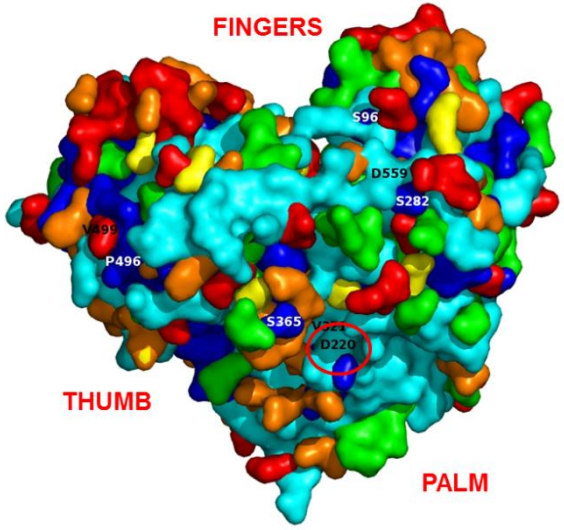
47% amino acids of HCV PROTEASE NS3 are conserved among all HCV-genotypes

Daclatasvir



46% amino acids of HCV NS5A are conserved among all HCV-genotypes

Sofosbusvir



55% amino acids of HCV POLYMERASE NS5B are conserved among all HCV-genotypes

Amino acid variability:

0% | ≤1% | 1-5% | 5-10% | 10-25% | >25%

Cento et al., PLoS ONE 2012

Love et al., J Vir 2009

Amino acid variability:

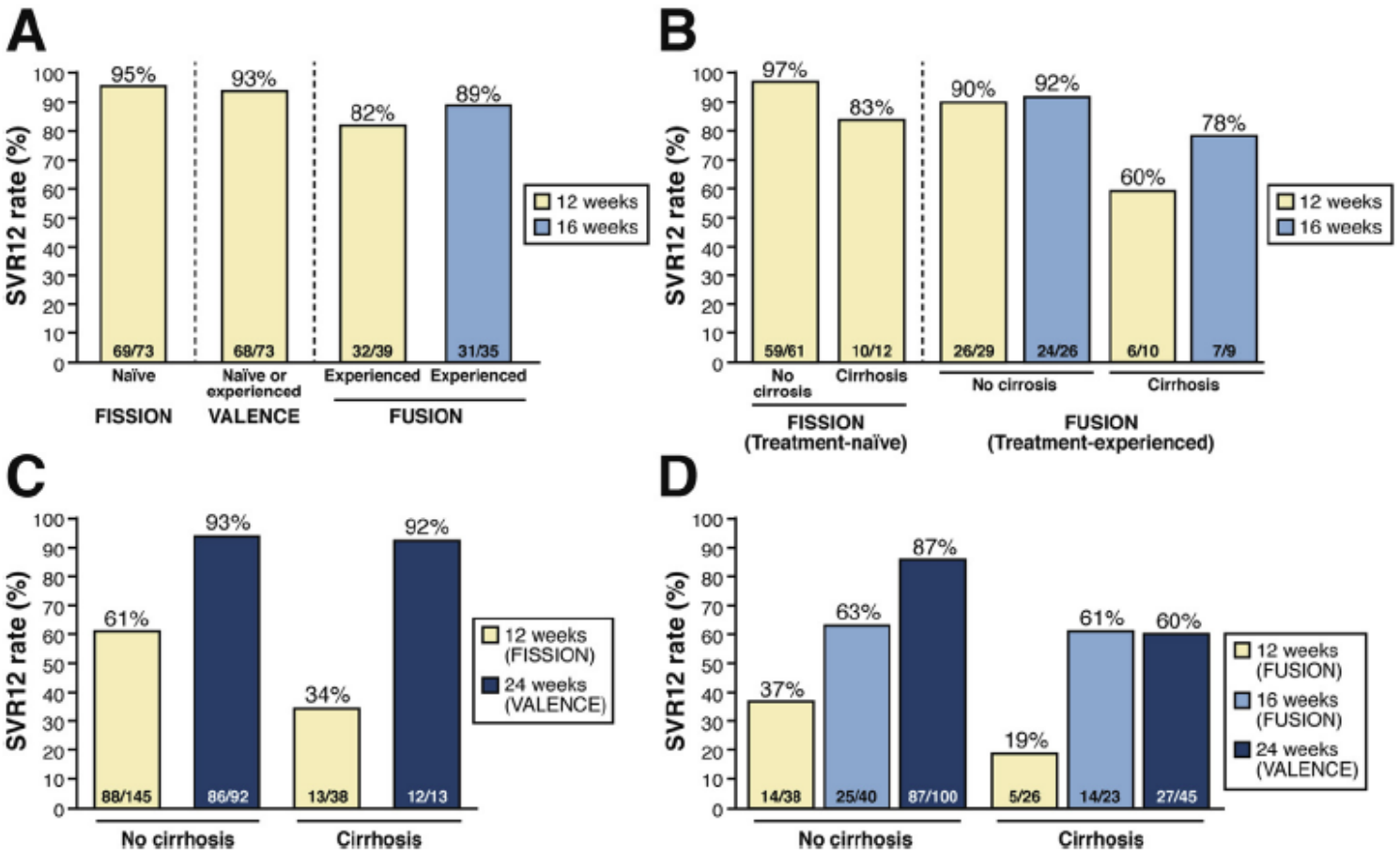
0% | ≤1% | 1-5% | 5-10% | 10-25% | >25%

Di Maio et al., AAC 2014

Genotype 3 HCV-infected patients had poor SVR rates following treatment for 12 or 16 weeks with Sofosbuvir+RBV

HCV-2

HCV-3

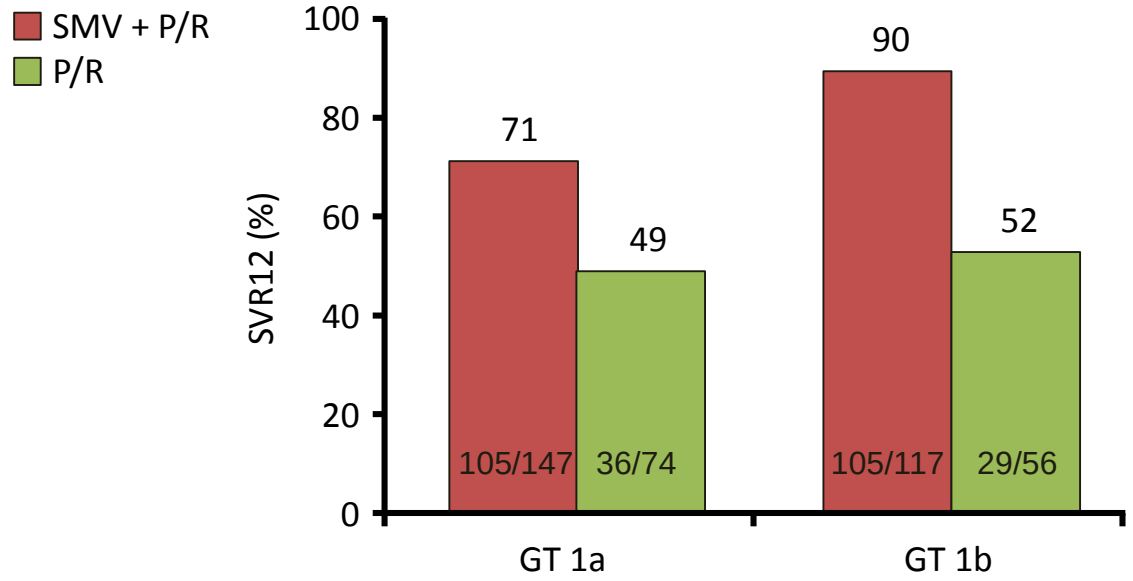


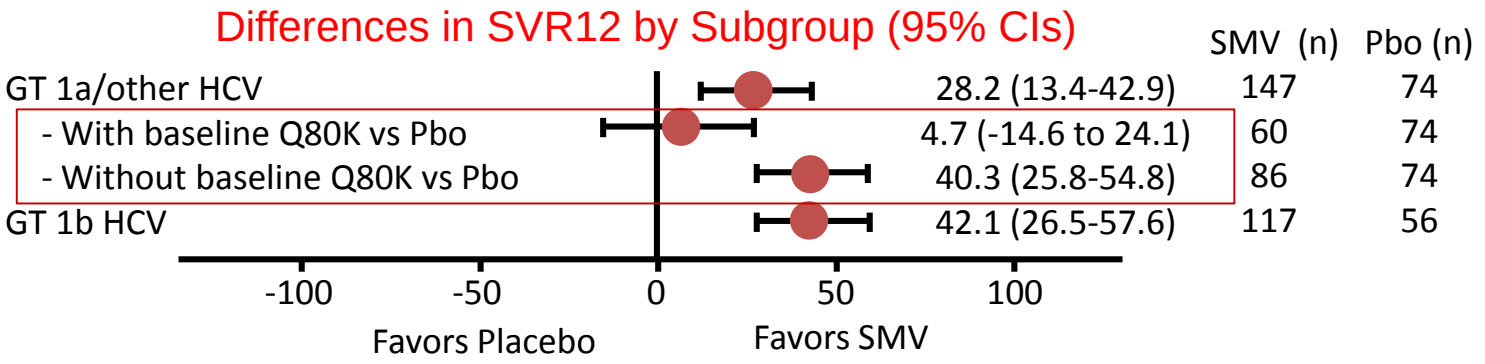
Rates of SVR12 in the FISSION, FUSION, and VALENCE phase III trials. Patients infected with HCV genotypes 2 or 3 received sofosbuvir (400 mg, once daily) plus weight-based ribavirin.60,70,71 These results were generated in different studies; although the inclusion and exclusion criteria were similar across the 3 studies, the different groups cannot be compared as if patients had been assigned randomly to groups in a single study. (A) Rates of SVR12 among treatment-naive and treatmentexperienced patients infected with HCV genotype 2, treated for 12 or 16 weeks in the FISSION, VALENCE, and FUSION trials. (B) Rates of SVR12 among treatment-naive and treatment-experienced patients infected with HCV genotype 2 according to fibrosis stage (cirrhosis vs no cirrhosis) and treatment duration (12 or 16 weeks) in the FISSION and FUSION trials. (C) Rates of SVR12 in treatment-naive patients infected with HCV genotype 3 according to fibrosis stage (cirrhosis vs no cirrhosis) and treatment duration (12 or 24 weeks) in the FISSION and VALENCE trials. (D) Rates of SVR12 in treatment-experienced patients infected with HCV genotype 3 according to the fibrosis stage (cirrhosis vs no cirrhosis) and treatment duration (12, 16, or 24 weeks) in the FUSION and VALENCE trials

Pawlotsky Gastroenterology 2014

Virologic failure during simeprevir treatment was more common in patients with genotype 1a with Q80K

No differences between 1b and 1a without Q80K





Jacobson I, et al. EASL 2013

Different Sustained Virologic Responses at Post-Treatment Week 12 according to duration of therapy, subtype, previous status of treatment……

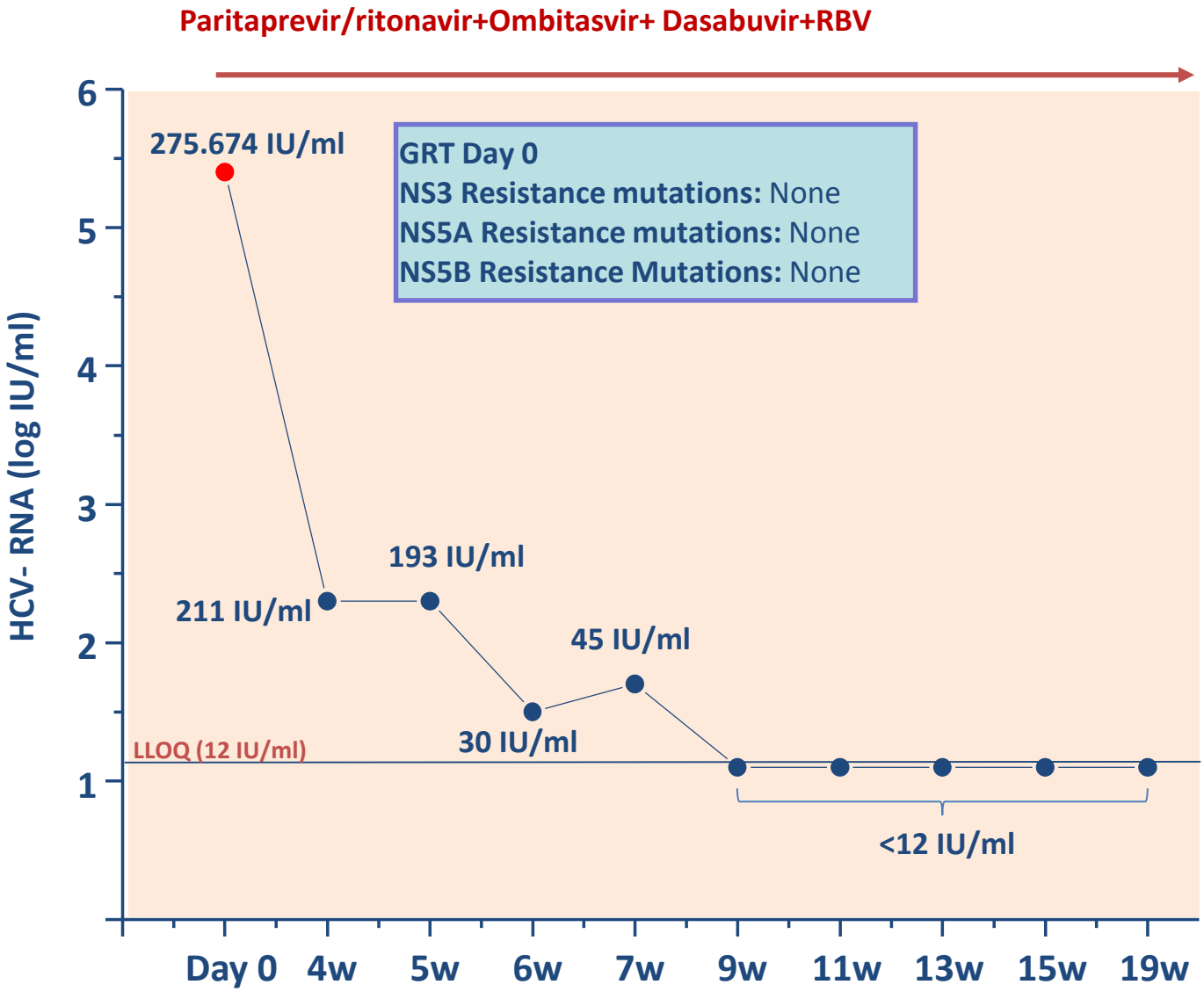
Table 2. Sustained Virologic Response at Post-Treatment Week 12 in Each Treatment Group, According to HCV Subgenotype and Status with Respect to Prior Treatment.*

Variable	12-Wk Group (N=208)	24-Wk Group (N=172)
	no./total no. (%)	
HCV genotype 1a infection		
No prior treatment	59/64 (92.2)	52/56 (92.9)
Prior treatment		
Null response	40/50 (80.0)	39/42 (92.9)
Partial response	11/11 (100)	10/10 (100)
Relapse	14/15 (93.3)	13/13 (100)
HCV genotype 1b infection		
No prior treatment	22/22 (100)	18/18 (100)
Prior treatment		
Null response	25/25 (100)	20/20 (100)
Partial response	6/7 (85.7)	3/3 (100)
Relapse	14/14 (100)	10/10 (100)

TURQUOISE-II, phase 3 trial, combination of coformulated paritaprevir–ombitasvir and dasabuvir + RBV for 12/24 weeks in previously untreated and previously treated adults with chronic HCV genotype 1 infection and compensated cirrhosis.

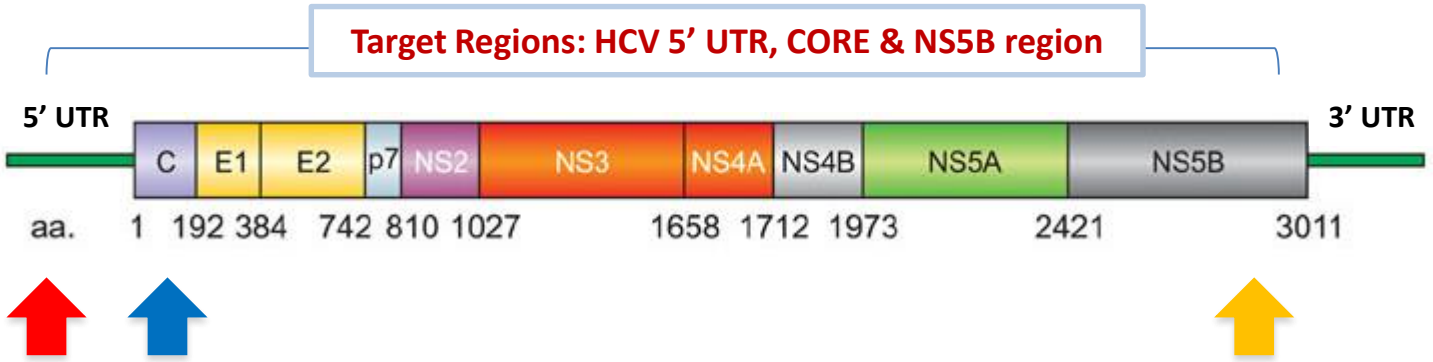
Poordad F. et al., NEJM 2014

ID_476	HCV genotype: 1b	Age: 55	Sex: M	Naive	Cirrhotic



A correct determination of HCV-genotype is relevant prior to treatment initiation

- Several commercial assays are available for determining genotype/subtype
- All assays target the 5'NCR gene for genotypes 1-6, in addition, the 2 assays more used in diagnostics, Abbott and INNO-LiPA-HCV-2.0, target also the NS5B and the core gene, respectively, providing additional information also in subtyping: for genotype 1 (1a/1b, both), and for all genotypes (only Innolipa)



Trugene HCV Genotyping assay — Direct sequencing

INNO-LiPA HCV 1.0 — Reverse hybridization

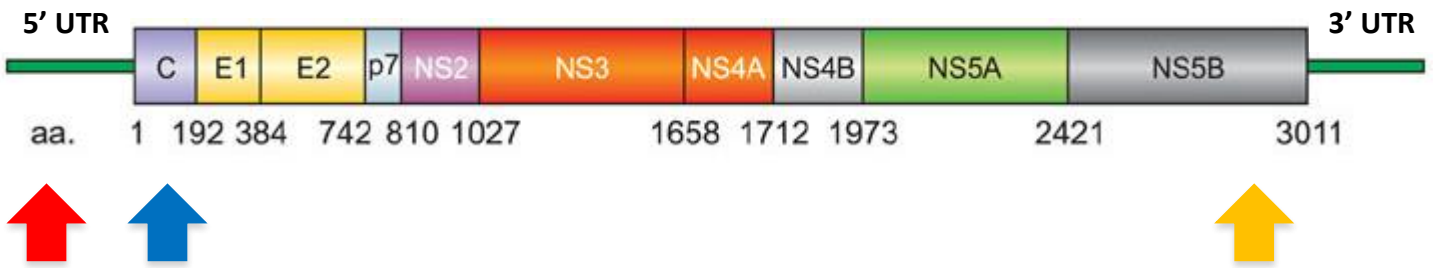
INNO-LiPA HCV 2.0 — Reverse hybridization

Abbott RealTime HCV Genotype II assay — Real time PCR

A correct determination of HCV-genotype is relevant prior to treatment initiation

- Several commercial assays are available for determining genotype/subtype

However, not all genotypes can be resolved, with results being reported as: 'indeterminate', 'mixed', 'genotype X reactivity with Y', or just the major genotype 1 alone.



Trugene HCV Genotyping assay	Direct sequencing
INNO-LiPA HCV 1.0	Reverse hybridization
INNO-LiPA HCV 2.0	Reverse hybridization
Abbott RealTime HCV Genotype II assay	Real time PCR

HCV sequencing and commercial genotyping assays were concordant in 91.8% of cases

	Patients analyzed (N)	Patients analyzed (%)
Geno/subtype confirmed	315	91.8%
Discordant genotypes	4	1.3%
Genotype 1 with discordant subtype	9	2.6%
Genotype 1 with no subtype*	6	1.7%
Mixed genotypes	8	2.3%
Indeterminate genotype*	1	0.3%
Total	343	100%

Number of HCV infected patients with available at least one NS3/NS5A/NS5B sequence. * Performed by using Abbott assay in 2013/2014.

A total of 662 HCV plasma samples with detectable HCV-RNA from 343 HCV infected patients candidate to start a treatment containing a DAA were analyzed between 2011-2014 in our laboratory.

NS3-sequencing and phylogenetic analysis provided additional information regarding HCV genotype…

Discordant genotypes

Pre sequencing subtype	Year genotyping	Post sequencing subtype	Abbott 2013/2014
1a	Unknown	2c	-
1b	1993	2c	2
1b	1994	4d	-
2a/2c	2005	1b	1b

Discordant subtypes

Pre sequencing	Post sequencing
1a=5	1g=1 1b=4
1b=4	1a=4
Indeterminate/1a =1	1g=1

Post sequencing subtype	Pre sequencing subtype	Year genotyping	Genotype assay
1g	1a	2013	Innolipa/Abbott
1b	1a	NA	Trugene
1b	1a	2011	Trugene
1b	1a	2011	Trugene
1a	1b	2009	NA
1b	1a	NA	NA
1a	1b	2014	NA
1a	1b	2012	NA
1a	1b	NA	NA
1g	Indeterminate/1a	2013/1999	Abbott/NA

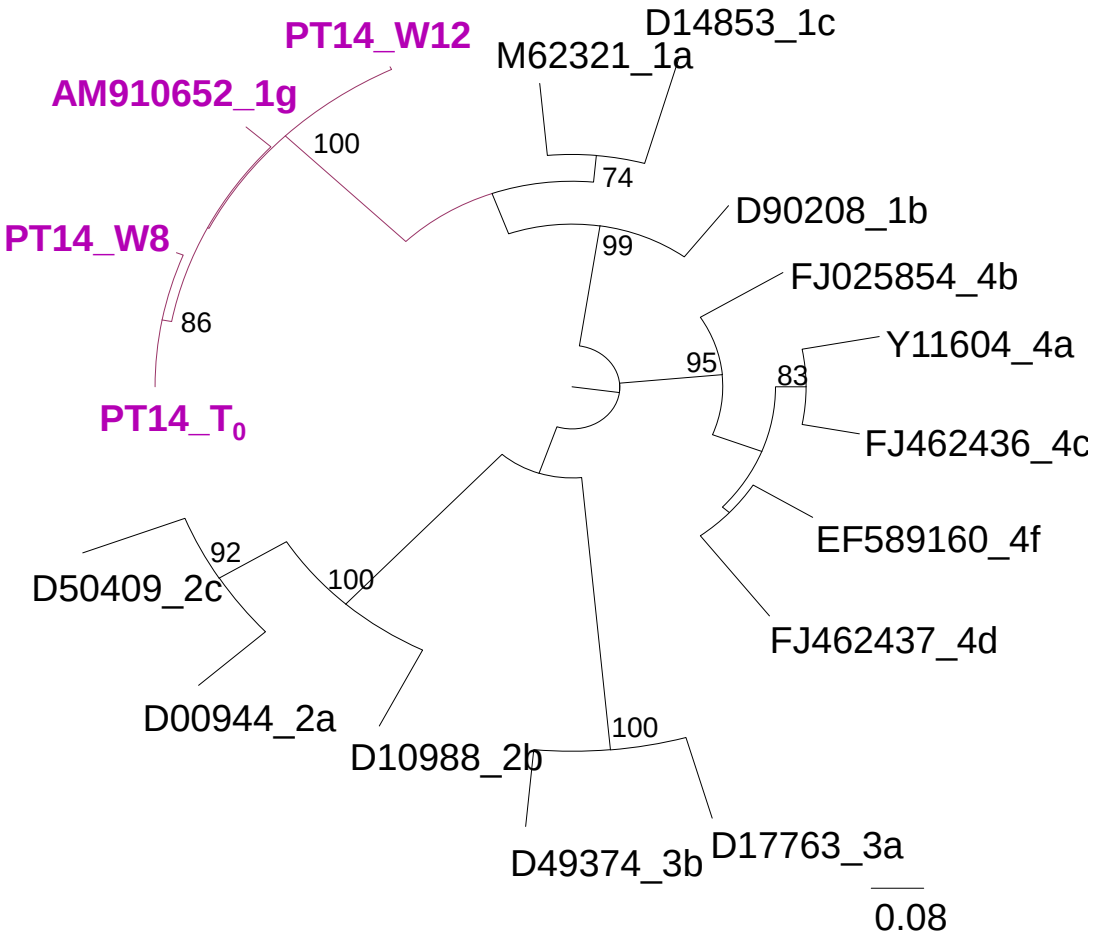
Discordant subtypes

Pre sequencing	Post sequencing
1a=5	1g=1 1b=4
1b=4	1a=4
Indeterminate/1a =1	1g=1

Overall, 14 out of 343 (4.1%) HCV infected patients candidate to start a treatment containing a DAA showed a discordant genotype or subtype according to the sequencing

1b	1a	2011	Trugene
1b	1a	2011	Trugene
1a	1b	2009	NA
1b	1a	NA	NA
1a	1b	2014	NA
1a	1b	2012	NA
1a	1b	NA	NA
1g	Indeterminate/1a	2013/1999	Abbott/NA

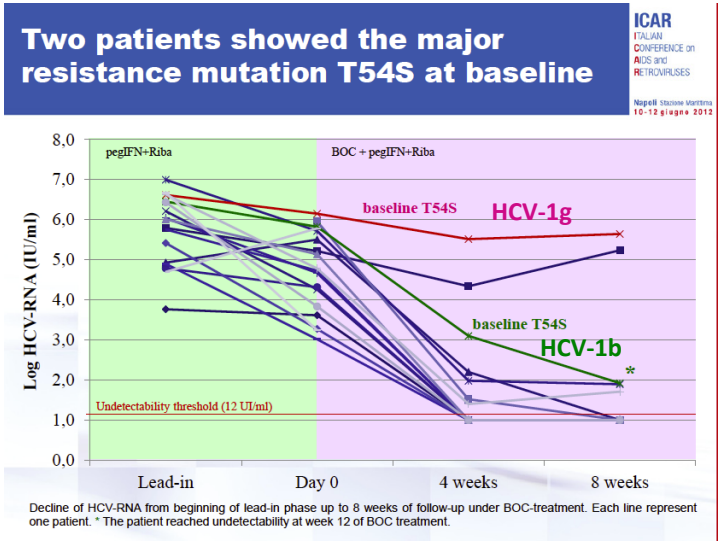
By phylogenetic analysis, a non-responder patient (previous non responder to SOC & IL-28:TT) resulted to be infected with HCV genotype 1 subtype g



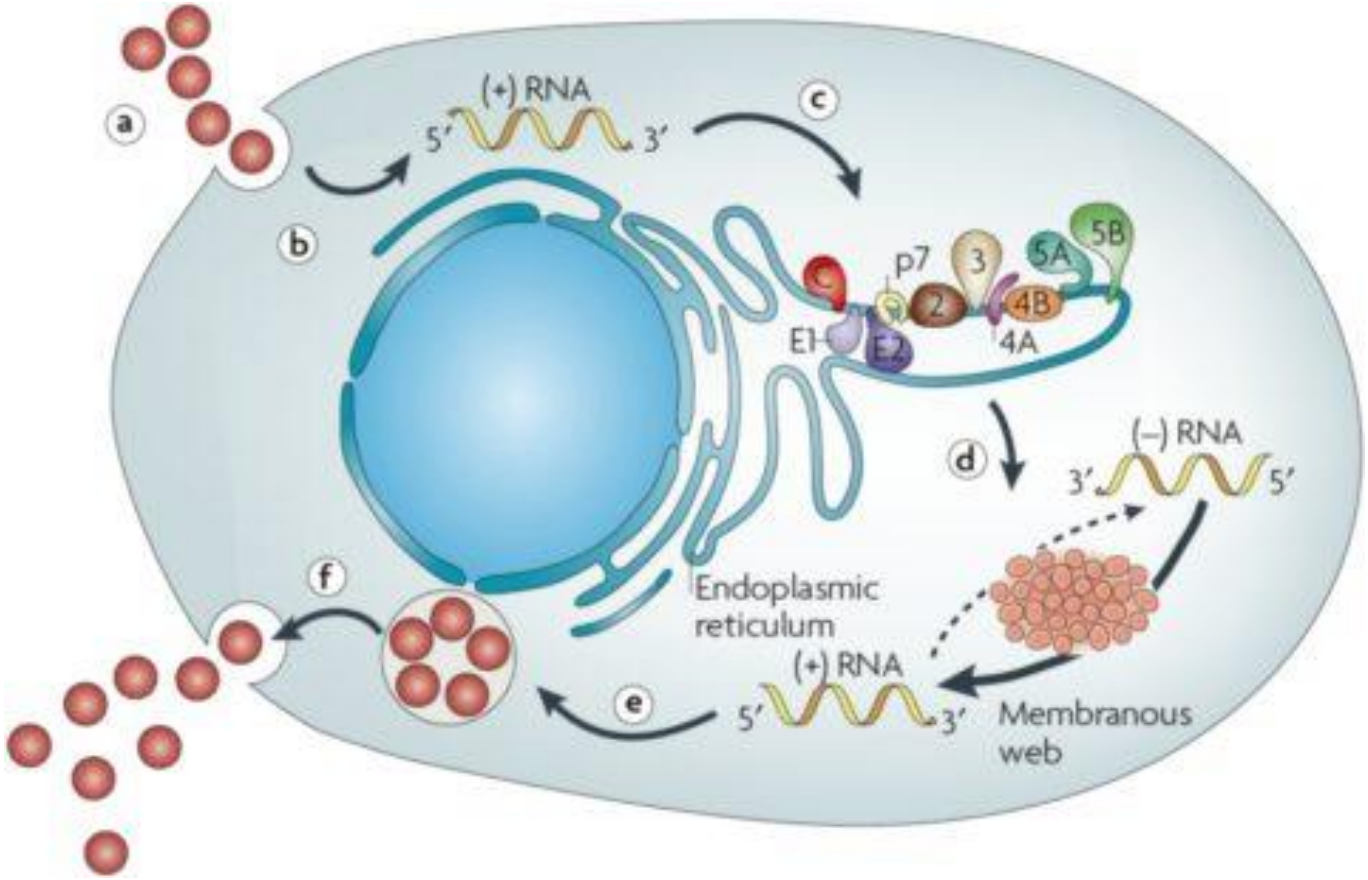
Cento V. et al., Antiv Therapy 2013

All HCV-1g sequences were closely related and formed a distinct cluster from other HCV-1 subtypes.

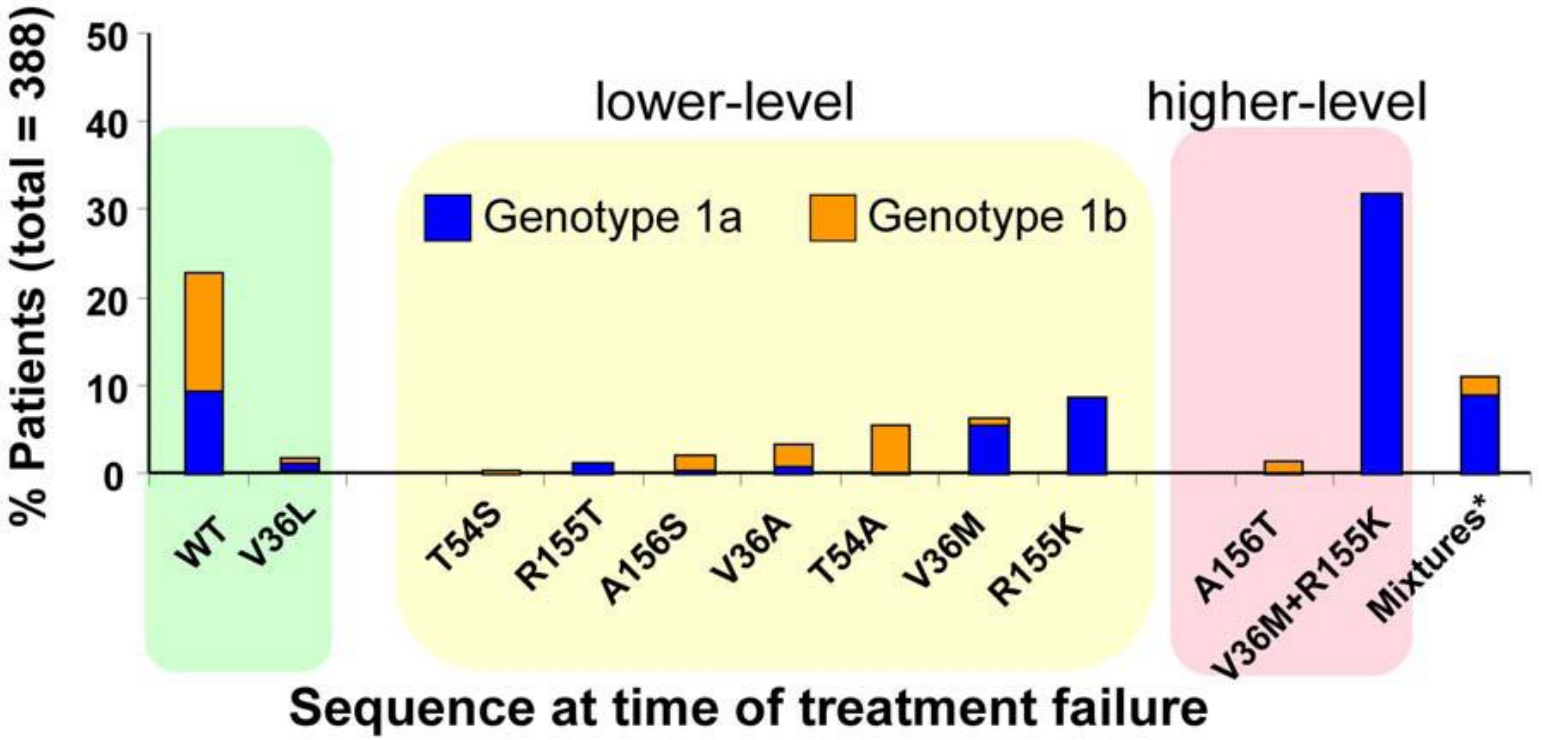
Notably, by **LiPA VERSANT Siemens 2.0 the patient was classified as infected with HCV-1a**…and the patient was **again classified as infected with HCV-1a by using Abbott RealTime HCV Genotype** II, thus confirming the incorrect result and indicating the **difficulty of the next generation diagnostic tools to discriminate HCV-1g sequences.**



Mutations occur frequently during the replication of HCV



Different distribution of TVR RAVs in TVR treated patients according to subtype



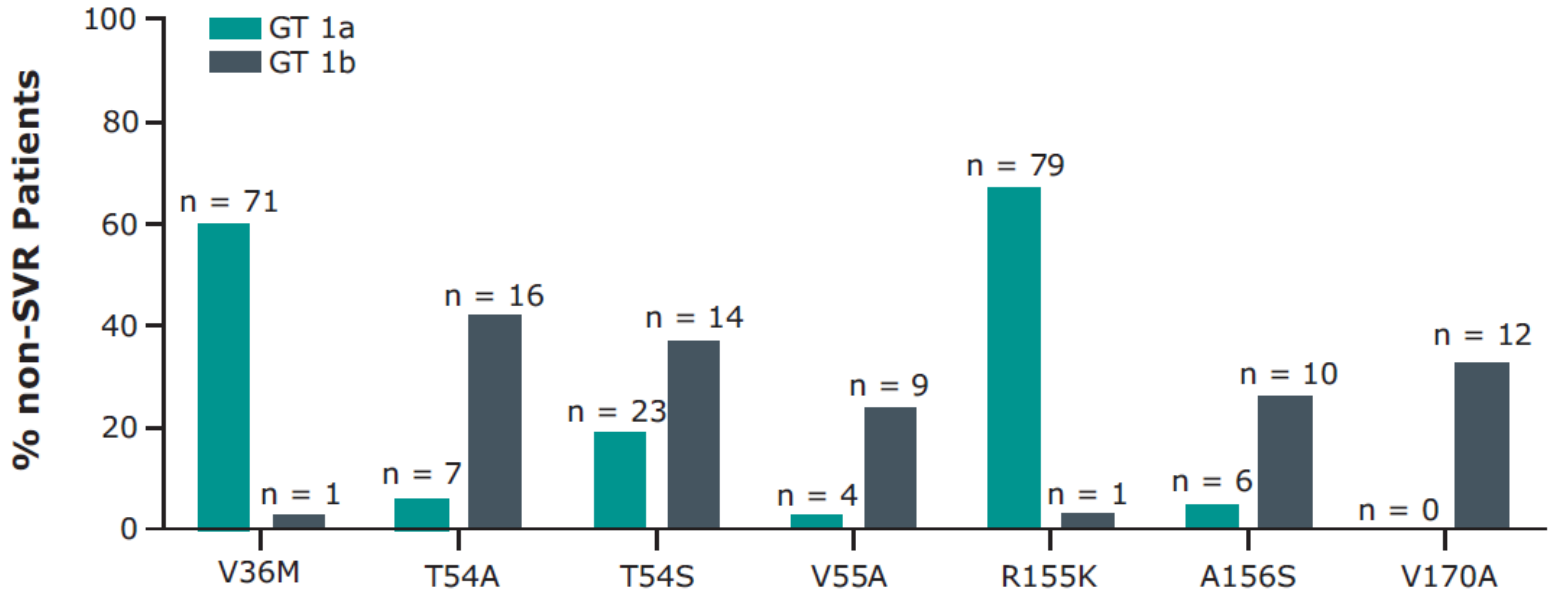
*Mixtures (n=43): V36A/M, T54A/S, A156S/T/V, V36V/A+T54T/A, V36M+T54S, V36A+I132V, V36L/M+R155K, V36L+R155K, V36V/A+R155R/K, V36A/M+R155R/K, V36V/I+R155R/K, V36M+A156S, V36V/M+A156A/S, V36V/M+A156A/T, T54S+R155K, T54T/S+R155R/K, T54S+A156T, I132I/V+R155K, R155G+D168N, R155T+D168N, V36L+T54S+I132V, V36M+T54S+I132V, V36V/M+T54T/S+R155K, V36A/M+T54T/S+R155R/K, V36M+R155K+A156A/S, V36V/M+R155R/K+A156A/S, V36V/M+R155R/K+A156V/T

Figure 3. Frequency of Resistance Profiles in Patients Who Did Not Achieve an SVR with a TVR-based Regimen. X-axis is the % of patients with a given resistant variant out of all patients who did not achieve an SVR and had available sequence data in Phase 3 trials (n = 388, includes all TVR arms of all 3 Phase 3 trials). Higher-level resistance (red) is defined as >25-fold increase in IC_{50} and lower-level resistance (yellow) is defined as 3- to 25-fold increase in IC_{50} from wild-type. Variants observed in only a single subject (ie, 0.26% of the failure population) are not displayed. These variants are: V36G/I, I132V (1a), and R155M.
doi:10.1371/journal.pone.0034372.g003

Kieffer, PLoS ONE 2012

Different emergency of resistance mutations in GT1a and GT1b also in BOC-failing patients

Figure 4. Frequency of the most common treatment-emergent RAVs in GT 1a- and GT 1b-infected patients treated with boceprevir



- V36M and R155K were primarily found in HCV GT 1a-infected patients
- T54A, V55A, and A156S were predominantly found in HCV GT 1b-infected patients
- V170A was exclusively found in GT 1b-infected patients
- T54S was frequently observed in both GT 1a- and GT 1b-infected patients
- A similar pattern of RAVs for GT 1a- and GT 1b-infected patients were observed in IVR, BT, and RL patients

Ogert RA et al., AASLD 2011 (poster n° 927)

New Approved DAAs

Sofosbuvir

Nucleotide analogue
400 mg qd
All genotypes
High barrier
January 2014

Simeprevir

Protease inhibitor
150 mg qd
Genotypes 1 and 4
Low barrier
May 2014

Daclatasvir

NS5A inhibitor
60 mg qd
All genotypes
Low barrier
September 2014

Harvoni

Nucleotide analogue
+ NS5A inhibitor
400 mg sofosbuvir +
90 mg of ledipasvir qd
HCV-1 and HCV-4
High barrier
September 2014

Beware of HCV-genotype for daclatasvir resistance ...

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

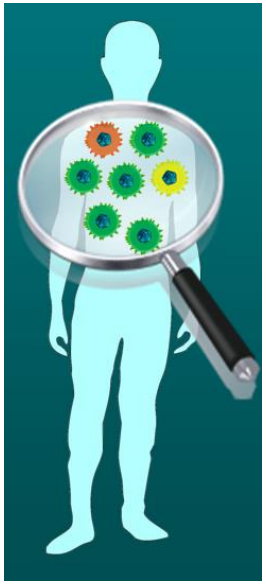
EC_{50}	< 10 pmol/L	< 100 pmol/L	< 1 nmol/L	< 10 nmol/L	< 100 nmol/L	< 1 μmol/L	> 1 μmol/L
DCV							
HCV GT							
1b	Wild (2.6 pmol/L) L28M L31M R30Q	R30E, H L31F, V P32L Y93H, N 37L or 54H/93H 23F/31F	23F/93H 30Q/31F 31V/58S 30H/31M		31F, M, V/93H 30Q/31M/93H		Δ30/32L
1a	Wild (6 pmol/L)			M28T Q30H, 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

Useful a HCV sequencing test before starting treatment?

Currently, all international guidelines list several new treatment options with similar high efficacy for initial treatment and retreatment of prior non-responders.
The new DAAs may be used with or without ribavirin for 12 or 24 weeks, depending on factors including HCV genotype, subtype (1a or 1b), presence of liver cirrhosis, and prior treatment history…..

CONSEQUENCES OF HCV VARIABILITY AT PATIENT'S LEVEL: QUASISPECIES POPULATION



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 t

Rong L et al., Sci Transl Med 2010

HCV resistant variants are naturally produced during the HCV life cycle

Table 1

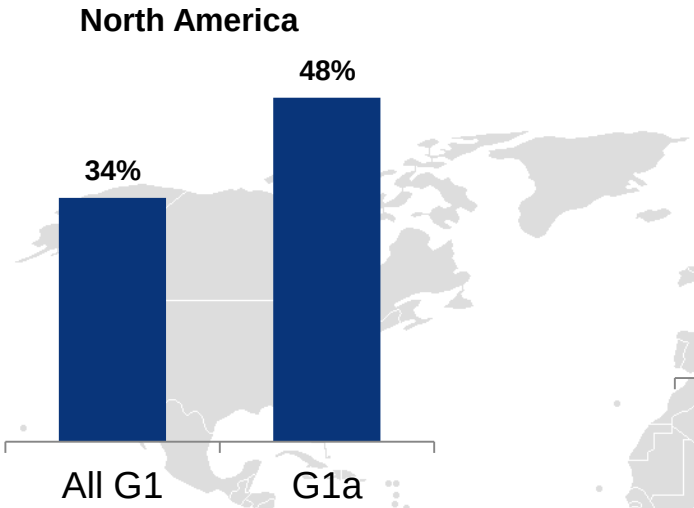
Prevalence of the most important natural occurring RAVs in DAA-naïve patients. N = 2111 for genotype 1a and 1336 for genotype 1b. n.o. = not observed prior treatment. Adapted from (Bartels et al., 2013).

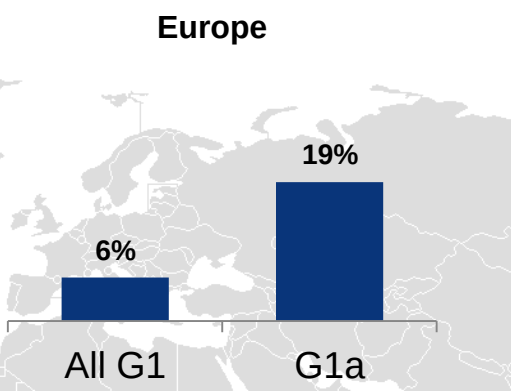
Amino acid site	Resistance associated to:	Observed amino acid changes	Cumulative prevalence of naturally occurring RAVs (%)	
			1a	1b
V36	TVR, BOC	I, L, M	2.5	1.3
T54	TVR, BOC	A, S	3.1	1.9
V55	BOC	A, I	4.9	0.4
Q80	SMV	K, R	38.3	1.42
V107	BOC	I	0.2	0.6
R155	TVR, BOC, SMV, FDV	K	0.9	n.o.
A156	TVR, BOC.	F, N, S, T, V	n.o.	n.o.
D168	TVR, BOC, SMV, FDV	E	0.2	0.6
I/V170	BOC	A, T	0.1	0.2

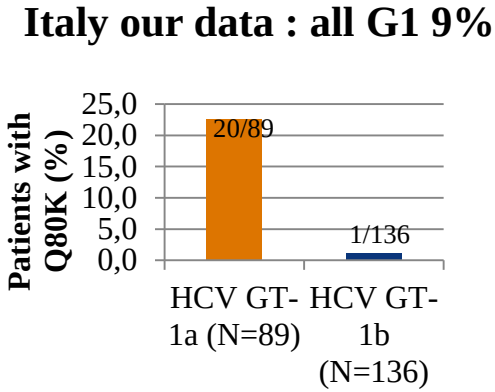
M.D. Schneider, C. Sarrazin Antiviral Research 2014

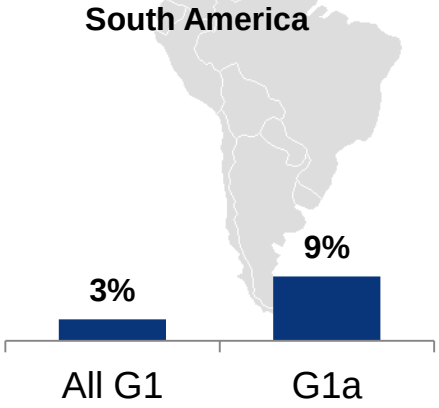
Overall prevalence of Q80K in G1 across different regions

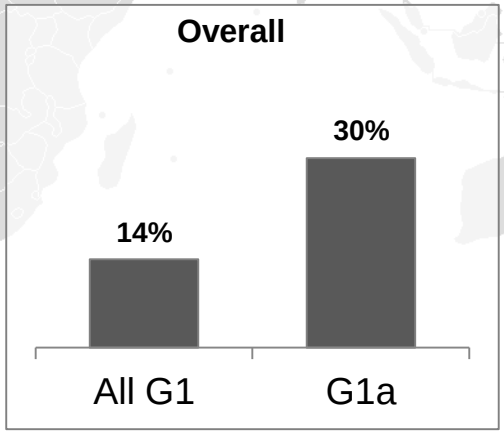
13.7% of patients (274/2007) all HCV G1
29.5% (269/911) of those with HCV GT1a and 0.5% (5/1096) of those with HCV GT1b









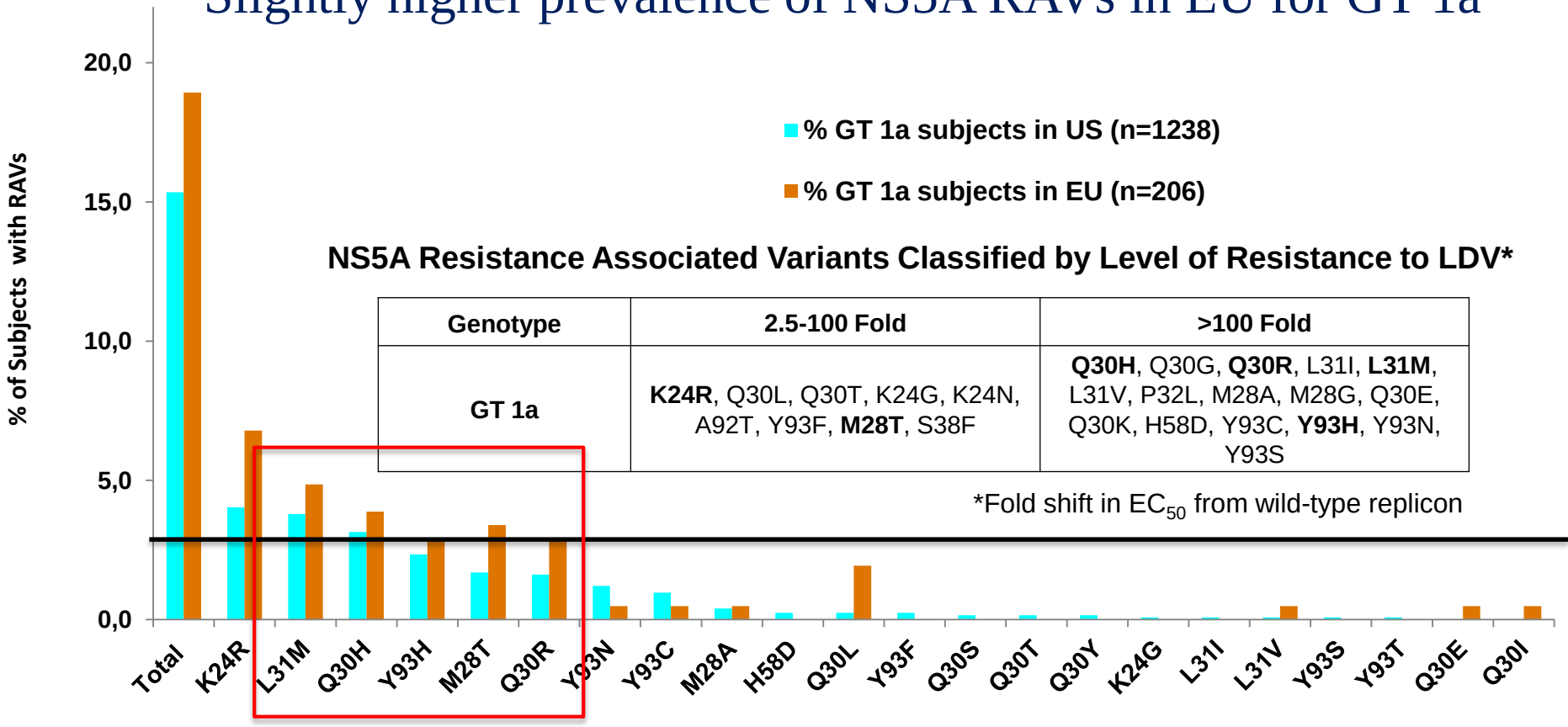


Includes 15 patients with non-1a/b genotype.

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

GT 1a Subjects with NS5A RAVs in US and EU

Slightly higher prevalence of NS5A RAVs in EU for GT 1a



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

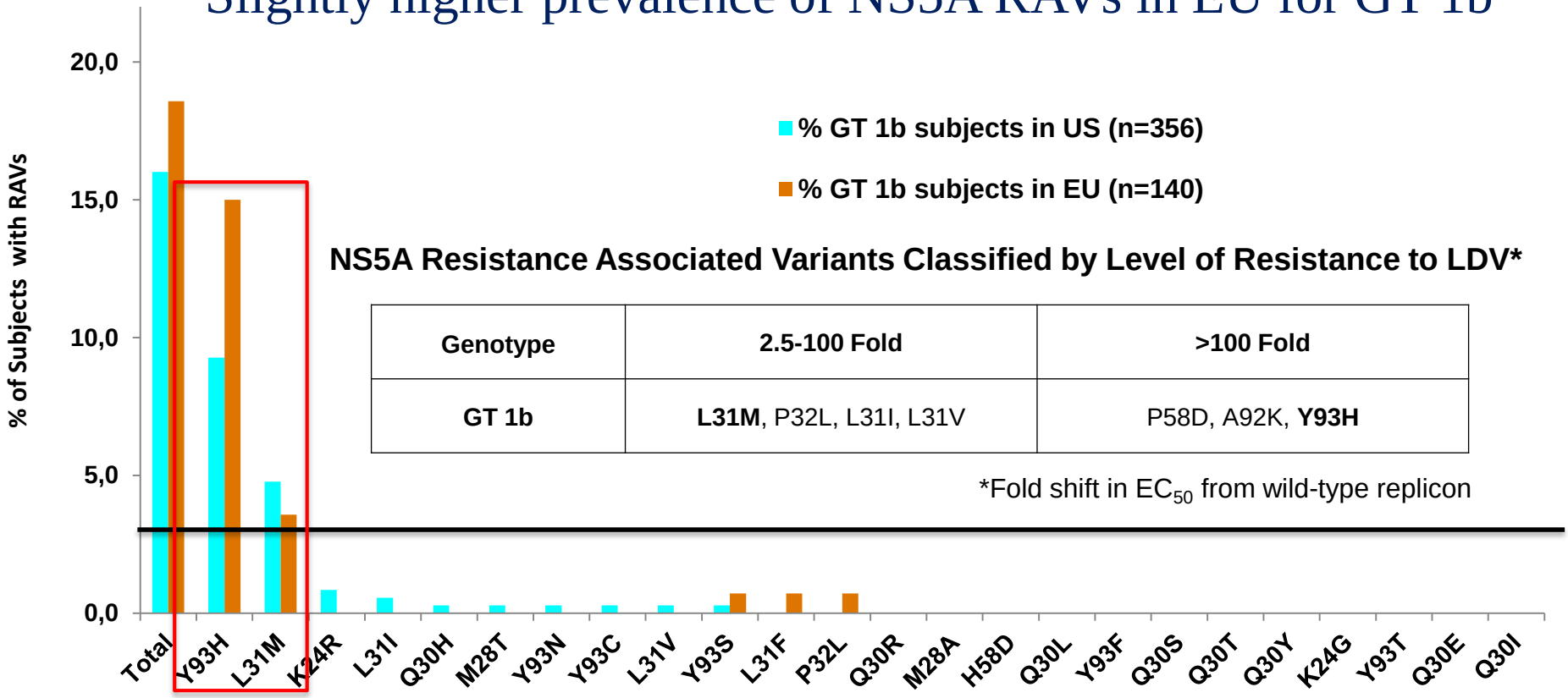
Genotype	2.5-100 Fold	>100 Fold
GT 1a	**K24R**, Q30L, Q30T, K24G, K24N, A92T, Y93F, **M28T**, S38F	**Q30H**, Q30G, **Q30R**, L31I, **L31M**, L31V, P32L, M28A, M28G, Q30E, Q30K, H58D, Y93C, **Y93H**, Y93N, Y93S

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

Abbvie (Dasabuvir) failure in **GT1a**: **M28T (2/7) and Q30R (3/7) in NS5A**

GT 1b Subjects with NS5A RAVs in US and EU

Slightly higher prevalence of NS5A RAVs in EU for GT 1b



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

Abbvie (Dasabuvir) failure in the single patient **GT1b: L31M + Y93H in NS5A**

Characterization of virologic escape in hepatitis C virus genotype 1b patients treated with the direct-acting antivirals daclatasvir and asunaprevir

5/10 patients with baseline NS5A-Y93H experienced virologic failure…

Hepatitis C virus genotype 1b Japanese patients (prior null responders to PegIFN-a/RBV [n = 21] or PegIFN-a/RBV ineligible or intolerant [n = 22]) were administered daclatasvir/asunaprevir for 24 weeks during a phase 2a open-label study

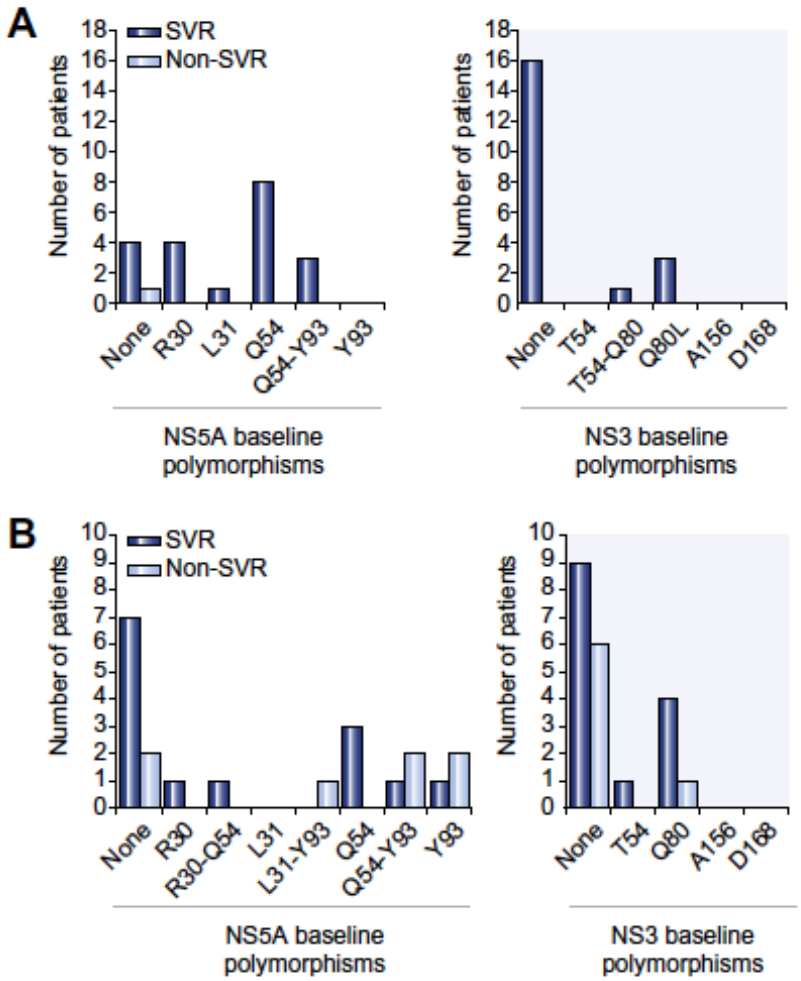


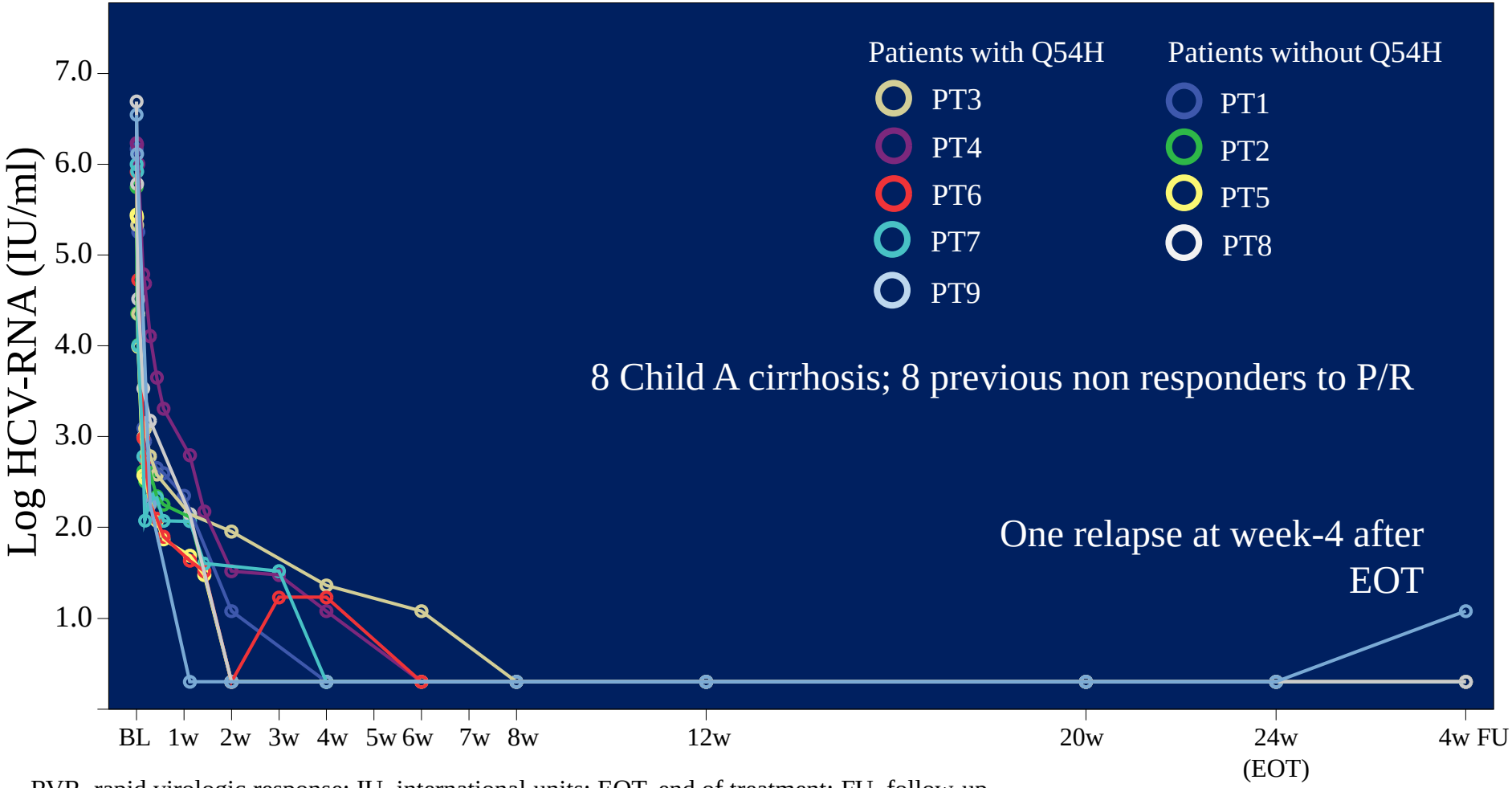
Fig. 2. Impact of baseline polymorphisms associated with resistance on virologic outcome among genotype-1b (A) null responders or (B) ineligible/intolerant patients. The ineligible/intolerant analysis excludes one patient (P-40) who discontinued therapy and was subsequently lost to follow-up. SVR, sustained virologic response.

Baseline NS5A polymorphisms (L28M, L31M, Y93H) associated with daclatasvir resistance (<25-fold) were detected in five null responders and six ineligibles. All three viral breakthroughs and 2/4 relapsers carried a baseline NS5A-Y93H polymorphism

Karino et al J of Hepatol 2013

The Q54H DCV RAV was detected at baseline in 5/9 GT-1b patients starting DCV+ASV

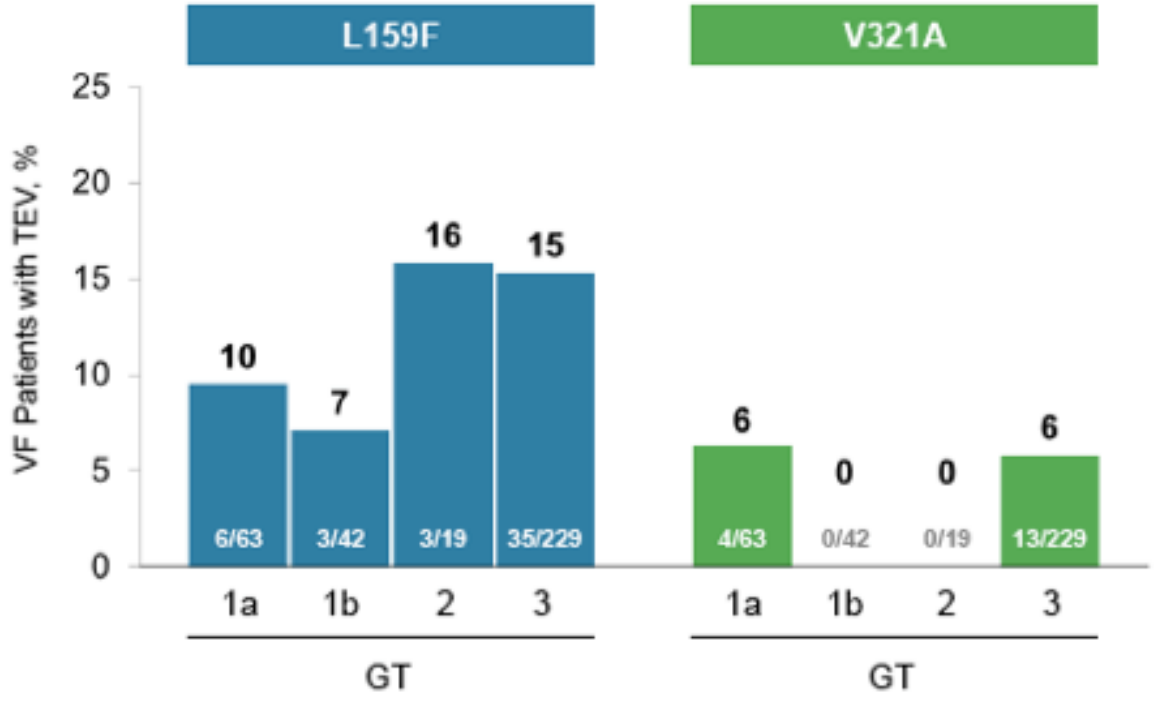
NS5A-RAV Q54H was detected at BL in all 3 non-RVR patients, in the only DCV+ASV relapser and in 1 RVR/SVR patient. No BL NS3- RAVs were detected.



RVR, rapid virologic response; IU, international units; EOT, end of treatment; FU, follow-up

Cento V., unpublished

L159F and V321A emergence was evaluated in 408 virological failures from 8 SOF and 5 LDV/SOF clinical trials using deep sequencing of NS5B (1% assay cut-off)



VF, virologic failure; TEV, treatment-emergent variant.

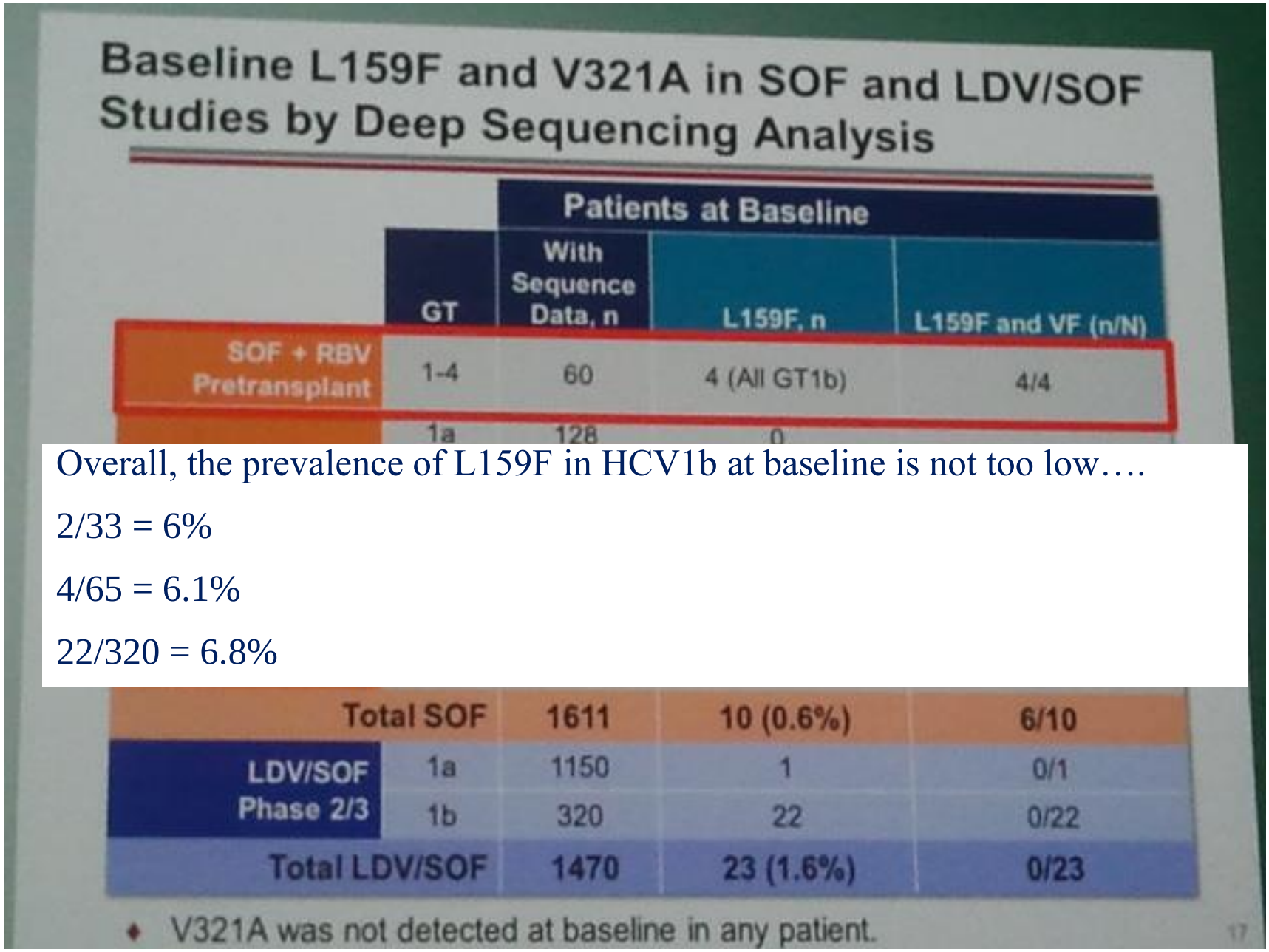
Svarovskaia ES et al., AASLD 2014

Baseline L159F and V321A in SOF and LDV/SOF Studies by Deep Sequencing Analysis

	GT	Patients at Baseline: With Sequence Data, n	L159F, n	L159F and VF (n/N)
SOF + RBV Pretransplant	1-4	60	4 (All GT1b)	4/4
SOF + RBV Phase 3	1a	128	0	
	1b	33	2	1/2
	2	402	0	
	3	699	0	
SOF + RBV + PEG Phase 3	1a	224	0	
	1b	65	4	1/4
Total SOF		**1611**	**10 (0.6%)**	**6/10**
LDV/SOF Phase 2/3	1a	1150	1	0/1
	1b	320	22	0/22
Total LDV/SOF		**1470**	**23 (1.6%)**	**0/23**

- V321A was not detected at baseline in any patient.

Svarovskaia ES et al., AASLD 2014



Svarovskaia ES et al., AASLD 2014

Useful a genotypic HCV resistance test after failure?

In the era of DAA…..

Treatment Failure

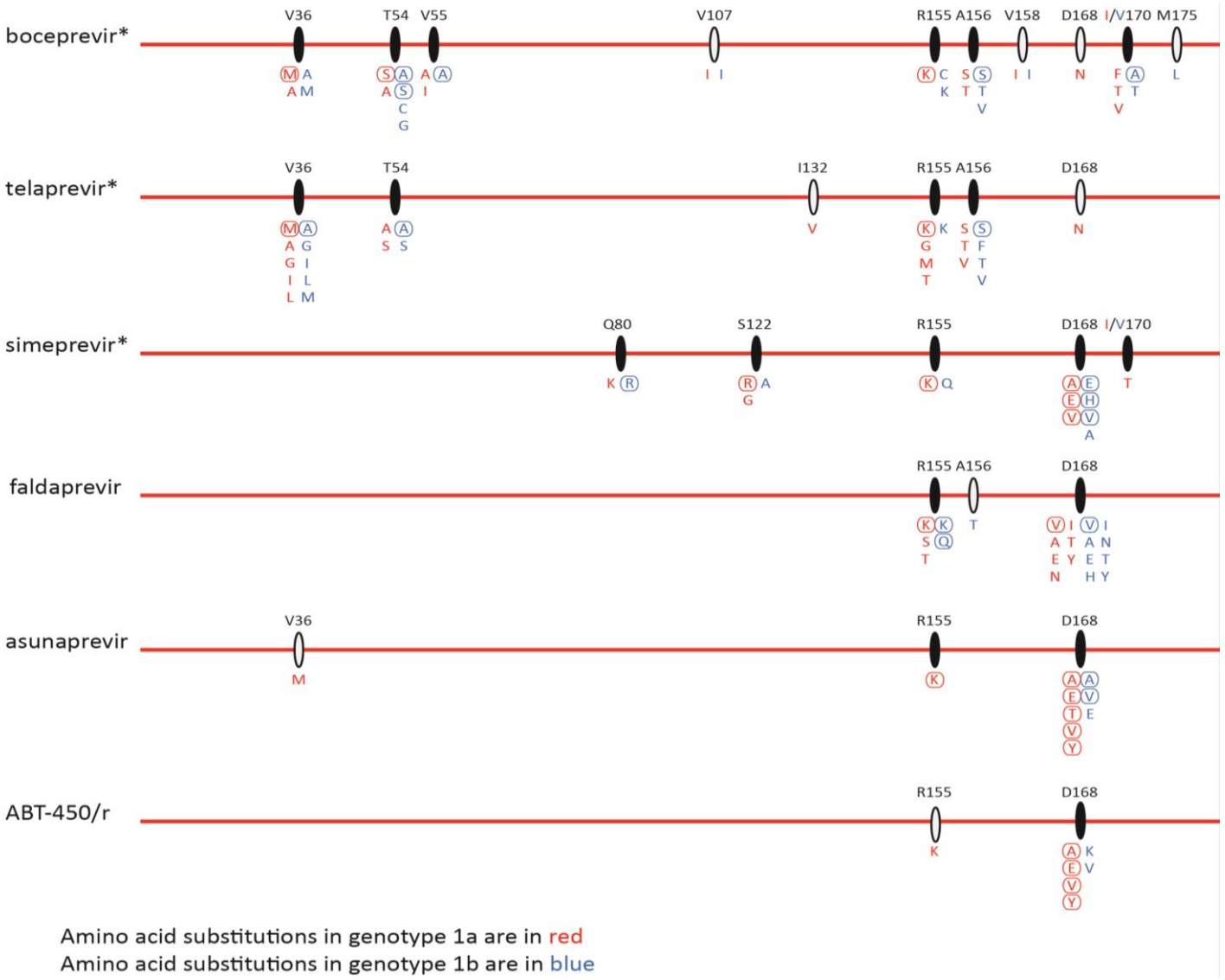
=

Failure to Cure HCV infection

=

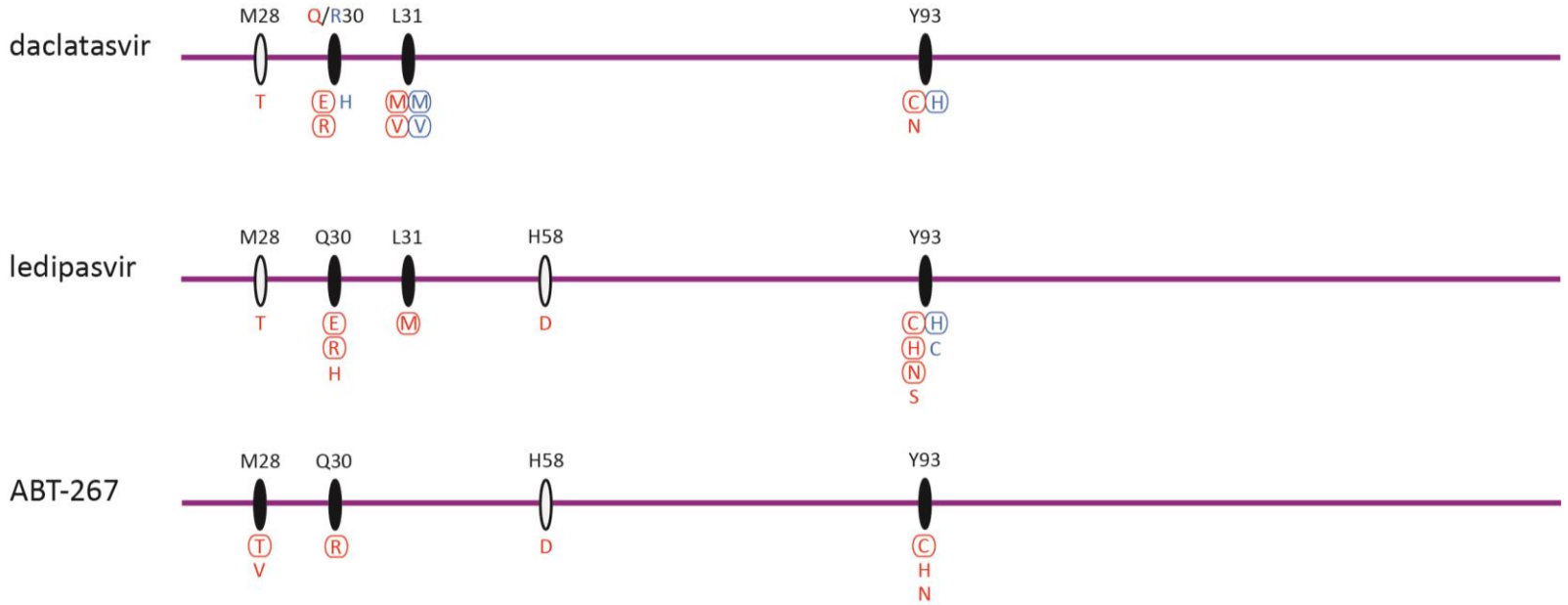
There remains hepatocytes in the liver that are infected with wt and/or resistant HCV viruses when treatment is stopped

Protease Inhibitor Resistance



HCV DRAG, Forum for Collaborative HIV Research, April 2014

NS5a Inhibitor Resistance



Amino acid substitutions in genotype 1a are in red
Amino acid substitutions in genotype 1b are in blue

HCV DRAG, Forum for Collaborative HIV Research, April 2014

SAPPHIRE I: Virologic Failure With 3 DAAs (peritrapevir/ombitasvir + dasabuvir) + RBV (12 weeks) in Treatment-Naive Patients

SVR_{12} 95% GT1a and 98% GT1b

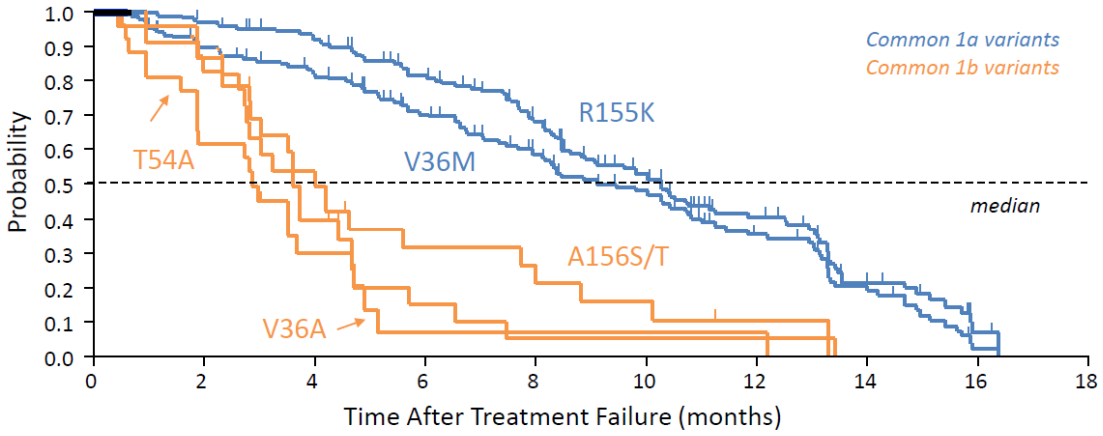
Outcome, n/N (%)	3 DAAs + RBV (n = 473)	GT1a	GT1b
Virologic failure			
▪ On-treatment breakthrough	1/473 (0.2)	1	0
▪ Posttreatment relapse	7/463 (1.5)	6	1

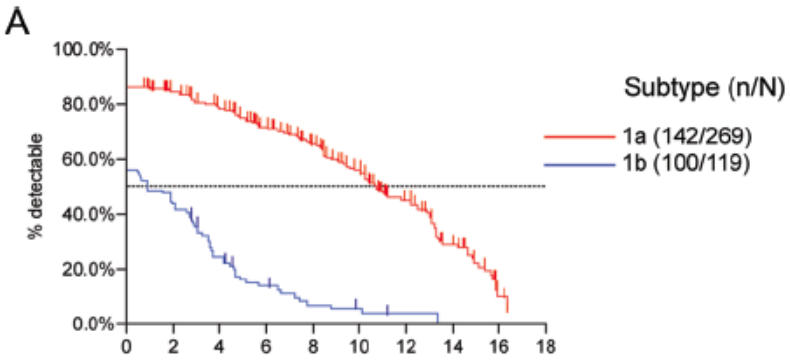
- **Virologic failure** occurred in **8/473 pts (1.7%):** 7 patients with GT1a and 1 patient with GT1b
- Relapses occurred at post-treatment Wk 2 (n = 3), Wk 8 (n = 3), and Wk 12 (n = 1)
- **Emergent resistance-associated variants uncommon: 8/473 pts (1.7%); 8/8 VF (100%)**
  - GT1a: D168V (6/7) in NS3; M28T (2/7) and Q30R (3/7) in NS5A; and S556G (3/7) in NS5B
  - GT1b: Y56H + D168V in NS3; L31M + Y93H in NS5A; and S556G in NS5B

Feld JJ, et al. EASL 2014; Feld JJ, et al. N Engl J Med. 2014

The rate of decline is specific to each drug resistant mutation and it is related to the replication capacity

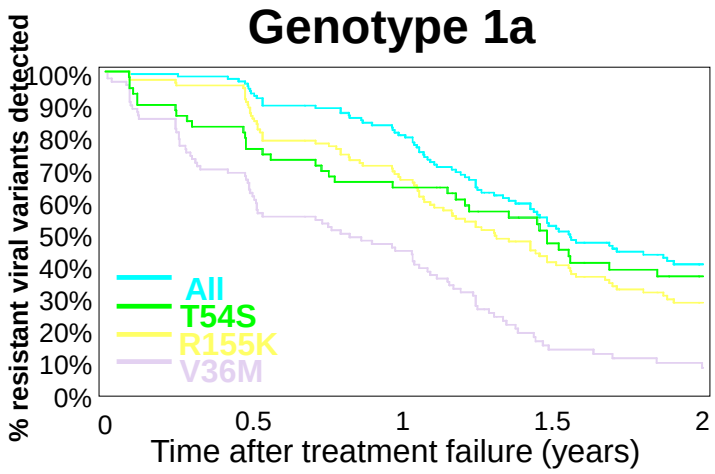
Dynamics of Resistant HCV Variants Post-Treatment (TVR)

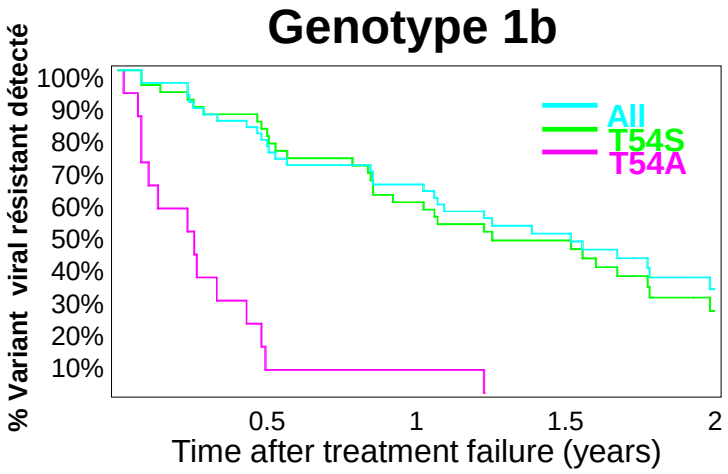




Sullivan et al., EASL 2011, Sullivan et al CID 2013

Dynamics of Resistant HCV Variants Post-Treatment (BOC)

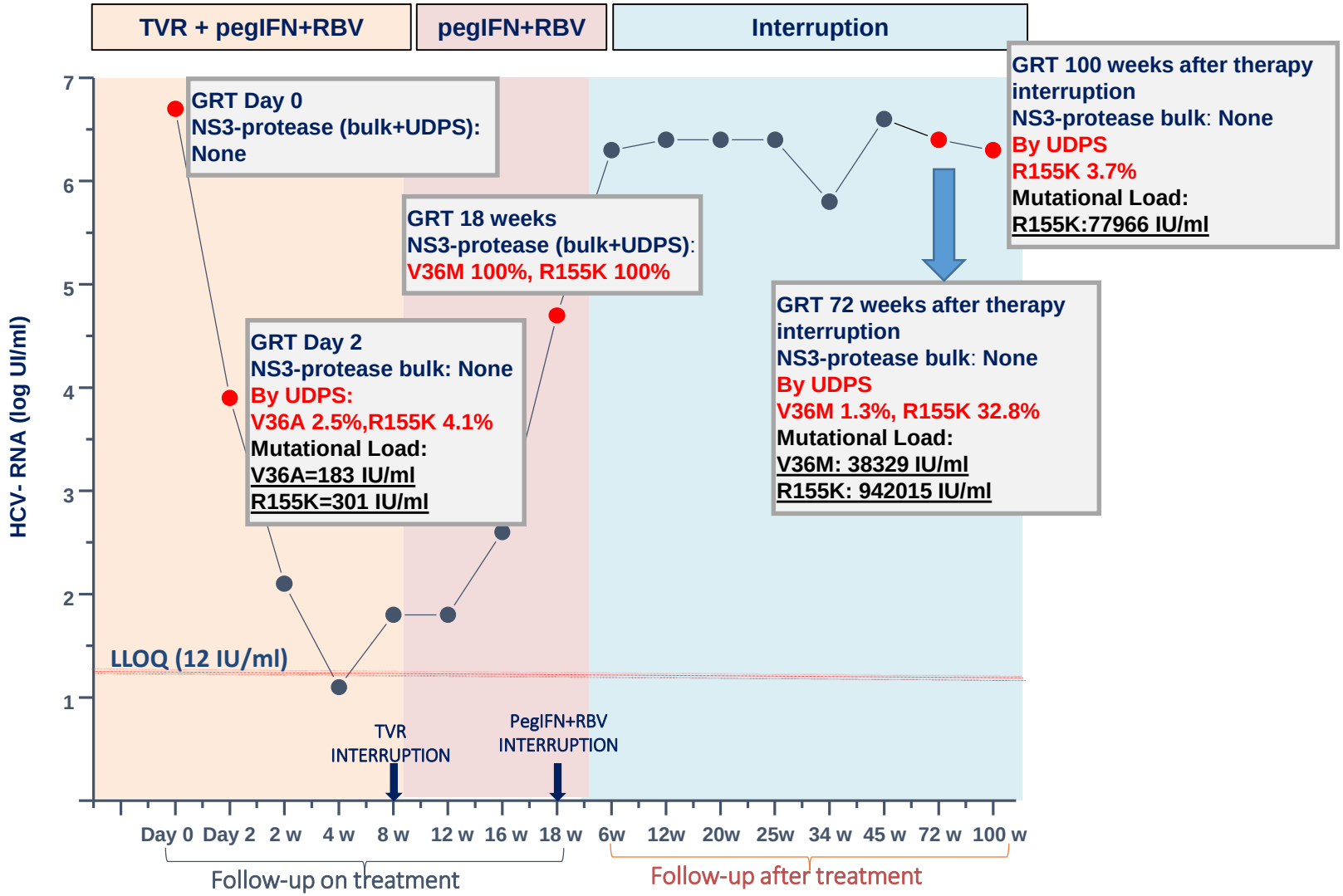




Barnard et al., Virology 2013

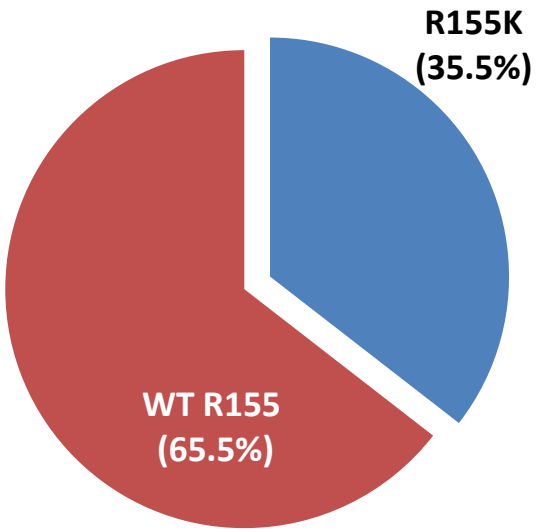
An HCV-1a infected patient who failed Telaprevir triple therapy with V36M+R155K still showed R155K at 100 weeks after therapy interruption

ID_13	HCV genotype: 1a	Age: 44	Sex: M	Null responder to SOC	IL-28: CT



2 HCV-1a patients, both failing telaprevir with V36M+R155K, still showed R155K after 100 weeks of therapy interruption

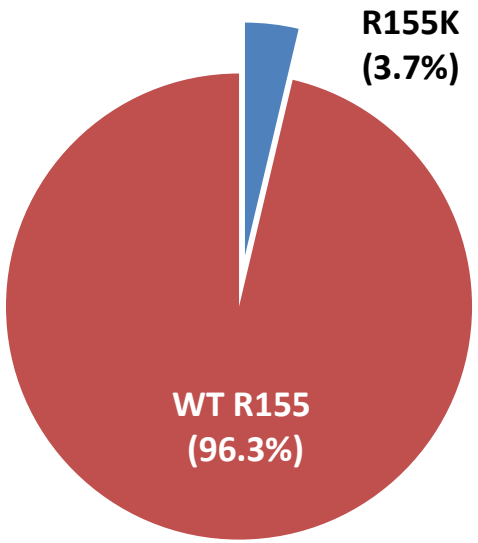
HCV-RNA : 1,671,926 IU/ml



PT 11

Mutational Load of R155K: 594,022 IU/ml

HCV-RNA: 2,090,903 IU/ml



PT 13

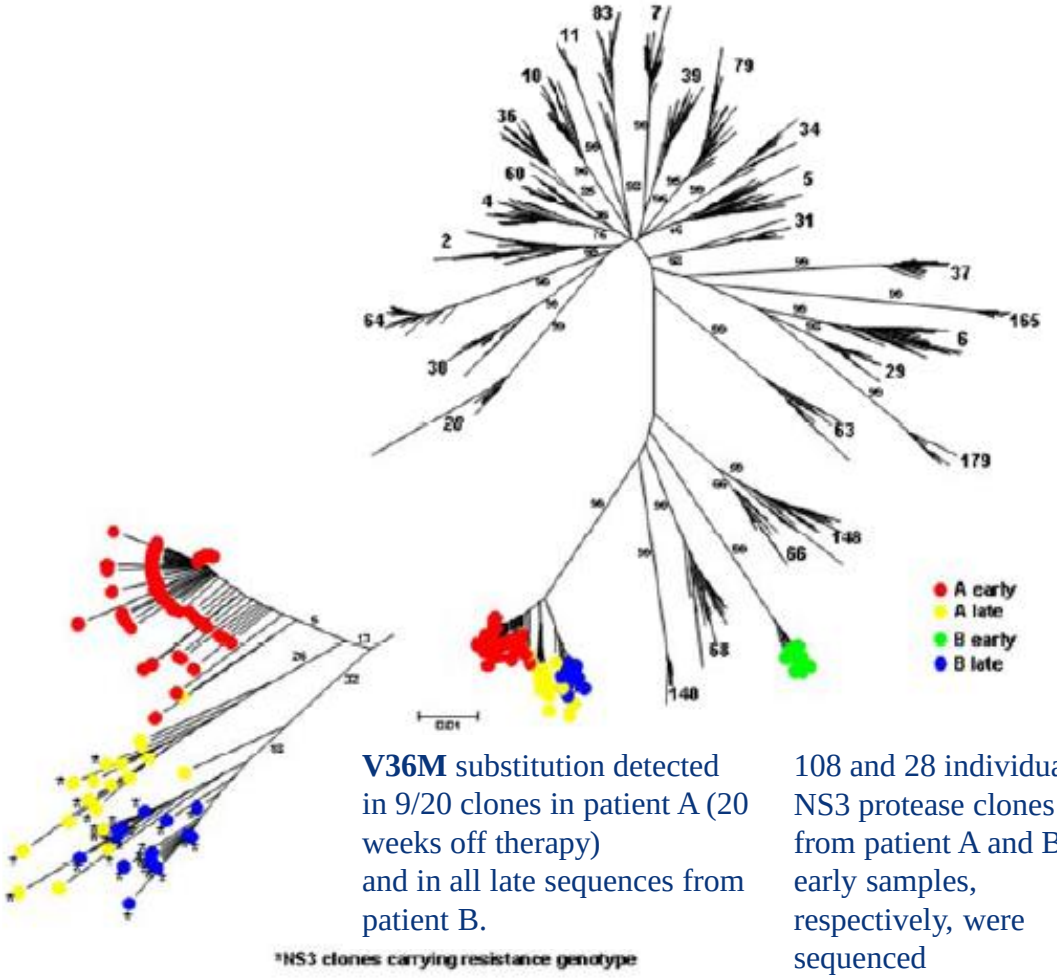
Mutational Load of R155K: 77,966 IU/ml

WT= wild-type

First documentation of a transmission of an HCV DAA resistant variant from a DAA treated patient to his sexual HIV-infected partner

Patient A, a man chronically infected with HCV genotype 1a and co-infected with HIV- 1, treated with pegIFN/RBV plus telaprevir in July 2012 with HCV breakthrough.
HCV NS3 protease sequences before treatment with telaprevir did not have any major substitution associated with NS3 PIs.

Patient B, a man also HIV-1 infected and sexual partner of patient A, diagnosed of acute HCV co-infection in January 2011, with HCV genotype 1a. This patient refused therapy with pegIFN/RBV during the acute phase of HCV infection.
In April 2012 he entered a clinical HCV trial and was treated for 24 weeks with pegIFN/RBV plus Daclatasvir, with undetectable HCV RNA at week 24 and 36 after the end of treatment.
However, at week 48 after stopping therapy, presented elevated transaminase and detectable HCV RNA, suggesting a HCV re-infection. The patient denied any known risk for HCV infection except unprotected sexual intercourse with his partner (patient A).
After 12 weeks, patient B tested negative for HCV infection.



V36M substitution detected in 9/20 clones in patient A (20 weeks off therapy)
and in all late sequences from patient B.

*NS3 clones carrying resistance genotype

108 and 28 individual NS3 protease clones from patient A and B early samples, respectively, were sequenced

Franco et al Gastroenterology May 2014

Conclusions

- **HIV and HBV have intracellular viral reservoirs. Differently, for HCV, there is no stable reservoir of genetic material.**
- The proper knowledge of the biological characteristics of HBV and HCV helps in selecting the best strategies aimed at obtaining the maximum achievable clinical result.

Current therapy of HBV infection based on PEG-IFN α-2a or NUCs does not guarantee sustained virologic response in many chronically infected persons. The current NUCs can efficiently inhibit the production of viral particles, however with only a minimal activity on the metabolic activity of cccDNA and integrated HBV genome. This implies to continue HCC surveillance. Treatment with some NUCs is associated with mutations and subsequent selection of resistant strains resulting with therapeutic failure, risk of cross-resistance, and selection of

Conclusions

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

To cure everyone with HCV we need to find it. When we have found it, we need to treat it properly.

Accurate diagnostics and treatment will be key to eliminating HCV from the planet and therefore to reduce the diseases HCV-related.

Conclusions

When treating patients with chronic viral hepatitis C **with direct antiviral agents** we should always remember:

The complexity: virus, host, clinical aspects, previous treatment outcome, DAA

The virus is very variable:

- At population level: different genotypes and subtypes, different response to PegIFN/RBV and to new DAA! Different PI, NS5A and NS5B resistance development and prevalence (1a vs 1b subtype, genotypes).
- At patient level: quasispecies, minor variants, pre-existing resistance.

Conclusions

The performance of a **baseline sequencing of HCV** can provide at the same time **two important virological information** for clinical management of patients with chronic HCV infection:

1) **a correct subtype assignment based on sequence analysis (**often incomplete, or even wrong, with old diagnostic methods).

2) **detection of variants that are potential non responders to therapy** (natural resistance or previous failure resistance).

In GT-1a patients: test for Q80K in patients eligible for simeprevir treatment.

What about NS5A natural resistance Y93H and other mutations?

What about NS5B L159F polymorphism in GT-1b patients for sofosbuvir?

Potential role for the duration of therapy?

HCV-RNA decay at **early time points** (i.e. 48h? week 2) may help in predicting treatment outcome: HCV-RNA >100 IU/ml at week 2 may identify patients at risk of failure in clinical practice. Attention to early resistance development/selection!

The cost-effectiveness and availability of HCV sequencing may deserve further attention in clinical practice.

Thanks for your attention