LE INFEZIONI VIRALI STORICHE, SARÀ POSSIBILE ERADICARLE?

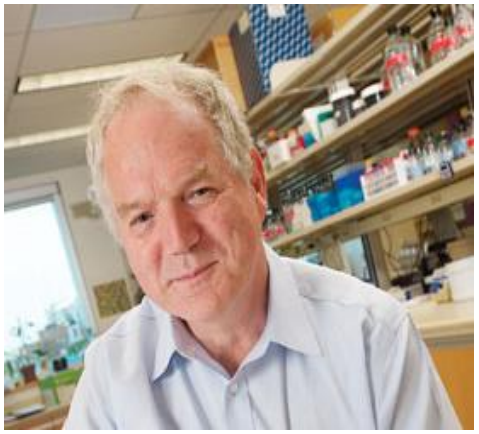
Francesca Ceccherini-Silberstein

HCV dal punto di vista virologico

Università degli Studi di Roma
"Tor Vergata"
Cattedra di Virologia

Ferrara, 20 Settembre 2018

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



Michael Houghton

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

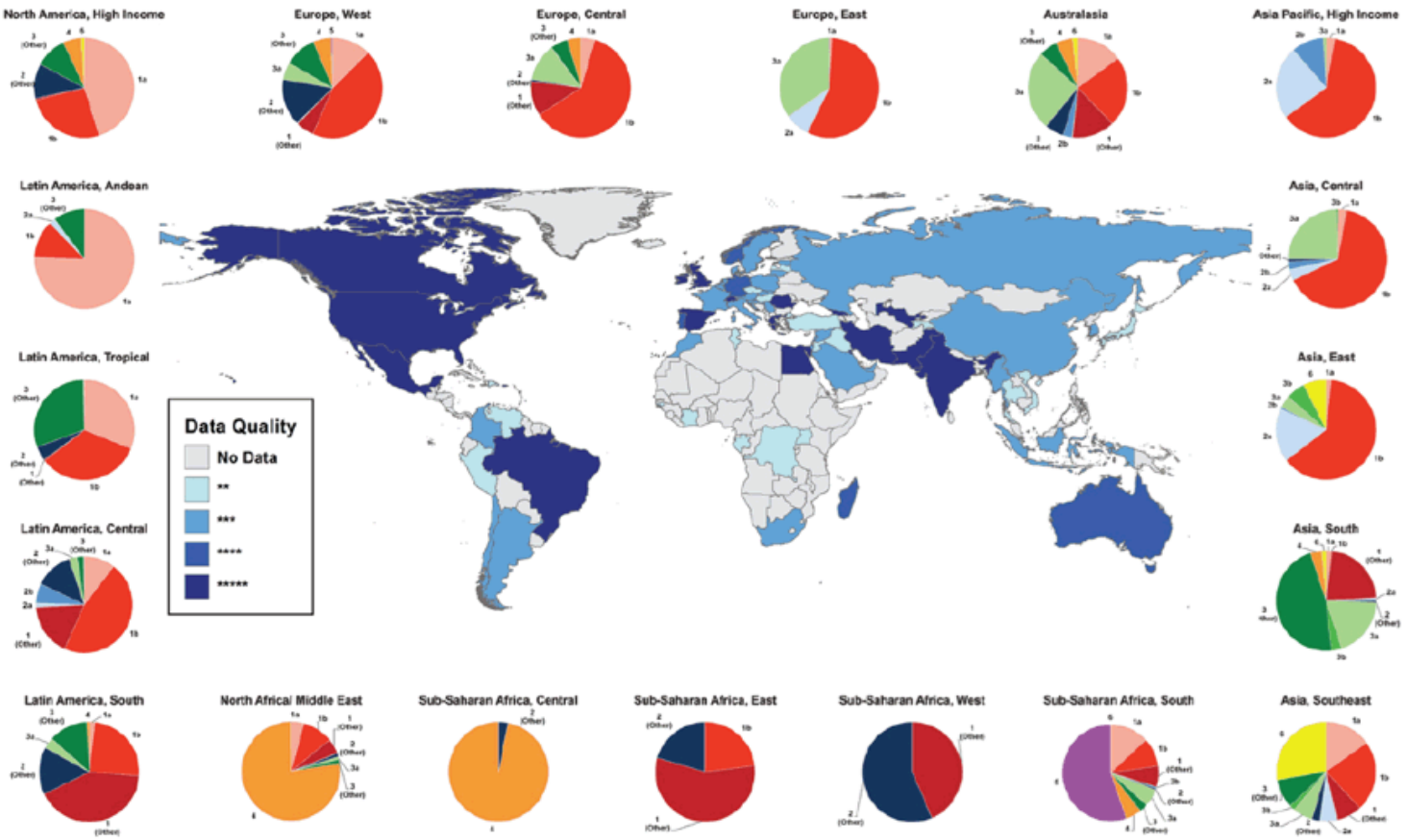
G. KUO, Q.-L. CHOO, H. J. ALTER, G. L. GITNICK, A. G. REDEKER, R. H. PURCELL, T. MIYAMURA, J. L. DIENSTAG, M. J. ALTER, C. E. STEVENS, G. E. TEGTMEIER, F. BONINO, M. COLOMBO, W.-S. LEE, C. KUO, K. BERGER, J. R. SHUSTER, L. R. OVERBY, D. W. BRADLEY, M. HOUGHTON

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 viremic HCV infection has been estimated at 1*-3%, which equates to 62*-170 million people

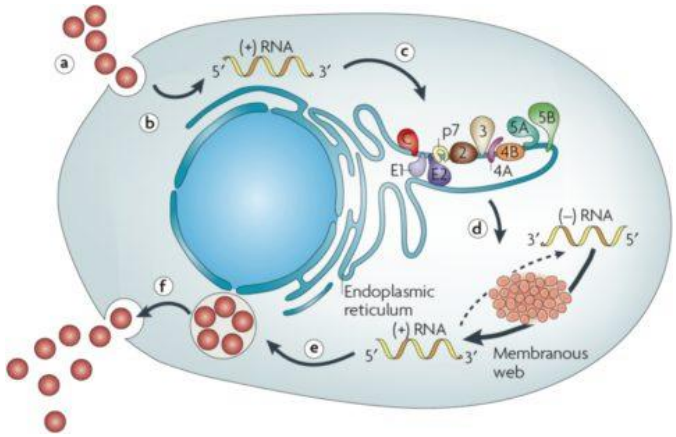
>350,000 mortality cases each year for HCV chronic disease related



*Manns et al Nature Rev 2017

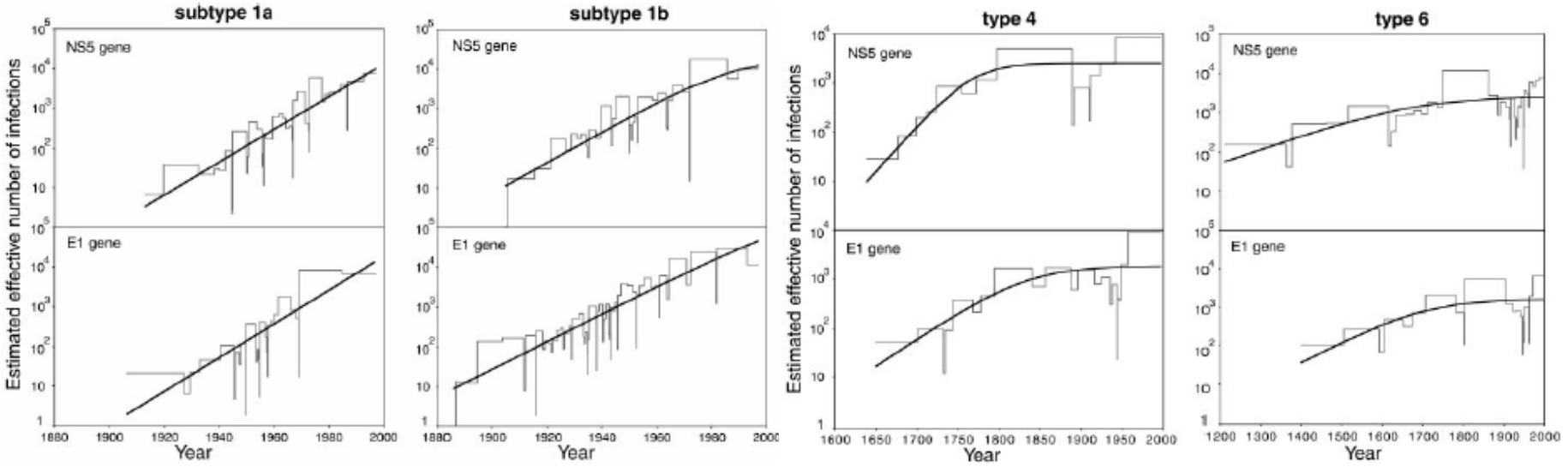
Messina JP, Hepatology 2014

HCV Flavivirus (genus epacivirus)



Identified in 1989 (nonA-nonB) cloned and sequenced.

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.**

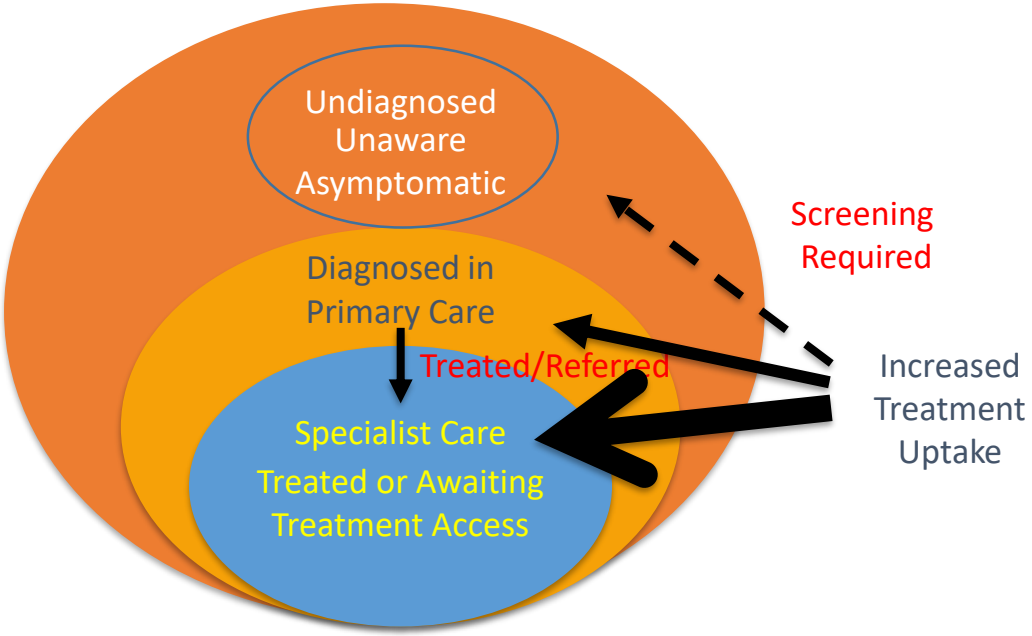


Pybus et al Science 2001

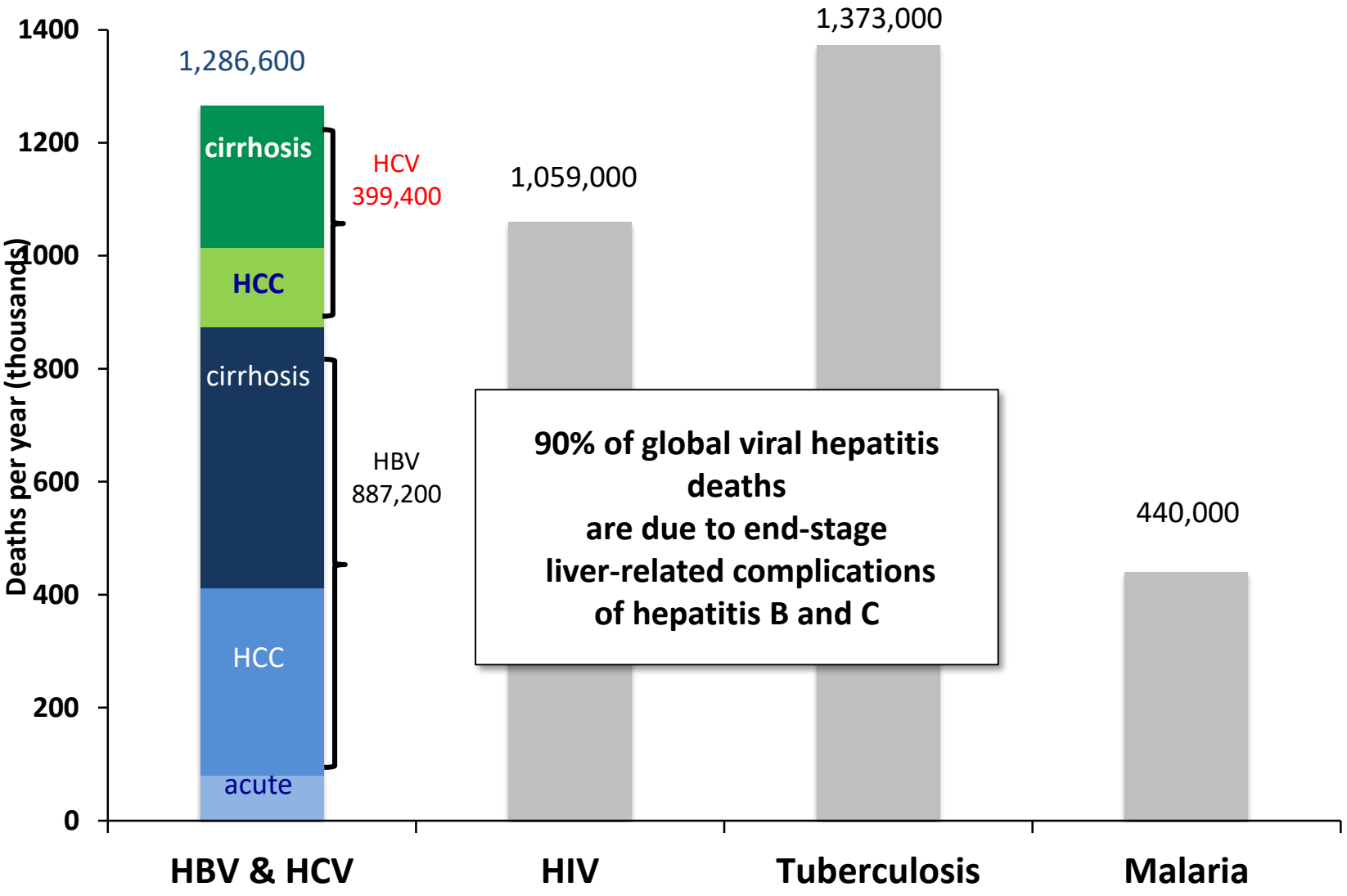
HCV Population

The number of chronically infected persons worldwide is estimated to be about 62-170 million, but most are unaware of their infection.

HCV prevalence and incidence data are needed to analyse the magnitude of the pandemic in different regions and to design public health interventions.

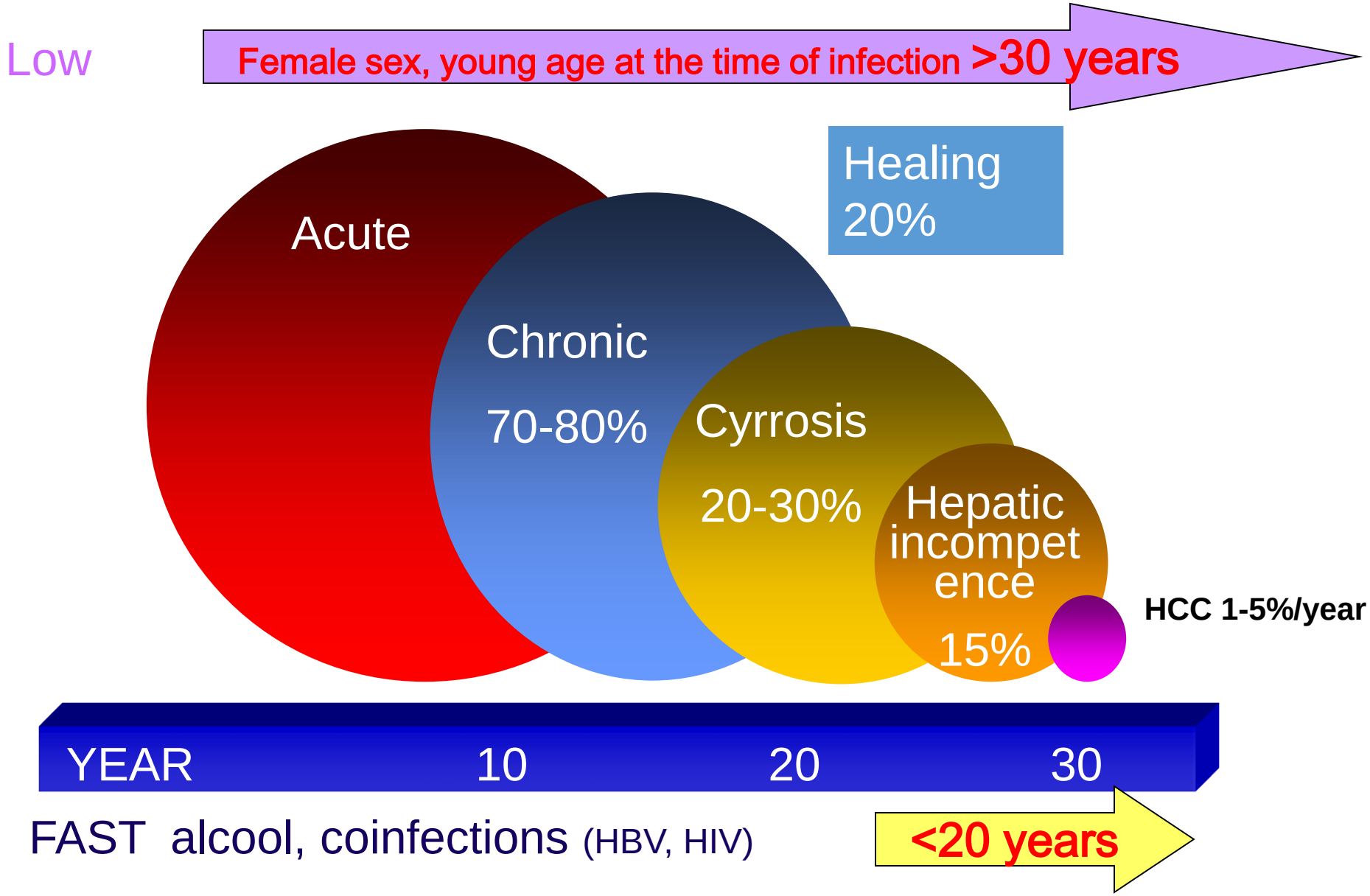


The burden of viral liver disease



WHO 2017 Global Hepatitis Report

HCV Natural history



Co-morbidities in HCV patients are relevant – bringing potential for competing risks

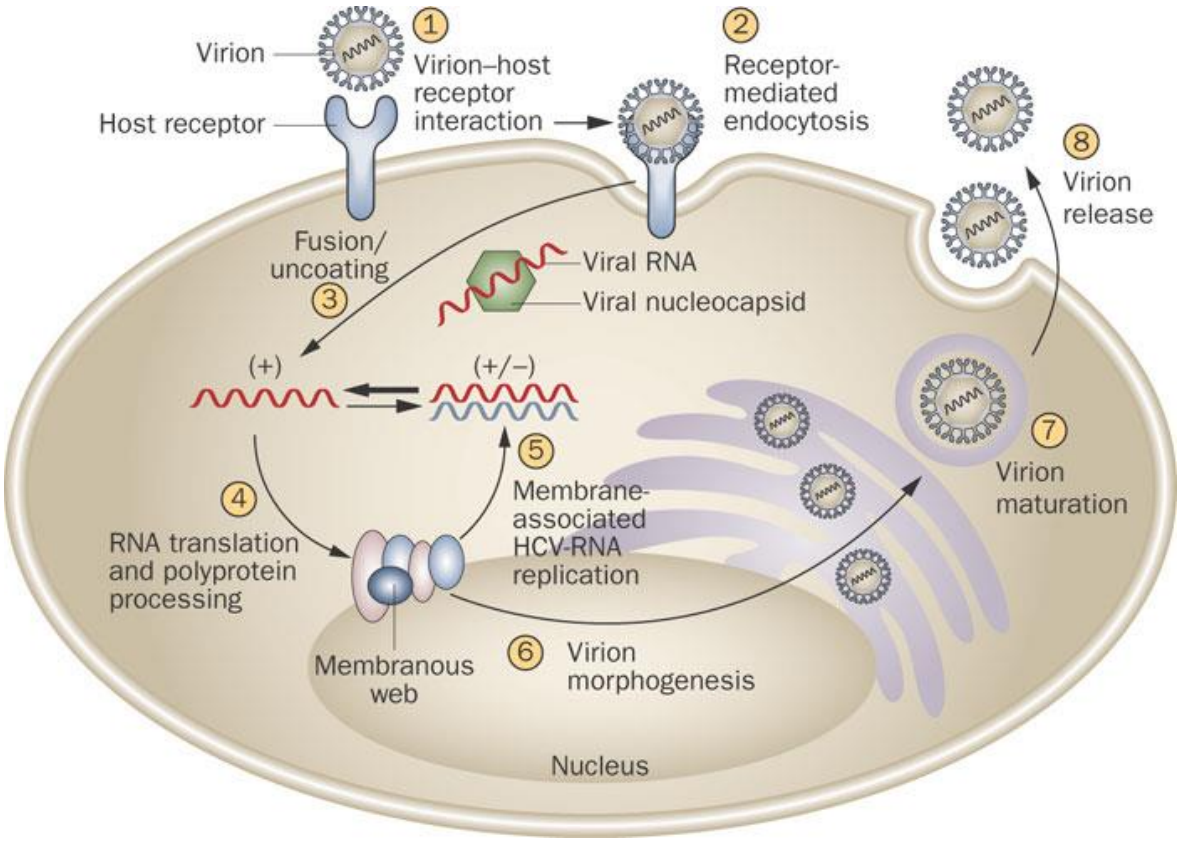
92% HCV patients had co-morbidities

40% had 5 or more co-morbidities

- Depression
- Substance misuse
- Psychosis
- Cognitive impairment
- Cardiovascular disease
- Respiratory disease
- Atherosclerosis
- Lymphoma
- Diabetes
- Dyslipidaemia
- Cancer
- Renal disease
- HIV co-infection

Vutien P, et al. AASLD 2014; Poster #1481.

The long-term persistence of HCV infection is unique among RNA viruses that replicate without a DNA form



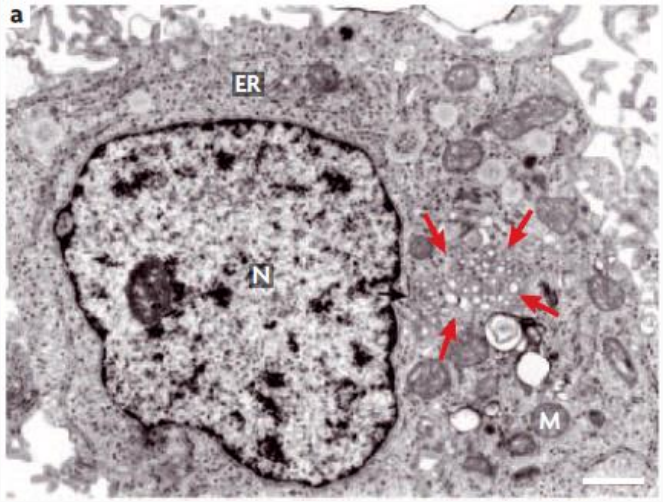
Pereira A A and Jacobson I M
Nat Rev Gastroenterol Hepatol, 2009

- **Unlike DNA viruses or retroviruses that are classically associated with latency no episomial or integrated form of HCV has been demostrated**
- **HCV replication occurs only in cytoplasm**

HIV, HBV and HCV share several biological similarities, but …

Differently from HIV and HBV:

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



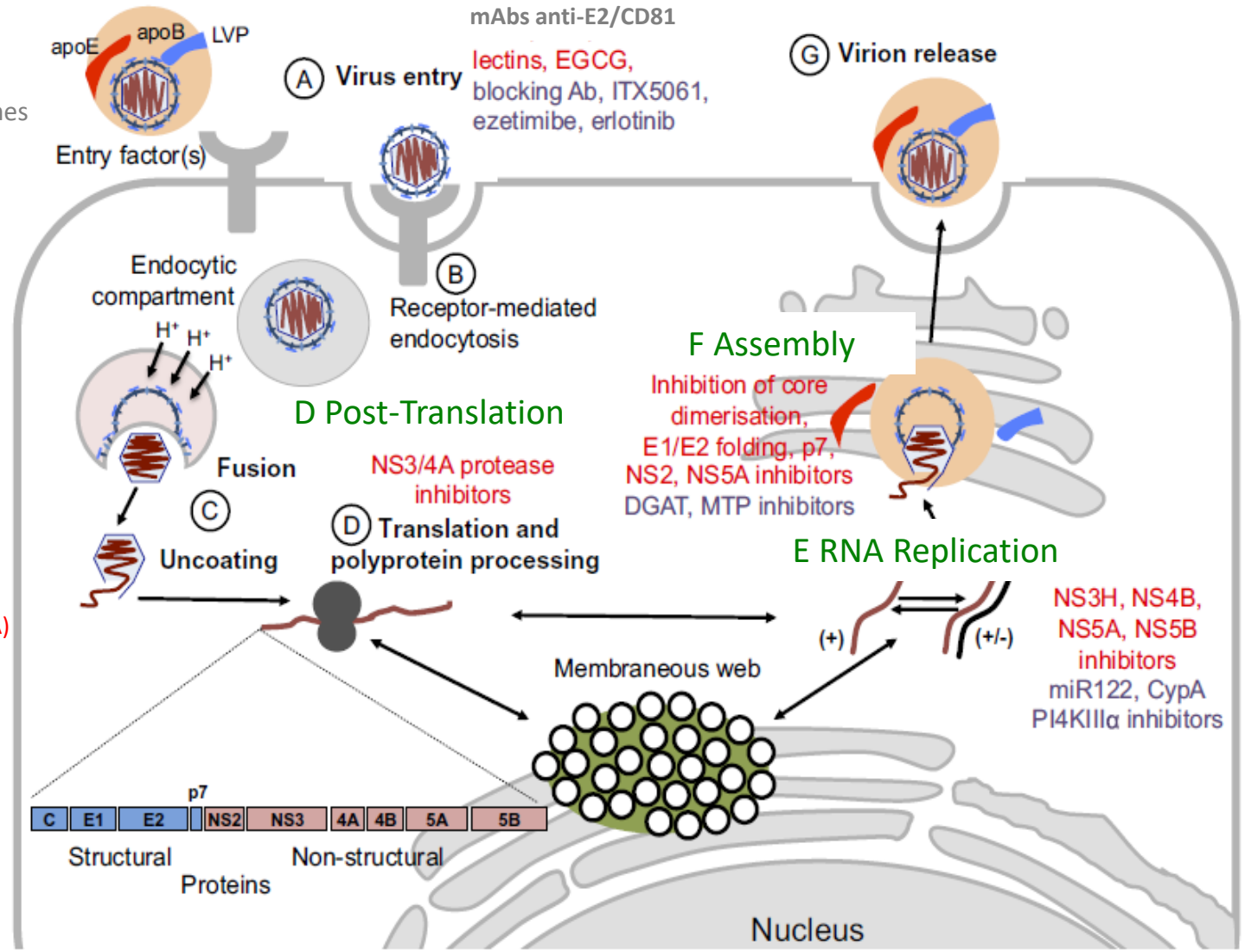
Moradpour D et al., Nature 2007

… This makes HCV curable!!!!

The better knowledge of HCV replication cycle allowed the identification of several targeted drugs

Drugs active on viral enzymes

Drugs active on host cell enzymes



D Translation

miRNA

ISIS 14803 (antisense)

AVI- 4066 (antisense)

Heptazyme (ribozyme)

VGX-410C (small molecules

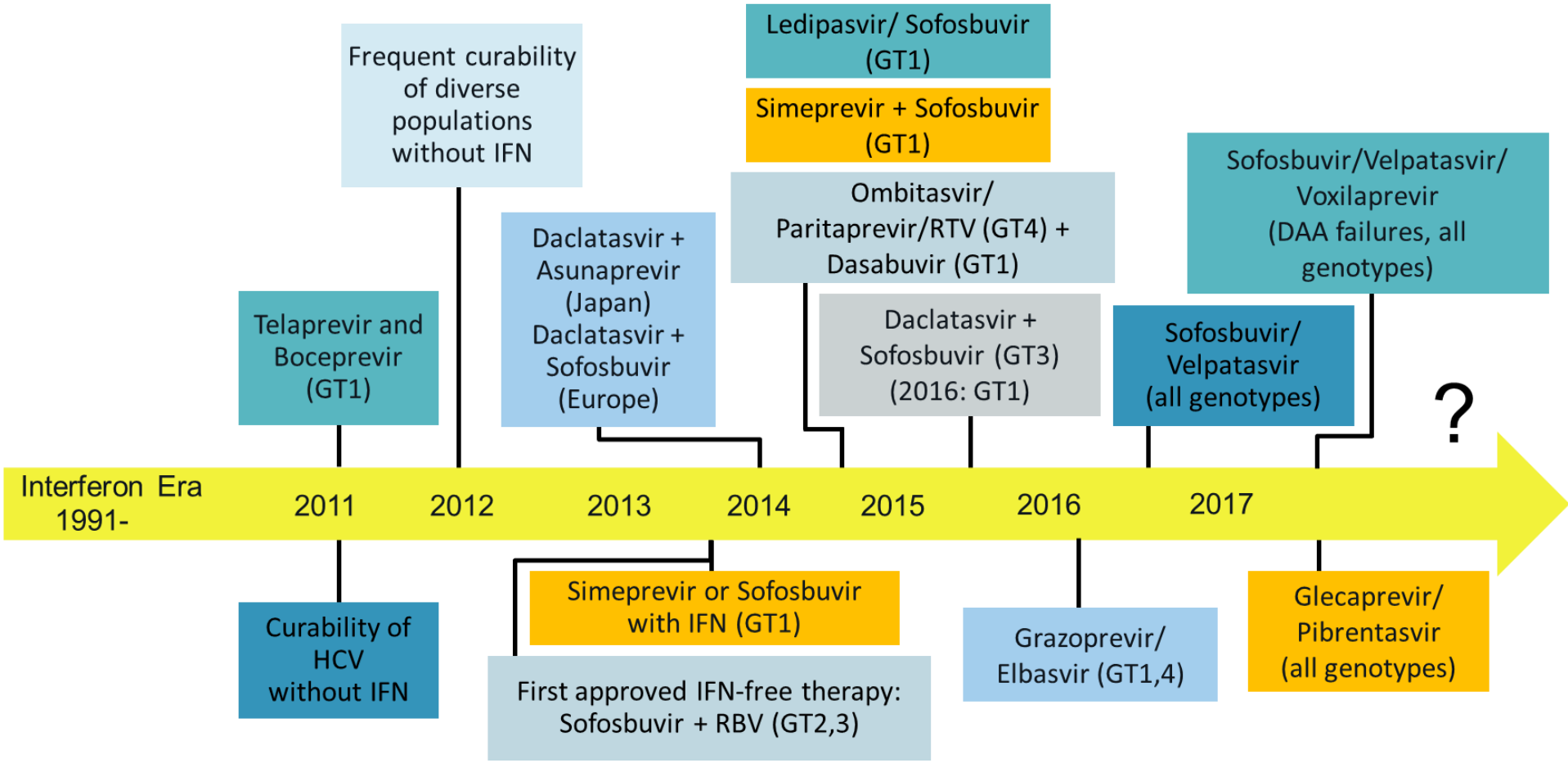
IRES inhibitor) TT 033 (sIRNA)

eIF2a phosphorilation

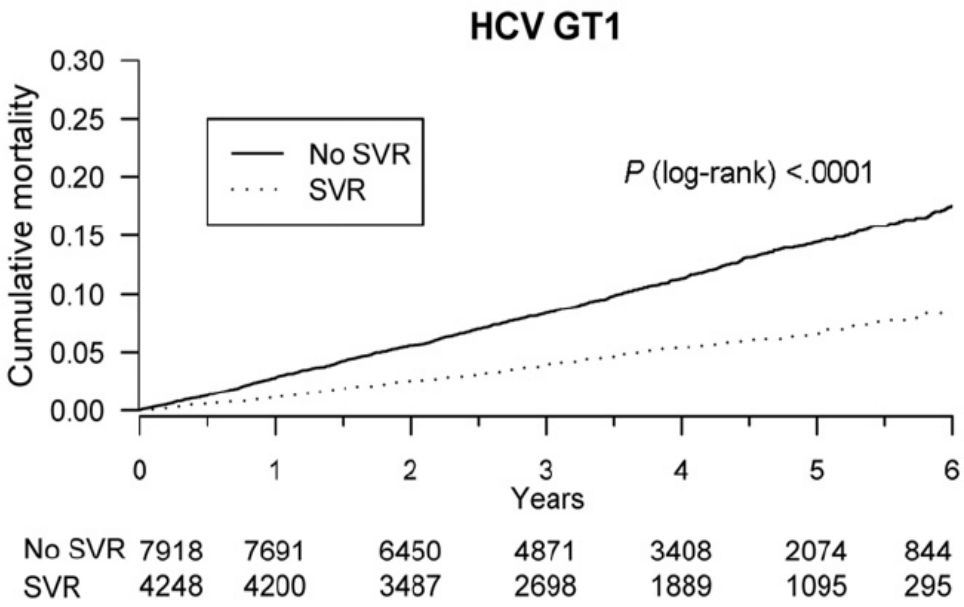
inhibitors: Nitazoxanide

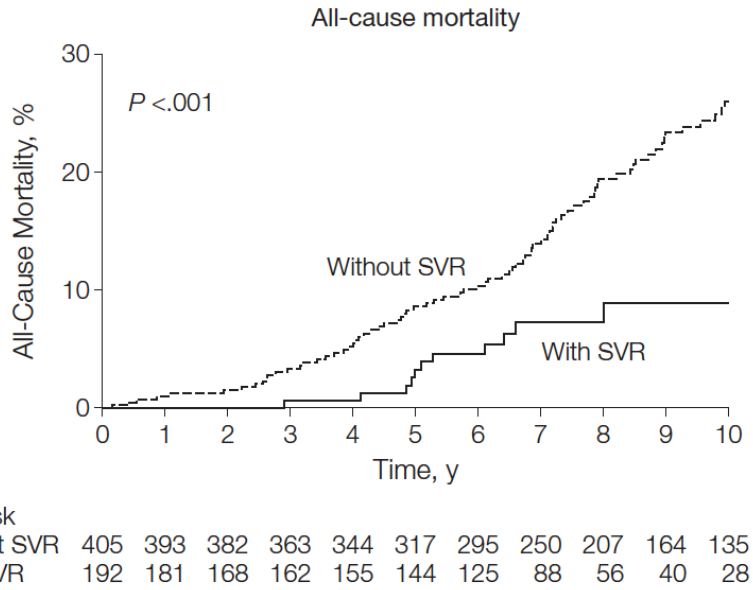
Ploss A Gut 2012

The evolution of HCV therapy



The elimination of the virus reduces mortality





SVR reduces mortality risk for each genotype:

Genotype-1 hazard ratio, 0.70; P < .0001 12,166 patients, SVR 35%
Genotype-2 hazard ratio, 0.64; P = .006 2904 patients, SVR 72%
Genotype-3 hazard ratio, 0.51; P = .0002 1794 patients, SVR 62%

Backus L, et al CGH 2011

SVR reduces mortality in patients with advanced hepatic fibrosis or cirrhosis (Ishak score 4-6)

192 patients (36%) SVR
10-year cumulative incidence rate of liver-related mortality or transplantation:
1.9% (95% CI, 0.0%-4.1%) with SVR
27.4% (95% CI, 22.0%-32.8%) without SVR
(P < .001) *Van der Meer AJ, et al JAMA 2012*

Patients who achieve SVR have substantially improved qualities of life, which include physical, emotional, and social health.

The standard of care for HCV patients has greatly improved

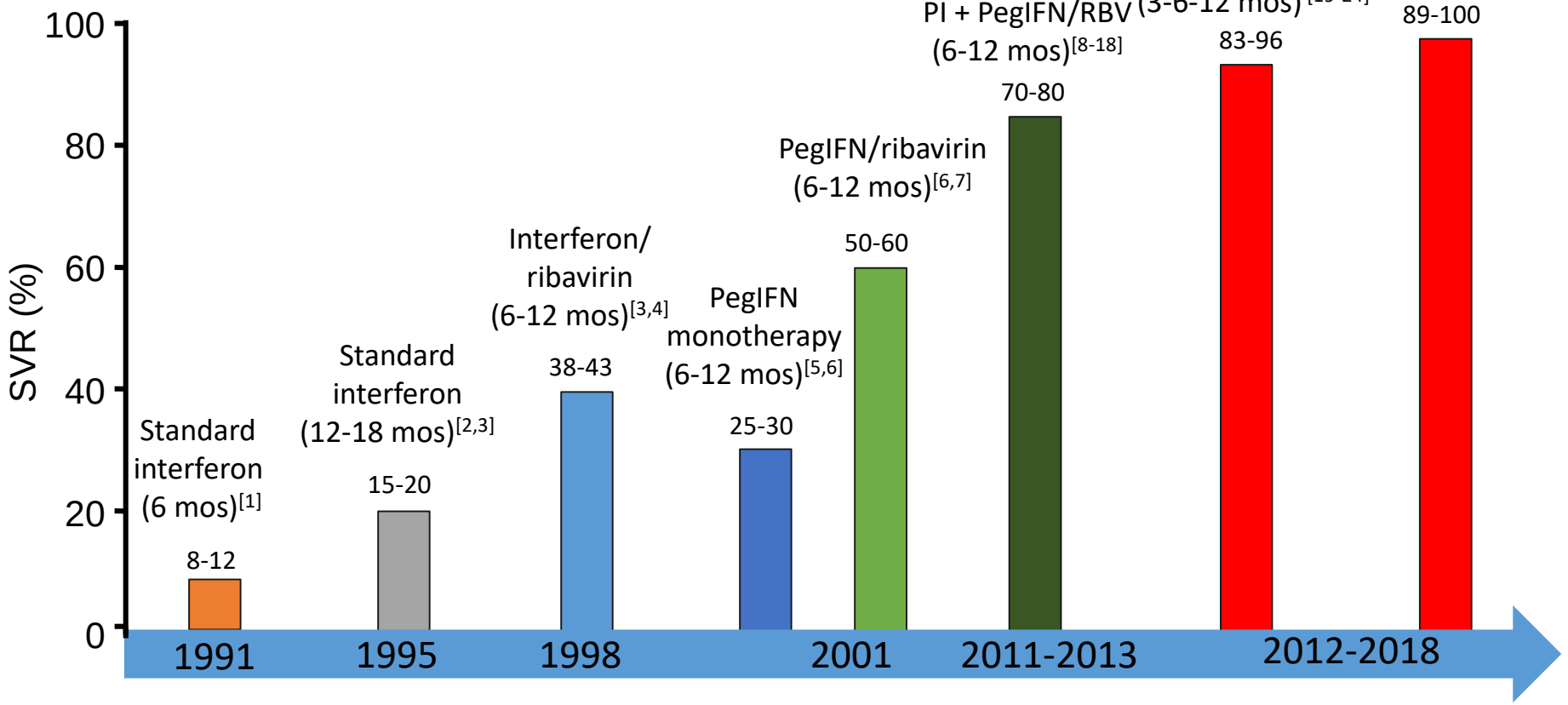
1974 Mystery virus

1989 HCV identified

1992 HCV Blood Test

1999 HCV Replicon

2005 HCV in-vitro Culture



1. Carithers RL Jr., et al. Hepatology. 1997;26(3 suppl 1):83S-88S. 2. Zeuzem S, et al. N Engl J Med. 2000;343:1666-1672. 3. Poynard T, et al. Lancet. 1998;352:1426-1432. 4. McHutchison JG, et al. N Engl J Med. 1998;339:1485-1492. 5. Lindsay KL, et al. Hepatology. 2001;34:395-403. 6. Fried MW, et al. N Engl J Med. 2002;347:975-982. 7. Manns MP, et al. Lancet. 2001;358:958-965. 8. Poordad F, et al. N Engl J Med. 2011;364:1195-1206. 9. Jacobson IM, et al. N Engl J Med. 2011;364:2405-2416. 10. Sherman KE, et al. N Engl J Med. 2011;365:1014-1024. 11. Jacobson IM, et al. 64th Annual Meeting of the American Association for the Study of Liver Diseases, 1-5 November 2013, Washington, DC. 12. Zeuzem S, et al. Gastroeneterology 2014;146:430-41. 13. Lawitz E, et al. Gastroenterology 2013;144:S-151. 14. Jensen D, et al. 64th Annual Meeting of the American Association for the Study of Liver Diseases, 1-5 November 2013, Washington, DC. 15. Jacobson I, et al. 64th Annual Meeting of the American Association for the Study of Liver Diseases, 1-5 November 2013, Washington, DC. 16. Marcellin P, et al. Gastroenterology 2013;145:790-800e3. 17. Bronowicki JP, et al. Antiviral Ther 2013;18:885-93. 18. Manns MP, et al. Hepatology 2012;56:884-93. 19. Hezode C, et al. Hepatology 2012;56:553A- 4A. 20. Dore G, et al. J Hepatol 2013;58:S570-1. 21. Lawitz E, et al. Lancet Infect Dis 2013;13:401-8. 22. Kowdley KV, et al. Lancet 2013;381:2100-7. 23. Lawitz E, et al. 64th Annual Meeting of the American Association for the Study of Liver Diseases. Washington, DC, 1-5 November 2013. 24. Lawitz E, et al. N Engl J Med 2013;368:1878-87. 25. Jacobson IM, et al. N Engl J Med 2013;368:1867-77. 26. Zeuzem S, et al. N Engl J Med 2014;370:1993-2001. 27. Osinusi A, et al. JAMA 2013;310:804-11. 28. Jacobson IM, et al. 64th Annual Meeting of the American Association for the Study of Liver Diseases. Washington, DC, 1-5 November 2013. 29. Sulkowski MS, et al. N Engl J Med 2014;370:211-21. 30. Zeuzem S, et al. N Engl J Med 2014;370:1889-9. 31. Afdhal N, et al. N Engl J Med 2014;370:1483-9. 32. Feld JJ, et al. N Engl J Med 2014;370:1594-603. 33. Zeuzem S, et al. N Engl J Med 2014;370:1604-14. 34. Ferenci P, et al. N Engl J Med 2014;370:1983-9. 35. Poordad F, et al. N Engl J Med 2014;370:1973-82. 36. Lawitz E, et al. 64th Annual Meeting of the American Association for the Study of Liver Diseases, Washington, DC, 1-5 November 2013. 37. Gane EJ, et al. Gastroenterology 2014;146:736-43e1.

Overall efficacy of different anti-HCV treatments in Italian real-life practice is 95-98%

Failure rate following the first DAA regimen in patients with advanced disease is similar to or lower than that reported in clinical trials (3.6%), although the majority of patients were treated with suboptimal regimens.

Table 3. Failure rates following the first DAA regimen, by HCV genotype and treatment regimen in patients who completed the 12 weeks post treatment evaluation (n = 3,830 patients).

	Overall	HCV genotype N. of failures/N. of treated patients (%)					
DAA regimen	N. of failures/N. of treated patients (%)	1a	1b	2	3	4	5
	139/3830 (3.6)						
SOF+RBV	68/710 (9.6)	5/15 (33.3)	20/56 (35.7)	8/499 (1.6)	32/132 (24.2)	3/8 (37.5)	-
SOF+SIM±RBV	38/683 (5.6)	8/99 (8)	24/520 (4.6)	1/2 (50)	1/1 (100)	3/60 (5)	1/1 (100)
SOF+LDV±RBV	16/1002 (1.6)	3/200 (1.5)	10/752 (1.3)	-	0/1 (0)	3/44 (6.8)	0/5 (0)
3D±RBV	9/894 (1)	3/86 (3.5)	6/806 (0.7)	-	-	0/2 0	-
2D+RBV	2/64 (3.1)	-	-	-	-	2/59 3.4%	0/5 (0)
SOF+DCV±RBV	6/471 (1.3)	0/47 0	1/115 (0.9)	0/55 (0)	5/244 (2)	0/10 (0)	
SIM+DCV	0/6 (0)	-	0/6 (0)	-	-	-	-

https://doi.org/10.1371/journal.pone.0185728.t003

Data on HCV genotype, liver disease severity, and first and second line DAA regimens were prospectively collected in consecutive patients who reached the 12-week post-treatment and retreatment evaluations from January 2015 to December 2016 in 23 of the PITER network centers.

Kondili, et al Plos One 2017

Treatment of chronic Hepatitis C: changing the horizon

High anti-viral effect: 90-95%
Great possibility to use DAAs across all spectrum of the disease
Mild-to-moderate-advanced decompensated-pre / post-transplant

Aim at individual level

Abolishing liver disease progression
Regression of the hepatic damage
Reducing liver and non-liver complications

At individual level: treat infection/ liver disease

Aim at community level

Reduce (abolish) the spread of HCV infection
Reduce disease burden
Elimination of HCV infection

At community level: treating infection; those with high potential for transmission

Disease Eradication *vs* Elimination *vs* Control

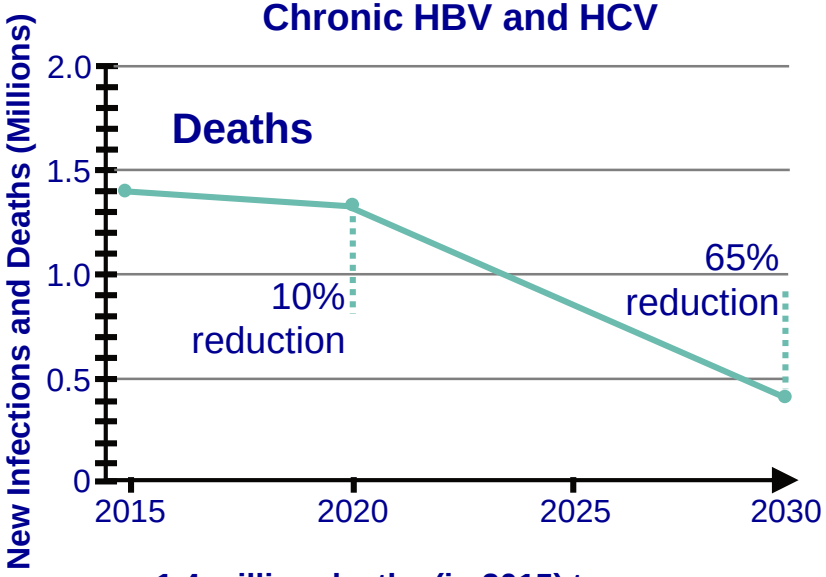
……From individual health to community health perspective……

- **Control:** reduction in the incidence, prevalence, morbidity, or mortality of an infectious disease to a locally acceptable levels; continued intervention measures required
- **Elimination:** reduction to zero of incidence in a defined geographical area as a result of deliberate efforts; continued intervention measures required
- **Eradication:** permanent reduction to zero of the worldwide incidence of infection; intervention measures no longer needed
  - Only 1 example: smallpox

Molyneux DH, et al. Trends Parasitol. 2004;20:347-351.

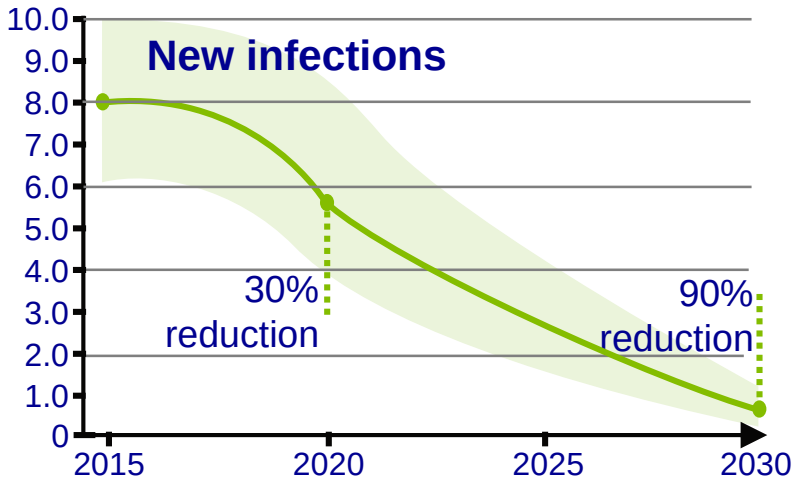
Proposed WHO targets for reducing new infections and stopping deaths

65% Reduction in Deaths from Chronic HBV and HCV



1.4 million deaths (in 2015) to under 500,000 deaths (by 2030)

90% Reduction in New Cases of Chronic HBV and HCV Infection

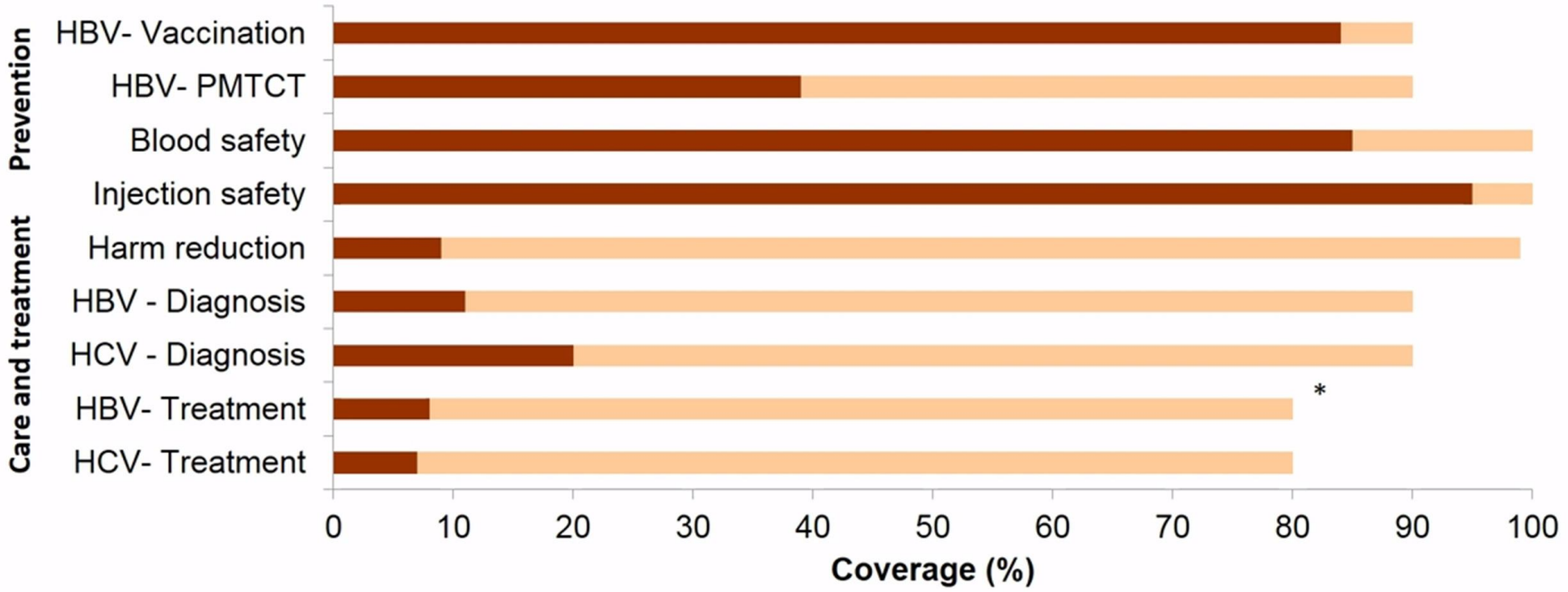


6–10 million (in 2015) to 900,000 infections (by 2030)
95% decline in HBV infections
80% decline in HCV infections

Elimination: reduction to zero of incidence in a defined geographical area as a result of deliberate efforts; continued intervention measures required.

WHO global health sector strategy on viral hepatitis 2016–2021. Available at: http://www.who.int/hepatitis/strategy2016-2021/ghss-hep/en/ (accessed March 2018).

GLOBAL ELIMINATION STRATEGY:
2015 BASELINE TOWARDS 2030 TARGETS

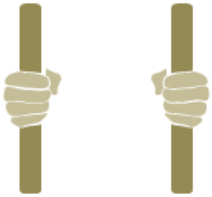


Where Are the Undiagnosed and Untreated?

PWID (people who inject drugs)[1]

MSM (men who have sex with men)[1]

Ethnic Minorities/Migrants[1]



Prisoners[1]

Certain birth cohorts[2]

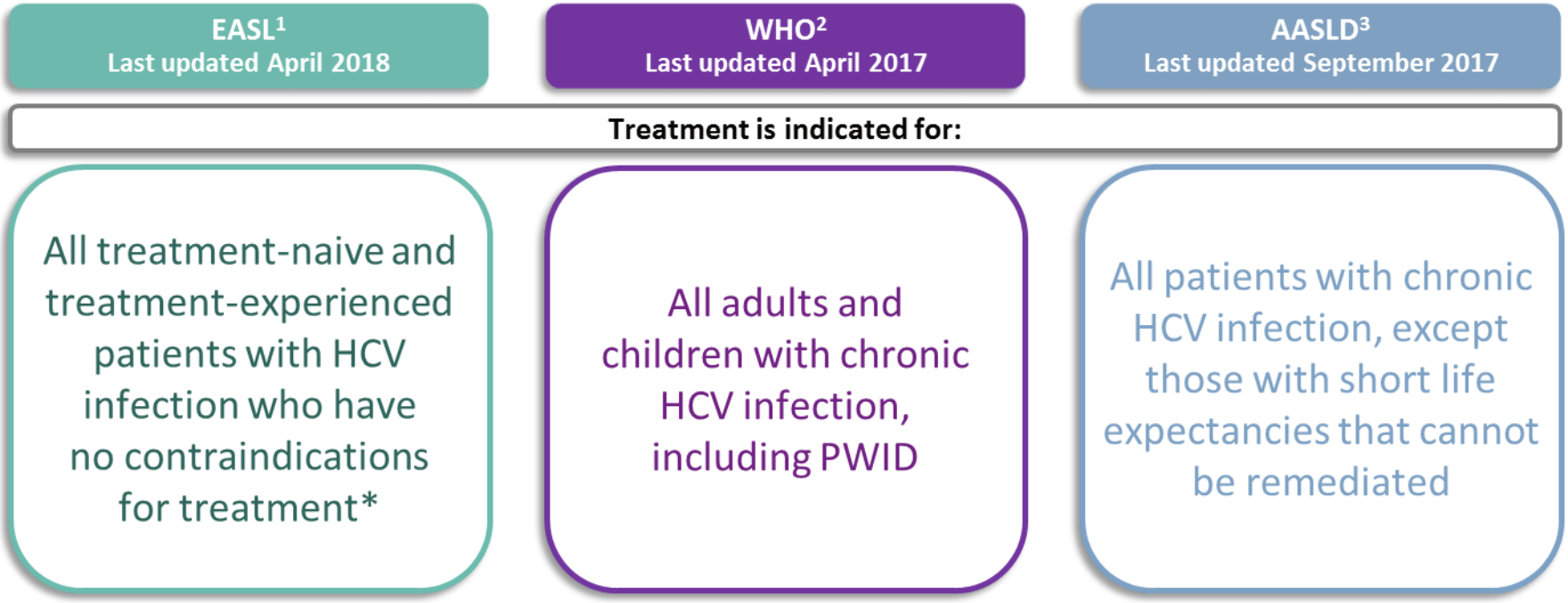
People living in countries with restricted access to treatment[1]

Patients who are lost to follow-up

Patients with chronic kidney disease[3]

1. Global Hepatitis Report 2017. Geneva: World Health Organization; 2017. License: CC BY-NC-SA 3.0 IGO. **2.** WHO guidelines on hepatitis B and C testing. Geneva: World Health Organization; 2017. License: CC BY-NC-SA 3.0 IGO. **3.** Ladino M et al. *J Am Soc Nephrol*. 2016;27:2238-2246.

Evolution of Treatment Guidelines: Treatment Is Now Indicated for All Patients

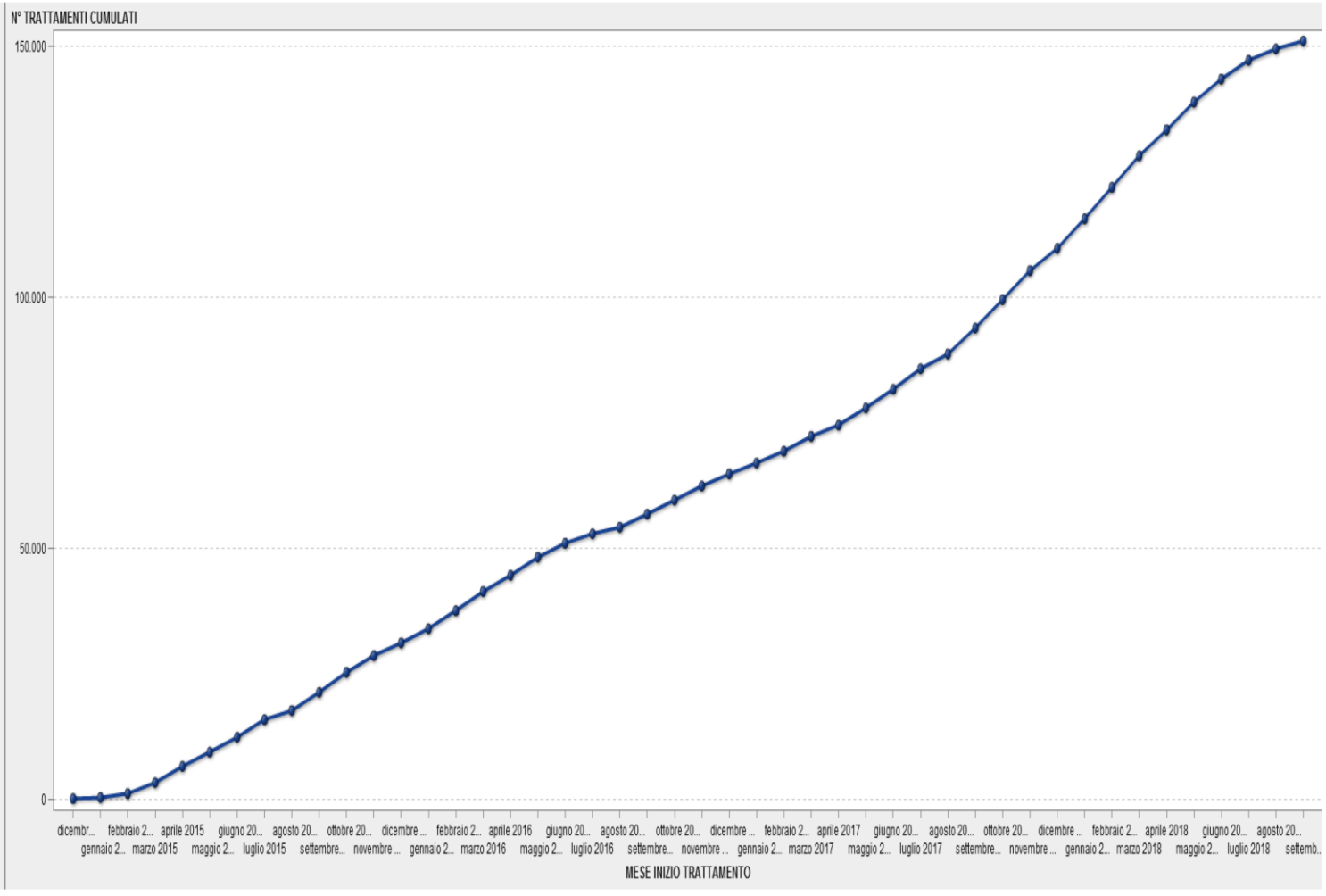


** Treatment is generally not recommended in patients with limited life expectancy because of non-liver-related comorbidities.*

EASL recommendations on the treatment of hepatitis C 2018. *J Hepatol* 2018; E-pub ahead of print (doi: 10.1016/j.jhep.2018.03.026).
WHO guidelines for the screening, care and treatment of persons with chronic HCV infection. Available at: http://apps.who.int/iris/bitstream/10665/205035/1/9789241549615_eng.pdf?ua=1 (accessed March 2018);
AASLD recommendations for testing, managing and treating hepatitis C. Available at: http://www.hcvguidelines.org/full-report-view (accessed March 2018).

Aggiornamento dati 17 settembre 2018

Trend cumulativo dei trattamenti avviati

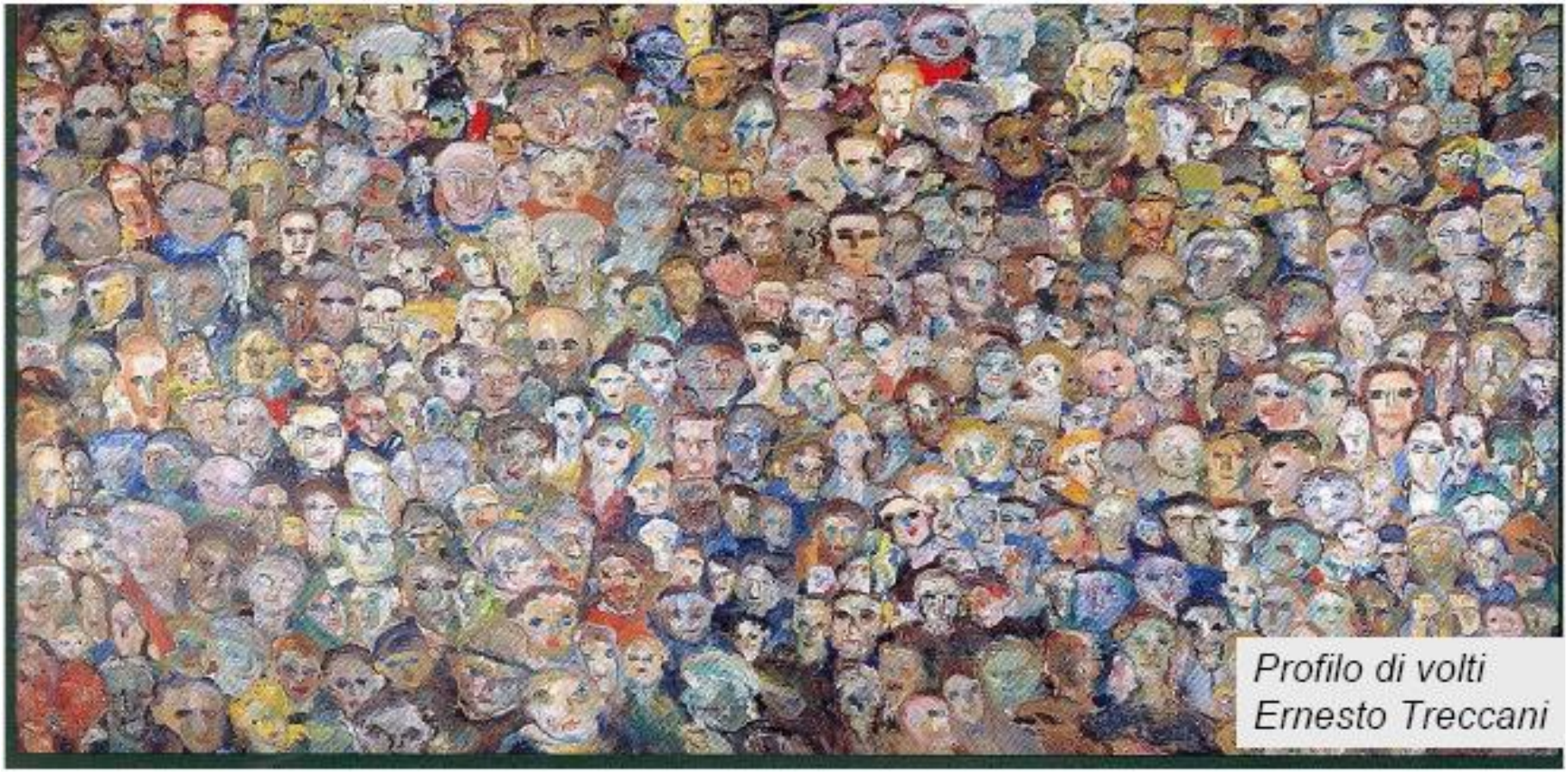


151.096 «avviati» sono i trattamenti (solo pazienti eleggibili)
con almeno una scheda di Dispensazione farmaco

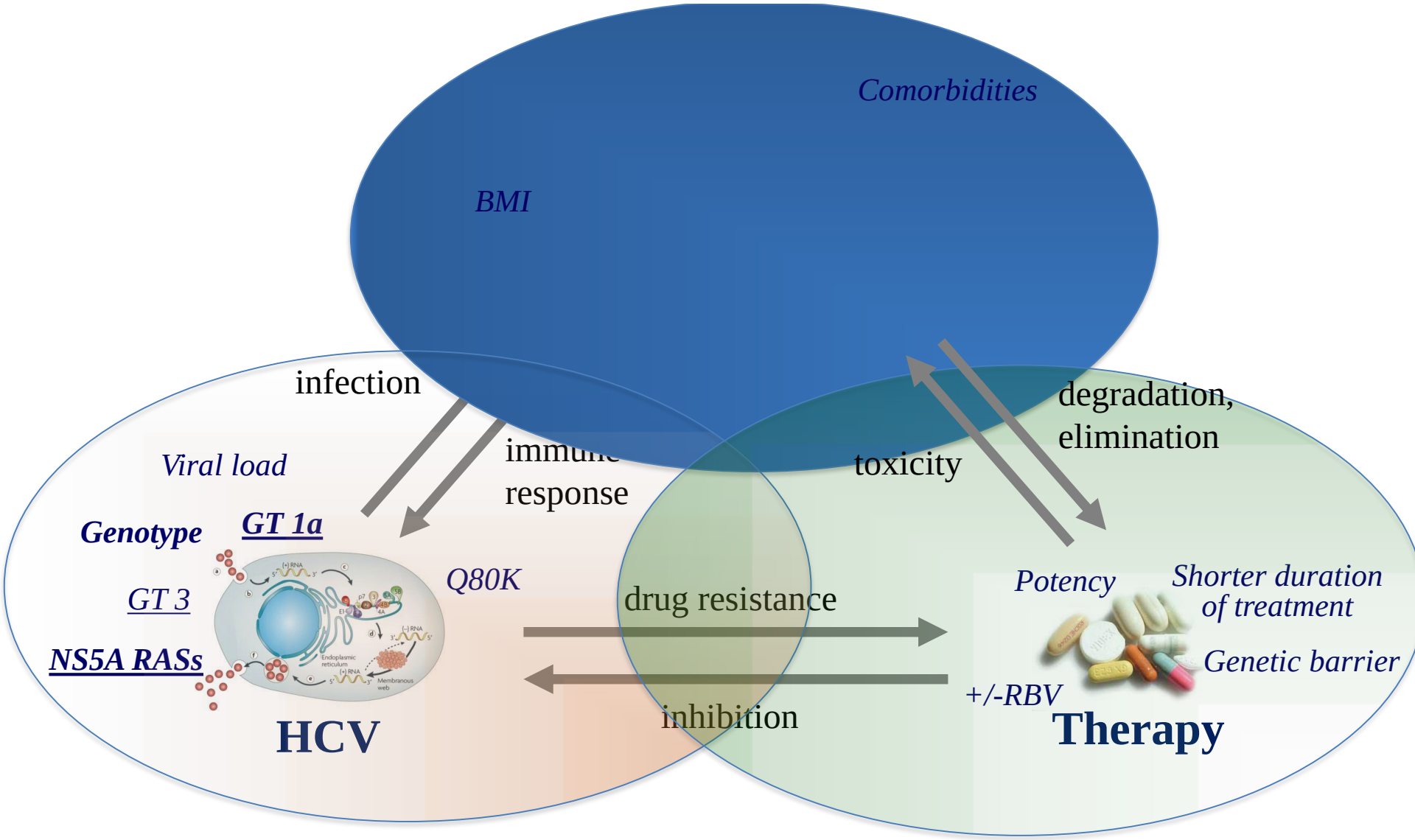
Many lessons learnt from HIV can be helpful for designing adequate treatment strategies against viral hepatitis such as HCV….

The personalized medicine

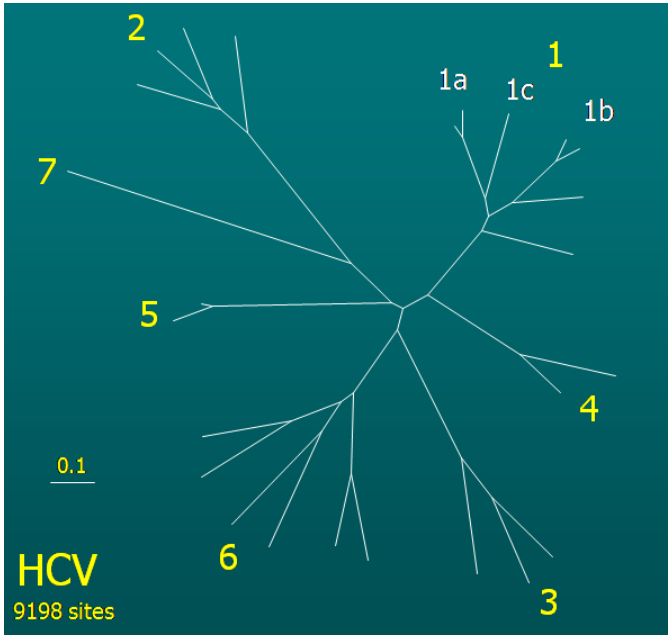
All international guidelines focus on the importance of **tailoring antiretroviral therapy** to the individual patient, on the basis of **HIV-1 genetic data**, integrated with clinical, laboratory and therapeutic information.

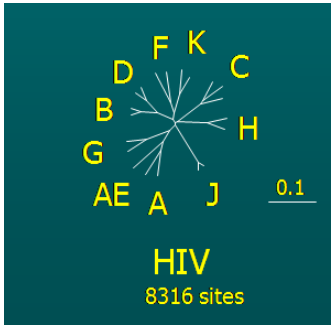


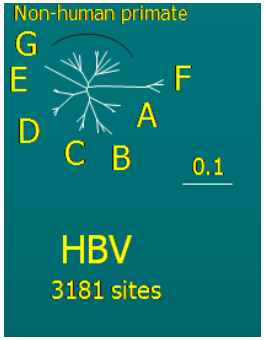
Many factors contribute to viral response to DAA-treatment

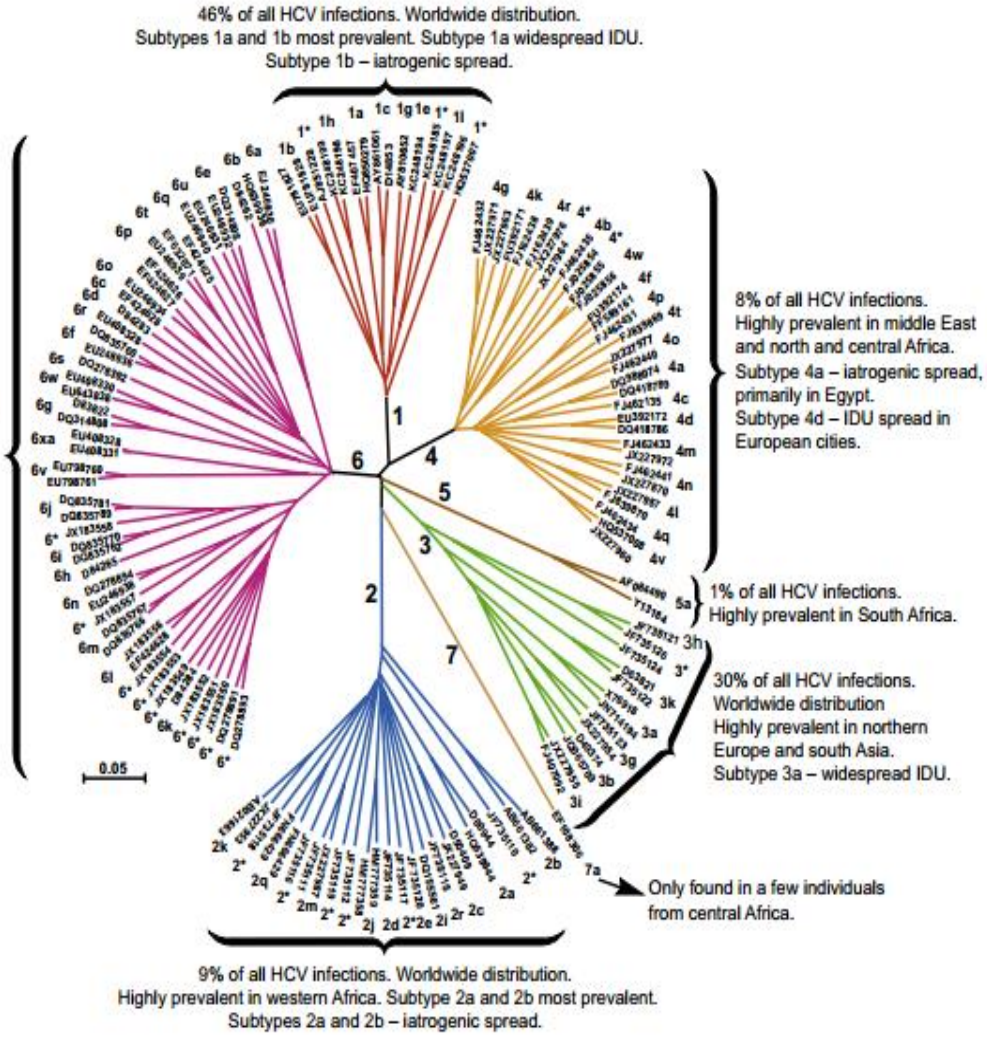


HCV genetic variability is higher than HIV's and HBV's





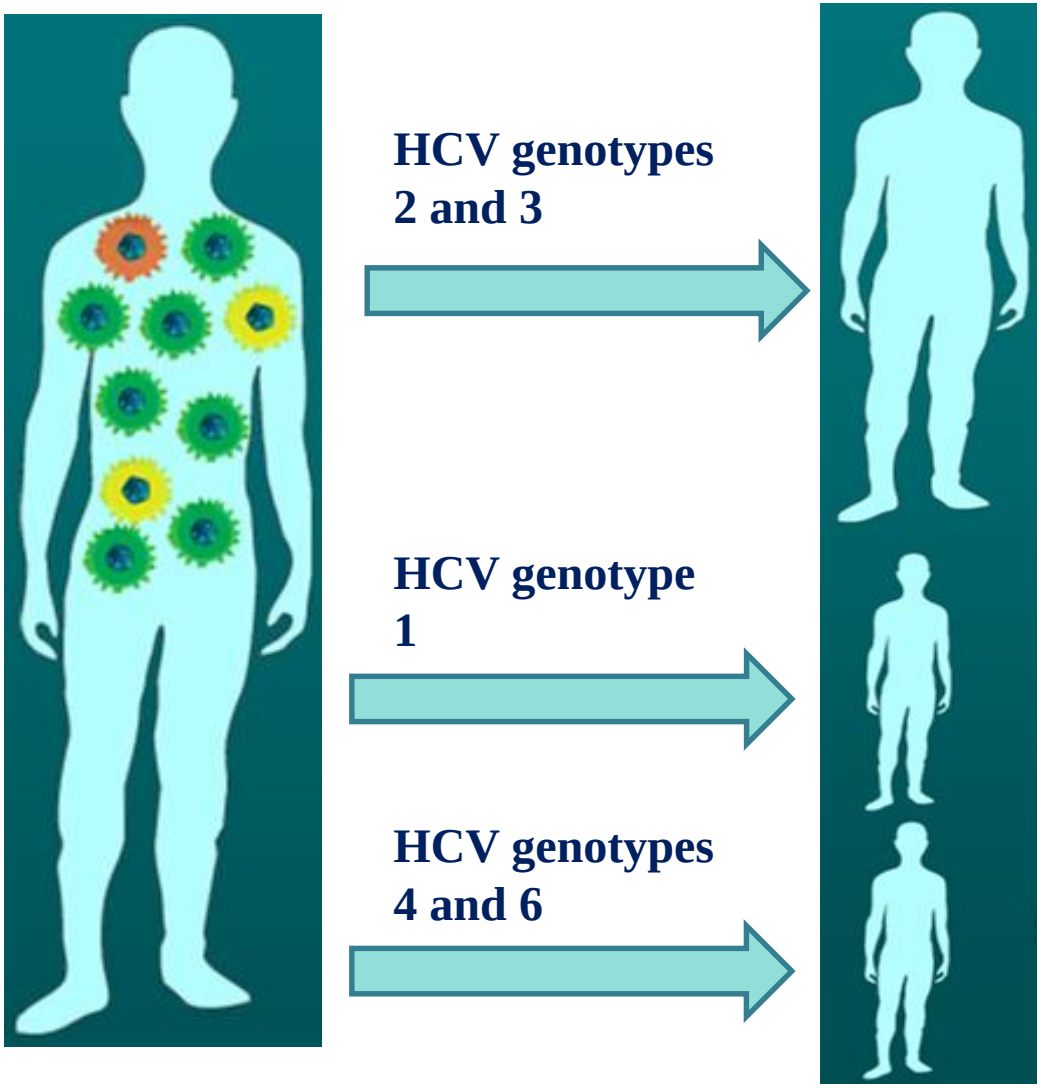




Jens Bukh 2016 J Hepatol

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

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%

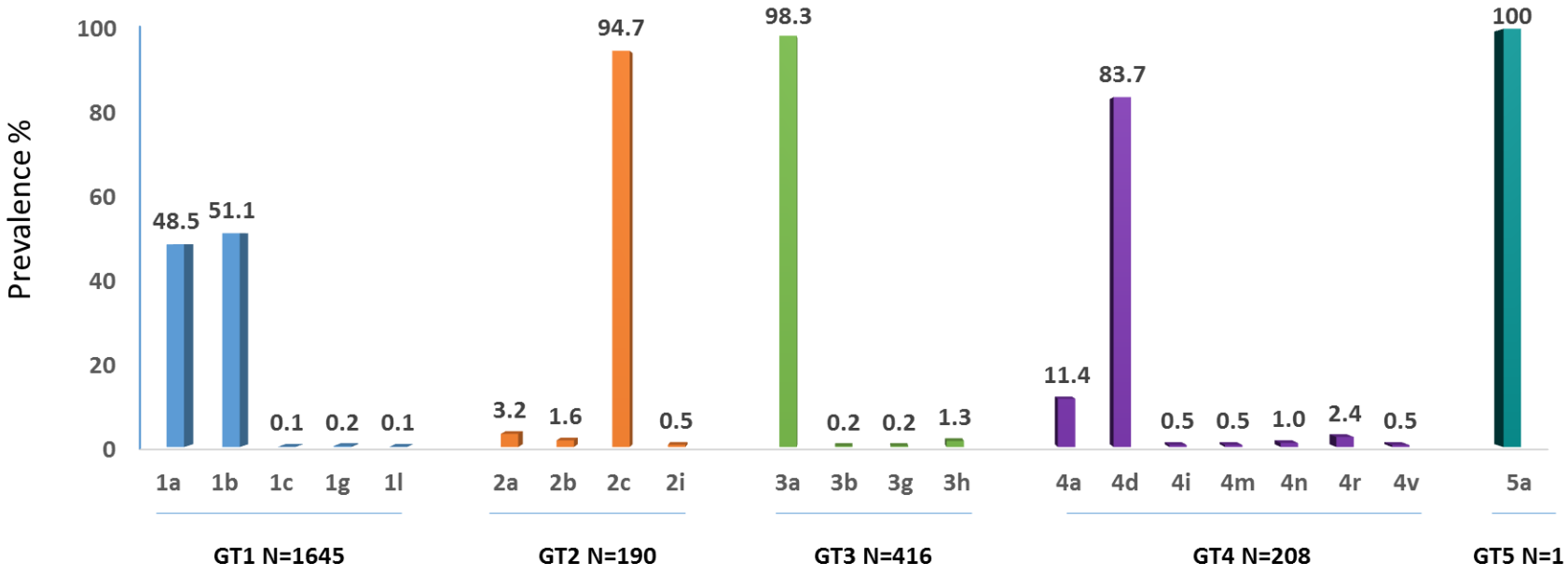
HCV genotypes 4 and 6

SVR = 42-52 %

Manns, Lancet 2001; Fried, N Engl J Med 2002; Hadziyannis, Ann Intern Med 2004; Alfaleh, Liver Int 2004

The underlying functional mechanisms for lower SVR rates of the different HCV genotypes were unknown

Distribution of HCV genotypes/subtypes within the Italian resistance database Vironet C (N=2460 patients)



The Journal of Infectious Diseases

MAJOR ARTICLE

Identification of a Novel Hepatitis C Virus Genotype From Punjab, India: Expanding Classification of Hepatitis C Virus Into 8 Genotypes

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A novel HCV GT was recently identified in 4 patients originating from Punjab, India

This novel HCV GT, **GT8**, is genetically distinct from previously identified HCV GT1–7 with >30% nucleotide sequence divergence to the established HCV subtypes.

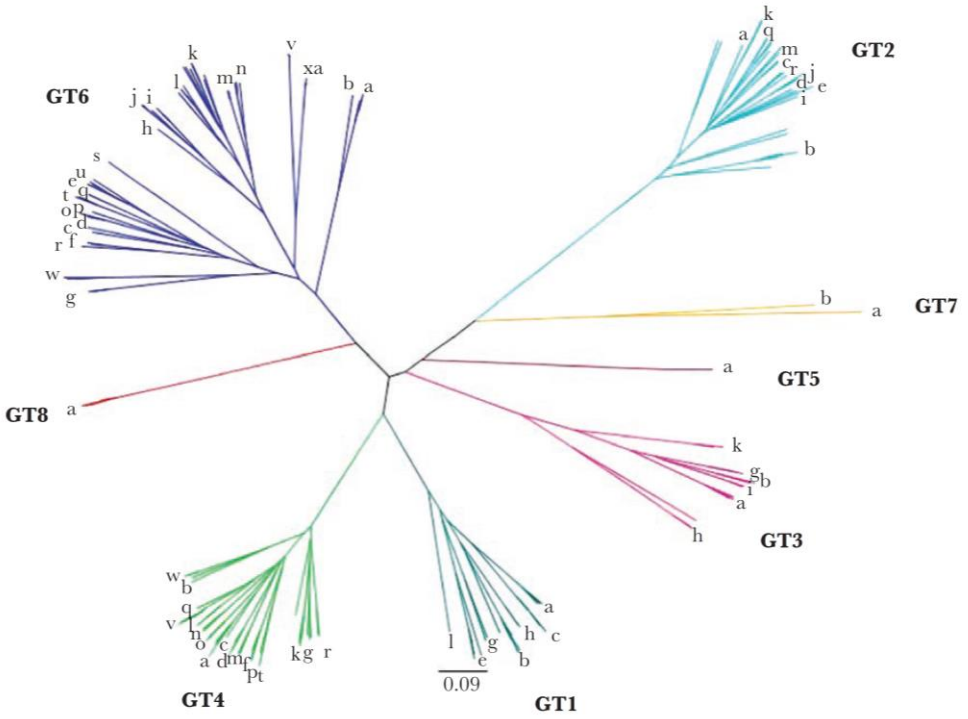


Figure 2. Punjabi districts from which patients with hepatitis C virus genotype 8 originated. Two patients were from the Ludhiana district, 1 from the Barnala district and 1 from the Sangrur district. Insert, Punjab State in red.

The estimated prevalence of HCV infection in India is approximately 0.5%–2.0%, with GT3 being most common. Despite the low prevalence of HCV, India with its large population accounts for a significant proportion of the global HCV burden with approximately 12–18 million people infected

Borgia SM et al., JID 2018

The four patients were previously identified to be infected with GT5 by LiPA or Abbott RealTime polymerase chain reaction assays

Despite presence of baseline resistance-associated substitutions within the GT8 virus of all 4 patients, all patients achieved a sustained virologic response; 2 treated with sofosbuvir/velpatasvir/voxilaprevir for 8 weeks, 1 with sofosbuvir/ledipasvir plus ribavirin for 24 weeks and 1 with sofosbuvir plus daclatasvir for 12 weeks.

Table 1. Characteristics of Patients with GT8 HCV Infection

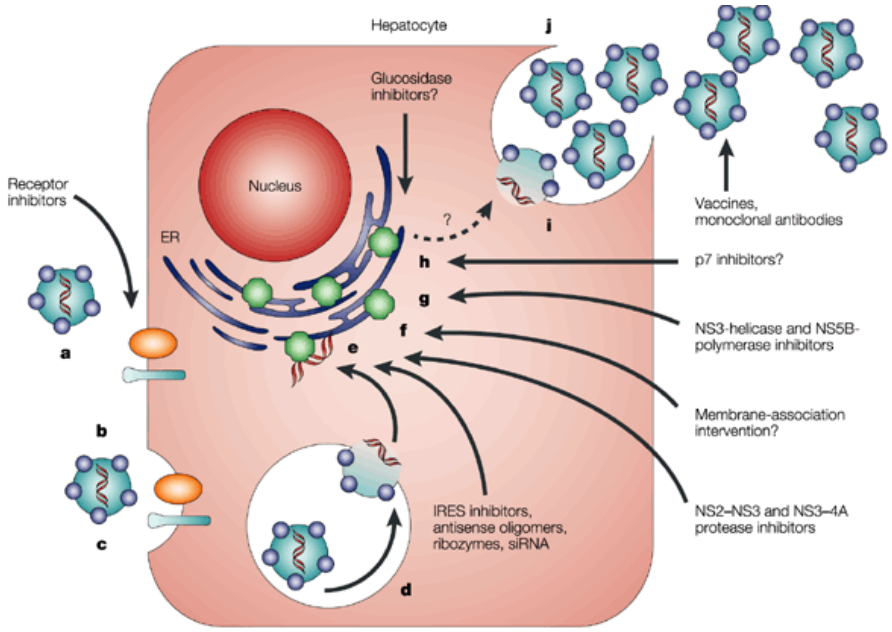
Patient	HCV VL (IU/mL)	Country (Origin)	Race	Age	Sex	GT by Abbott or LiPA	GT by Phylo Analyses	Resistance-Associated Substitutions[a]			Treatment	SVR12
								NS3 RASs	NS5A RASs	NS5B RASs		
1	20 100 000	CAN (Kalala village, Barnala District, Punjab, India)	Asian	28	Male	GT5	GT8	V36L Q80K	Q30S Y93S	None	SOF/VEL/VOX 8 wks	Yes
2	8 710 000	CAN (Rampura village, Sangrur District, Punjab State, India)	Asian	31	Male	GT5	GT8	V36L Q80R	Q30S Y93S	None	SOF/VEL/VOX 8 wks	Yes
3	4 735 001	CAN (Ludhiana City, Ludhiana District, Punjab, India)	Asian	40	Male	GT5	GT8	V36L Q80R	Q30S Y93S	None	SOF + DCV 12 wks	Yes
4	4 200 000	CAN (Raikot City, Ludhiana District, Punjab State, India)	Asian	66	Female	GT5	GT8	V36L Q80K	Q30S Y93S	None	LDV/SOF + RBV 24 wks	Yes

Abbreviations: CAN, Canada; DAA, direct-acting antiviral; DCV, daclatasvir; GT, genotype; HCV, hepatitis C virus; LVD, ledipasvir; RAS, resistance-associated substitution; RBV, ribavirin; SOF/VEL/VOX, sofosbuvir/velpatasvir/voxilaprevir; SVR, sustained virologic response; VL, viral load.

[a]RASs are defined as substitutions that confer reduced susceptibility to any approved DAA inhibitor with >2.5-fold change compared with GT1a reference (HCV1a H77 NC AF009606).

Borgia SM et al., JID 2018

Mutations occur frequently during the replication of 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.

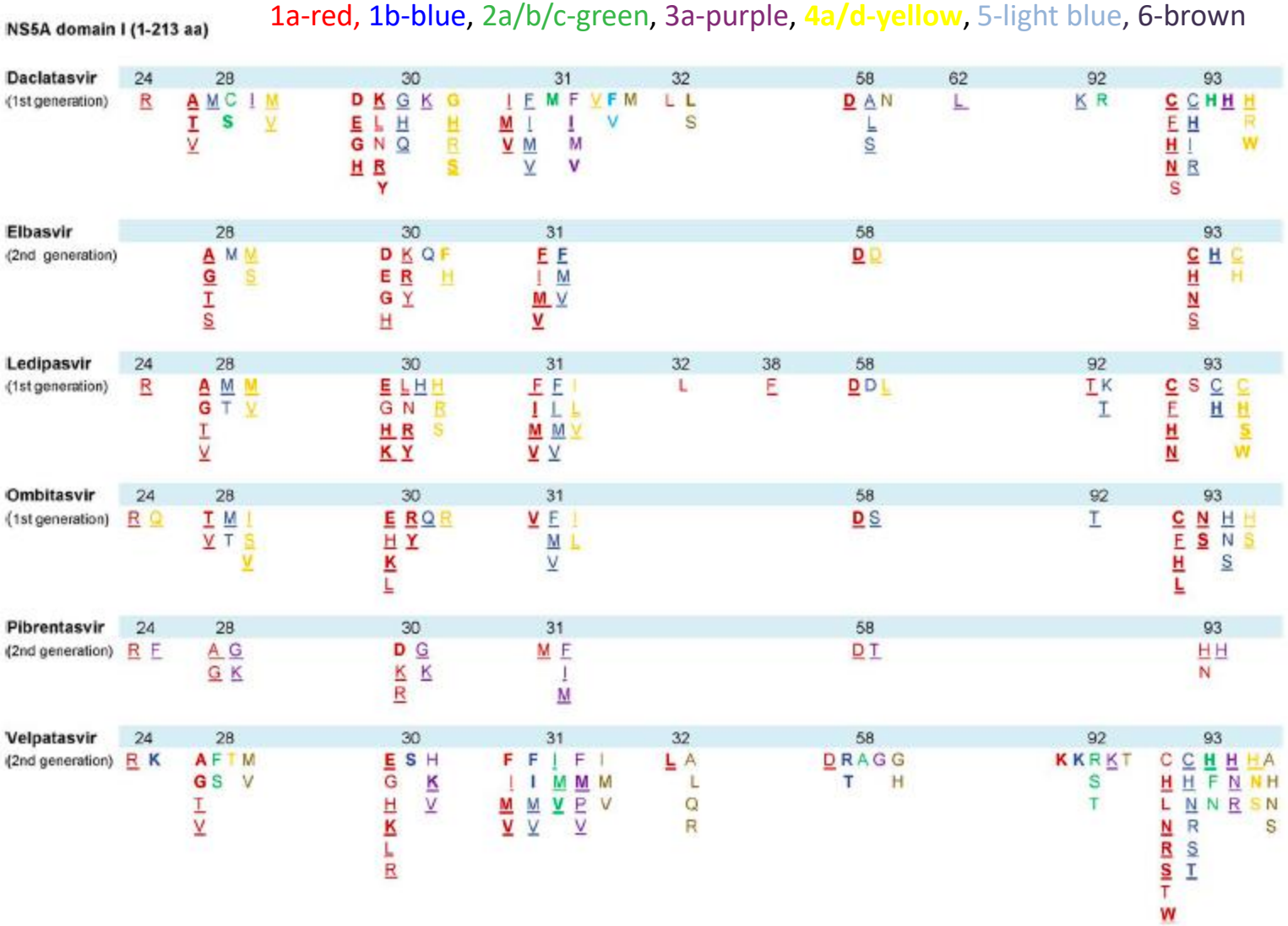
Time	Number of nucleotide changes	Probability	Number of virions generated per day	Number of all possible mutants	Fraction of all possible mutants created per day
Before therapy	0	0.91	9.1×10^{11}		
	1	0.087	8.7×10^{10}	2.9×10^{4}	1
	2	0.0042	4.2×10^{9}	4.1×10^{8}	1
	3	0.00013	1.3×10^{8}	4.0×10^{12}	3.4×10^{-5}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

Not all NS5A RASs are equally clinical relevant

1a-red, 1b-blue, 2a/b/c-green, 3a-purple, 4a/d-yellow, 5-light blue, 6-brown



Summary of NS5A substitutions associated with resistance to NS5A inhibitors. HCV genotypes and subtypes are represented by different colors: 1a-red, 1b-blue, 2a/b/c-green, 3a-purple, 4a/d-yellow, 5-light blue, 6-brown. Amino acid substitutions detected *in vivo* in DAA failing patients are underlined, independently of *in vitro* data information. In addition, NS5A RASs detected only *in vitro* but associated with fold-change in drug activity compared to the wild-type replicons ≥100 (1st generation NS5A-inhibitors,) or ≥3 (2nd generation NS5A-inhibitors) are also included in the figures. For 1st generation NS5A-inhibitors, *in vivo* substitutions with fold-change ≥100, and *in vitro* substitutions with fold-change > 000 are represented in bold. For 2nd generation NS5A-inhibitors, in vivo and/or in vitro substitutions with fold-change >10 are represented in bold.

Sorbo MC , et al Drug Resistance Update 2018

HCV genotype still dictates the choice of anti-HCV drugs and can modulate the duration of treatment in infected patients with chronic hepatitis C

The HCV genotype, including genotype 1 subtype (1a or 1b), should be assessed prior to treatment initiation.

EASL Recommendations on Treatment of Hepatitis C 2018

Genotype	Pangenotypic regimens			Genotype-specific regimens		
	SOF/ VEL	GLE/PIB	SOF/ VEL/ VOX	SOF/ LDV	GZR/ EBR	OBV/ PTV/r + DSV
Genotype 1a	Yes	Yes	No*	Yes[a]	Yes[b]	No
Genotype 1b	Yes	Yes	No*	Yes	Yes	Yes
Genotype 2	Yes	Yes	No*	No	No	No
Genotype 3	Yes[c]	Yes	Yes[d]	No	No	No
Genotype 4	Yes	Yes	No*	Yes[a]	Yes[e]	No
Genotype 5	Yes	Yes	No*	Yes[a]	No	No
Genotype 6	Yes	Yes	No*	Yes[a]	No	No

DSV, dasabuvir; EBR, elbasvir; GLE, glecaprevir; GZR, grazoprevir; IFN, interferon; LDV, ledipasvir; OBV, ombitasvir; PIB, pibrentasvir; PTV, paritaprevir; r, ritonavir; SOF, sofosbuvir; VEL, velpatasvir; VOX: voxilaprevir.

* Triple combination therapy efficacious but not useful due to the efficacy of double combination regimens.

[a] Treatment-naïve patients without cirrhosis or with compensated (Child-Pugh A) cirrhosis.

[b] Treatment-naïve and treatment-experienced patients without cirrhosis or with compensated (Child-Pugh A) cirrhosis with an HCV RNA level ≤800,000 IU/ml (5.9 Log_{10} IU/ml).

[c] Treatment-naïve and treatment-experienced patients without cirrhosis.

[d] Treatment-naïve and treatment-experienced patients with compensated (Child-Pugh A) cirrhosis.

[e] Treatment-naïve patients without cirrhosis or with compensated (Child-Pugh A) cirrhosis with an HCV RNA level ≤800,000 IU/ml (5.9 Log_{10} IU/ml).

Rating: Class I, Level A

Testing for HCV genotype is recommended to guide selection of the most appropriate antiviral regimen.

AASLD AMERICAN ASSOCIATION FOR THE STUDY OF LIVER DISEASES

HCV Guidance: Recommendations for Testing, Managing, and Treating Hepatitis C

Update 21 Sept 2017

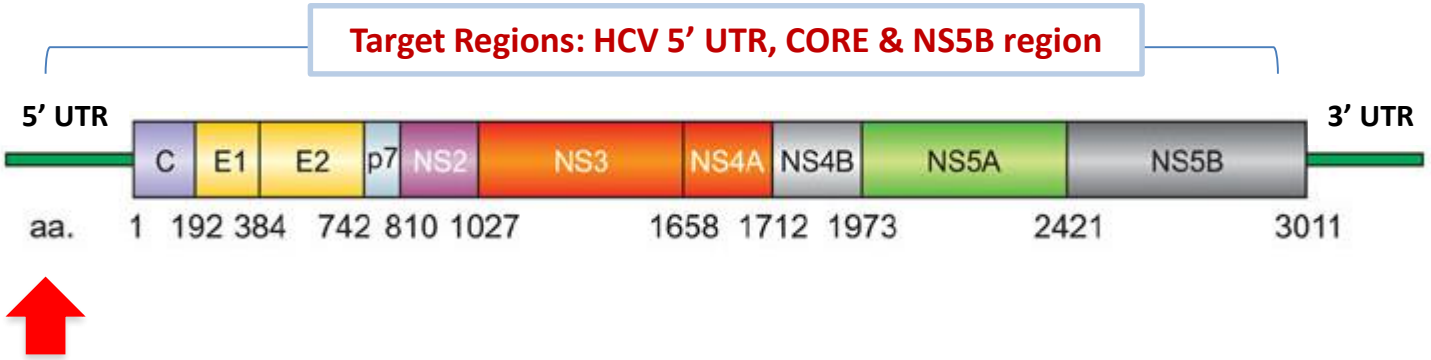
IDSA Infectious Diseases Society of America

HCV GT	Regimen	Duration, Wks	
		No Cirrhosis	Compensated Cirrhosis
1	GLE/PIB	8	12
	GZR/EBR	12	12
	SOF/LDV	8 or 12	12
	SOF/VEL	12	12
2 or 3	GLE/PIB	8	12
	SOF/VEL	12	12
4	GLE/PIB	8	12
	SOF/VEL	12	12
	GZR/EBR	12	12
	SOF/LDV	12	12
5 or 6	GLE/PIB	8	12
	SOF/LDV	12	12
	SOF/VEL	12	12

Several commercial assays are available for determining genotype/subtype

First-generation assays target the **5'UTR** for genotypes 1-6.

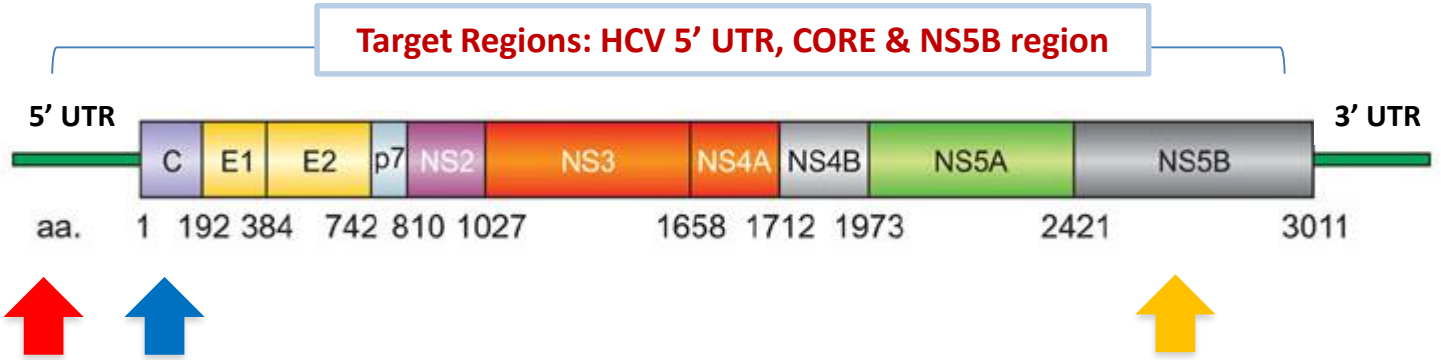
Concordance of the TRUGENE assay with NS3/4A or NS5B sequence-based genotype subtyping assays on 1461 samples tested was **79.6%** (*Sarrazin C et al., Antivir Res 2015*)



Trugene HCV Genotyping assay	Direct sequencing
INNO-LiPA HCV 1.0	Reverse hybridization

Several commercial assays are available for determining genotype/subtype

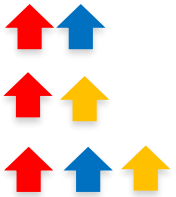
In addition to **5'UTR**, INNO-LiPA-HCV-2.0, Abbott and Cobas HCV genotyping target also the **NS5B** and/or the **core** genes, providing additional information for a correct subtyping.



INNO-LiPA HCV 2.0 Reverse hybridization

Abbott RealTime HCV Genotype II assay Real time PCR

Cobas Roche HCV genotyping Real time PCR



Issues in HCV genotyping

HCV Sanger sequencing confirmed the previous genotype by commercial-assays in 89.7% of cases analysed

	Patients (N)	Patients (%)
Genotype/subtype confirmed	1627	89.7
"Problematic" genotype no assignment		

Overall, 95 out of 1813 (5.2%) HCV infected patients candidate to start a treatment containing a DAA showed a discordant genotype or subtype according to the sequencing

Discordant cases		
Discordant genotypes	37	2.0
Genotype 1 with discordant subtype	58	3.2
Total	**1813**	**100**

Aragri M et al., et al., 15th European Meeting on HIV & Hepatitis 2017

HCV sequencing is useful for identifying RASs but also the "correct" genotype: 15/310 (4.8%) patients were found infected with a different HCV genotype at failure

Notably, 10 patients previously classified as infected with HCV-1 were actually infected with HCV-2 and HCV-3, 9/10 failed a 3D+RBV regimen and all presented RASs at failure

ID Patient	Pre-therapy genotype by commercial assay	Genotype by sequencing at failure	DAA regimen	DAA response	Failure RASs		
					NS3	NS5A	NS5B
1497	**1a**	**3a**	3D+RBV	Non-responder		**Y93H**	
2150	**1a**	**3a**	3D+RBV	Breakthrough	**Q80K**	**Y93H**	
2068	**1b**	**3a**	3D	Non-responder	**Q80K**	**Y93H**	
1424	**1b**	**3a**	3D+RBV	Non-responder		**Y93H**	
2140	**1b**	**3a**	3D+RBV	Non-responder		**A30K**	
2353	**1**	**3a**	3D	Non-responder		**Y93H**	
1823	**1b**	**2c**	3D+RBV	Non-responder	**D168V**		
2020	**1b**	**2c**	3D	Non-responder	**D168V**	**F28C**	
2623	**1b**	**2c**	3D	Relapse		**F28C**	
2890	**1b**	**2c**	SMV+SOF	Relapse		**L31M**	
2204	2	1b	LDV+SOF+RBV	Relapse		R30Q+L31I+Y93H	C316N
2886	2	1b	SOF+RBV	Relapse	Y56F		C316N
2153	2	3a	SOF+RBV	Relapse		A30K+L31F	
1111	4	1a	2D+RBV	Breakthrough	V36M+Y56H	M28T	
45	4	3a	SMV+SOF	Relapse	D168K		

Di Maio VC et al., EASL 2017

PrecisionMedicineapproach

HCV Resistance Testing Prior to (First-Line) DAA Therapy

Available, reliable, Interpretable, understandable*

⇩

Presence of NS5As RASs conferring high-level resistance (pop seq or >15%)

⇩

Add ribavirin and/or increase treatment duration

*Recommended for GZR/EBR for patients with GT1a but also for LDV/SOF for patients with GT1a and DCV/SOF and VPV/SOF patients with GT3

Not available

⇩

Optimize therapy to avoid treatment failure

⇩

- ✓ **Add RBV in TE patients with SOF/LDV, SOF/DCV, SOF/SMV**
- ✓ **With 3D, use RBV in GT1a, treat NR cirrhotics 24 weeks**
- ✓ **Use GZR/EBR 16 weeks with RBV in GT1a patients**

Pawlotsky et al, Gastroenterology 2016
NEW EASL Guidelines Sept 2016

Precision Medicine approach

HCV Resistance Testing Prior to (First-Line) DAA Therapy

Available, reliable, Interpretable

Not available

THERE'S THE NEED TO STANDARDIZE HCV RESISTANCE EVALUATION AND INTERPRETATION … ONLY AFTER THAT, HCV RESISTANCE TESTING CAN BE EFFICIENTLY APPLIED INTO CLINICAL PRACTICE

…….VIRONET C……..

RBV in GT1a patients

**Recommended for GZR/EBR for patients with GT1a but also for LDV/SOF for patients with GT1a and DCV/SOF and VPV/SOF patients with GT3*

Pawlotsky et al, Gastroenterology 2016
NEW EASL Guidelines Sept 2016

EASL THE INTERNATIONAL LIVER CONGRESS 2018

National Quality Control and Validation of Hepatitis C NS3, NS5A and NS5B Genotypic Resistance Testing

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National Quality Control and Validation of Hepatitis C NS3, NS5A and NS5B Genotypic Resistance Testing in Italy

16/21 labs generated all the 30 expected sequences, while the remaining 5 generated a mean of 23.4 ± 3.9 SD sequences.

The majority of the participating labs detected all the NS3, NS5A, NS5B RASs identified by NGS with prevalence >15%. Among the 16 labs that provided all 30 HCV sequences, with respect to NGS results, 12 labs had 0-1 RAS discordance, 3 labs had RAS discordances in 2 samples, and 1 lab had 4 RAS discordances in 2 samples.

The Geno2pheno tool was used for detection of resistance associated substitutions (RASs).

Center	N Sequences TOT	N Seq NS3	N Seq NS5A	N Seq NS5B	Discordances N samples
1.	30	10	10	10	1
2.	30	10	10	10	1
3.	30	10	10	10	2
4.	10	4	6	9 but short	1
5.	30	10	10	10	1
6.	30	10	10	10	2
7.	30	10	10	10	1
8.	27	10	10	7	1
9.	30	10	10	10	1
10	30	10	10	10	0
11.	22	9	7	6	0
12.	30	10	10	10	1
13.	30	10	10	10	0
14.	27	8	9	10	1
15.	30	10	10	10	1
16.	30	10	10	10	1
17.	30	10	10	10	1
18.	30	10	10	10	2
19.	30	10	10	10	2
20.	21	5	10	6	4
21.	30	10	10	10	1

Ruggiero T et al European Drug Resistance Workshop 2018

Different impact according to specific baseline NS5A RASs in HCV-1 patients treated with Ledipasvir/Sofosbuvir

First-line regimen

1a vs 1b

1% cutoff
No RAS, SVR:

GT1a: 98.3%
1306/1329 pts

GT1b: 98.6%
1741/1770 pts

15% cutoff
No RAS, SVR:

GT1a: 98%
1416/1445 pts

GT1b: 98.7%
1880/1915 pts

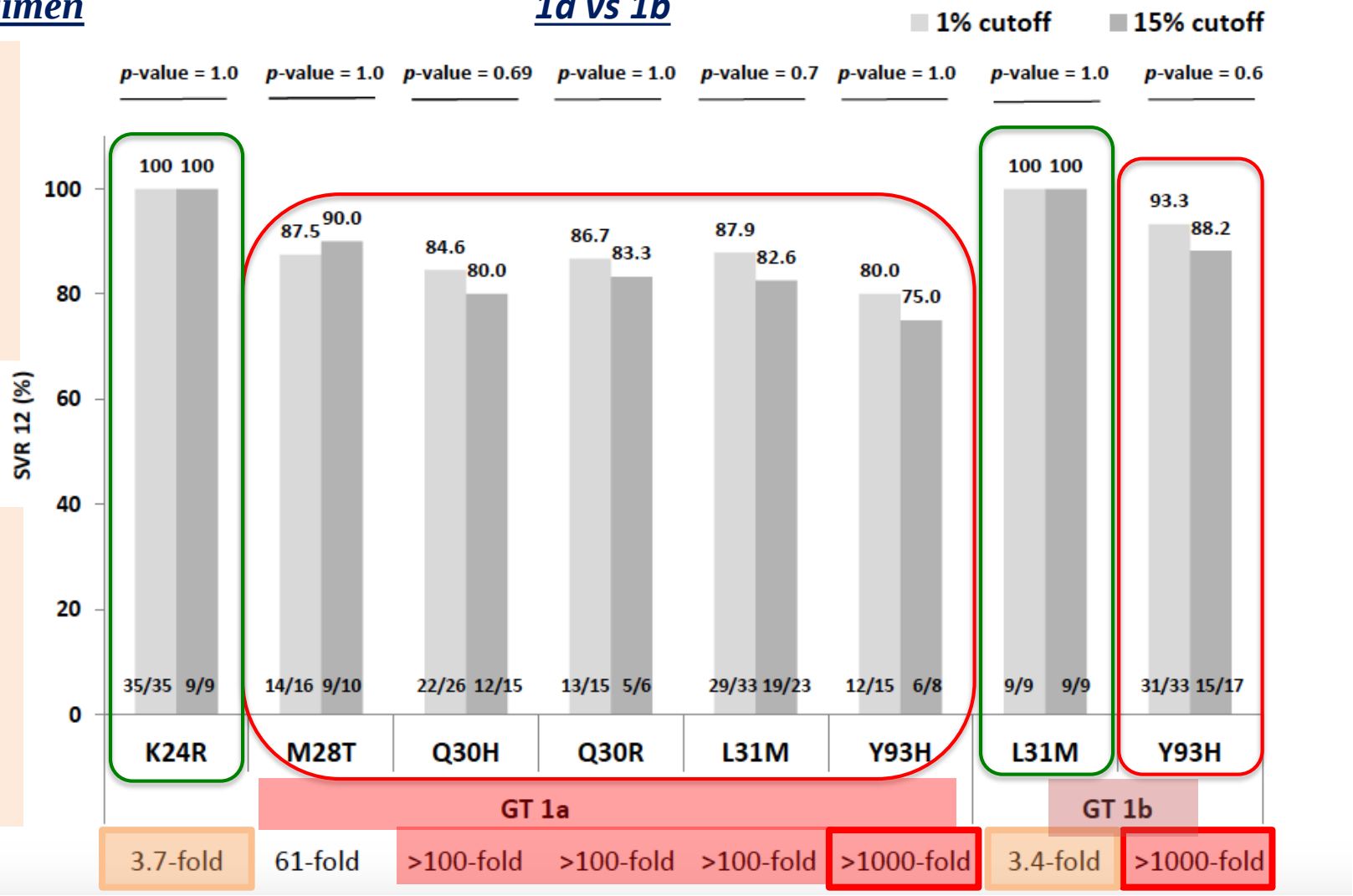


Figure 4. Treatment Outcome in Patients with NS5A RASs. Substitution analyses were conducted on deep sequencing data (population sequences were not included). (a) SVR12 by specific baseline NS5A RASs and cutoff (1 percent and 15 percent) in patients treated with ledipasvir/sofosbuvir.

Sarrazin et al Gastroenterology 2016

Clinically relevant NS5A RASs that can be used to guide treatment decisions in GT1a and GT3 patients

If present: add RBV and/or increase treatment duration

Clinically relevant NS5A RASs that can be used to guide treatment decisions in GT1a and GT3 patients

NS5A amino acid position	Ledipasvir RASs Genotype 1a Sofosbuvir/ Ledipasvir treatment	Elbasvir RASs Genotype 1a Grazoprevir/ Elbasvir treatment	NS5A RASs Genotype 3 Sofosbuvir/ Velpatasvir treatment
M28	M28A M28G M28T	M28A M28G M28T	
Q30	Q30E Q30G Q30H Q30K Q30R	Q30D Q30E Q30G Q30H Q30K Q30L Q30R	
L31	L31M L31V	L31F L31M L31V	
P32	P32L P32S		
H58	H58D	H58D	
Y93	Y93C Y93H Y93N Y93S	Y93C Y93H Y93N Y93S	Y93H

Adapted from EASL Guidelines Sept 2016

Clinically relevant NS5A RASs that can be used to guide treatment decisions in GT1a, Gt1b and GT3 patients

DAA Regimen	Genotype		
	1a	1b	3
Ledipasvir/sofosbuvir	Q30H/R L31M/V Y93C/H/N	L31V ?Y93H	n/a
Elbasvir/grazoprevir	M28A/T Q30H/R L31M/V Y93C/H/N	Y93H	n/a
Paritaprevir/ritonavir/ombitasvir with dasabuvir ± ribavirin	n/a	n/a	n/a
Sofosbuvir/velpatasvir	n/a	n/a	Y93H

Adapted from AASLD Guidelines Oct 2017

Y93H fold change for approved NS5A-inhibitors across genotypes 1a, 1b, 2, 3, 4

NS5A-inhibitors	Fold-change *in vitro*[a]				
	GT-1a	GT-1b	GT-2	GT-3	GT-4
Daclatasvir	1400-5432	19-145	749-1750	2154	45-169
Elbasvir	220-600	12-67	-	157	-
Ledipasvir	1677-3309	1319	-	30[b]	1000
Ombitasvir	41383	77	4710	6728	20-100
Pibrentasvir	7	0.6	-	2-3	-
Velpatasvir	609	3	46	724	3

[a]Y93H fold change value in comparison with wild type strains; maximum and minimum values are reported [26, 27, 39, 42, 57, 65, 71, 96, 105, 125, 132, 134, 144, 146, 157, 161]. For 1st generation NS5A-inhibitors, RASs with fold-change >100x are reported in red (resistance likely); RASs with fold-change 20-100 are reported in yellow (resistance possible); RASs with fold-change 3-20x are reported in green (likely susceptible); only *in-vivo* RAS, with no fold-change available are reported in violet (resistance possible). For 2nd generation NS5A-inhibitors elbasvir and velpatasvir, RASs with fold-change ≥10x are reported in red (resistance likely); RASs with fold-change 2.5-9 are reported in yellow (resistance possible); RASs with fold-change ≤2.5x are reported in green (likely susceptible); only *in-vivo* RAS, with no fold-change available are reported in violet (resistance possible). [b] Ledipasvir exhibited an EC50 value of 141 nM, affording a >670-fold reduction in potency when compared to daclatasvir [134]. GT, genotype. "-" indicates no data available.

Sorbo MC , et al Drug Resistance Update 2018 in press

Broad cross-resistance among NS5A Inhibitors, but not all single RASs and patterns are the same

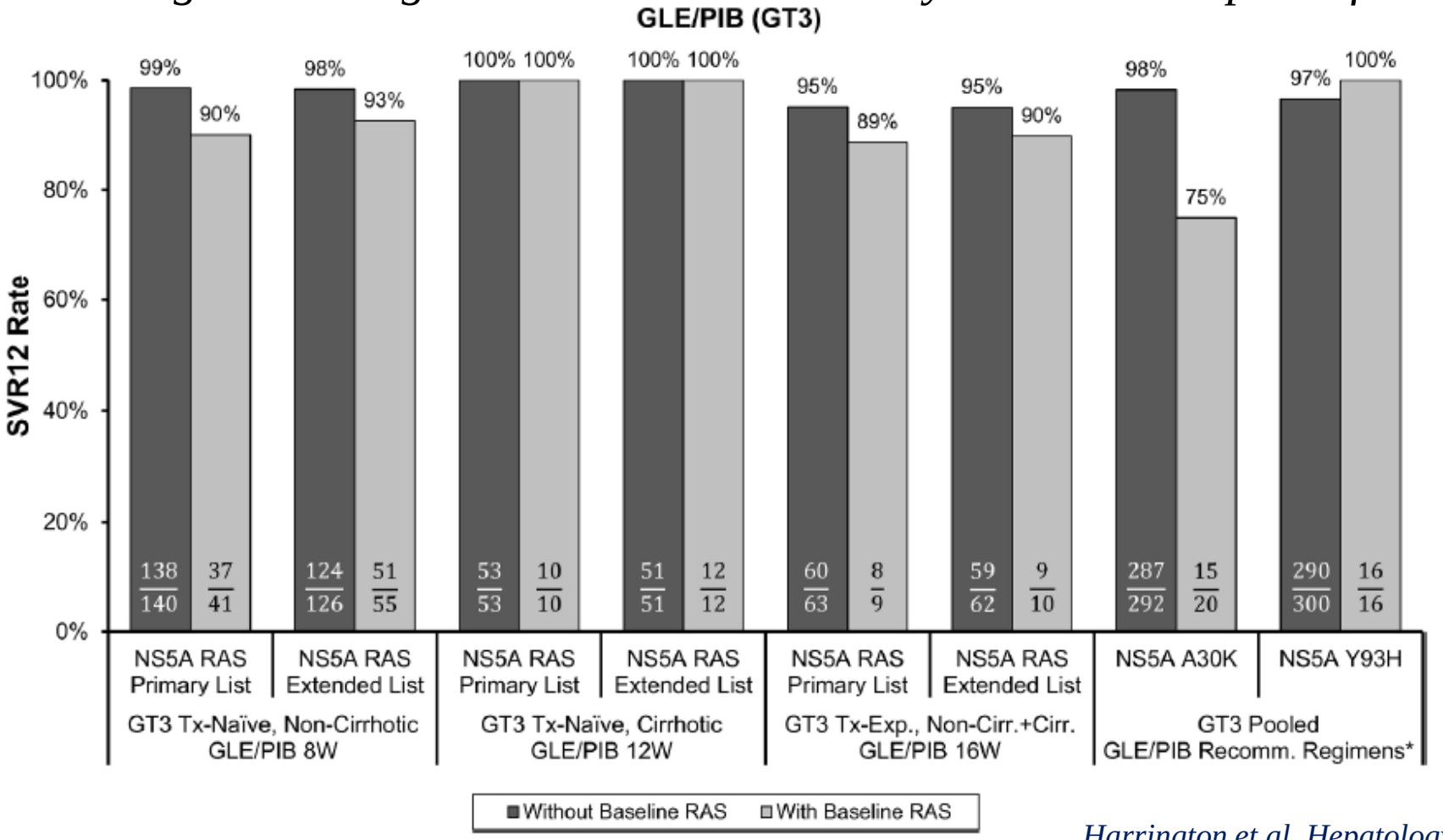
Table 1. Examples of NS5A and NS3 RASs and their reported phenotypic effect on DAA activity in transient HCV replicons.

	NS5A Inhibitors (Fold-Change in EC_{50} Values)					
NS5A RAS[1]	Ledipasvir	Ombitasvir	Daclatasvir	Elbasvir	Velpatasvir	Pibrentasvir
GT1a-K24R	4	≤1	2	≤1	≤1	≤1
GT1a-M28T	61	8965	205	15	8	2
GT1a-M28V	≤1	58	1	1	≤1	2
GT1a-Q30H	183	3	435	6	2	≤1
GT1a-Q30R	632	800	365	16	2	2
GT1a-L31M	554	2	105	10	16	≤1
GT1a-H58D	1127	243	367	6	7	≤1
GT1a-H58P	≤1	≤1	≤1	ND	≤1	≤1
GT1a-Y93H	1677	41383	1600	220	609	7
GT1a-M28T+Q30H	ND	ND	76,833	2286	ND	ND
GT1a-Q30H+Y93H	34,960	ND	98,167	ND	2835	17
GT1a-Q30R+Y93H	33,691	354,981	52,667	ND	18,698	260
GT1b-L31M	3	≤1	3	1[2]	2	2
GT1b-Y93H	1807	77	12	17[2]	3	≤1
GT1b-L31M+Y93H	20,270	142	16,000	ND	44	≤1
GT3a-A30K	n/a	n/a	117	n/a	50	≤1
GT3a-Y93H	n/a	n/a	3733	n/a	724	2
References	(18, 32, 67)	(18, 37)	(18, 48, 49, 67)	(19, 51, 68)	(55, 67, 69)	(59, 70)

Harrington et al, Hepatology 2017

No BL GRT recommendations are included in U.S. labeling for GLE/PIB, although available data on the impact of the GT3 NS5A A30K BL RAS are described for consideration by clinicians on a case-by-case basis

Indicating that a longer treatment duration may reduce the impact of A30K



Harrington et al, Hepatology 2017

SCIENTIFIC REPORTS

OPEN

Prevalence of Single and Multiple Natural NS3, NS5A and NS5B Resistance-Associated Substitutions in Hepatitis C Virus Genotypes 1–4 in Italy

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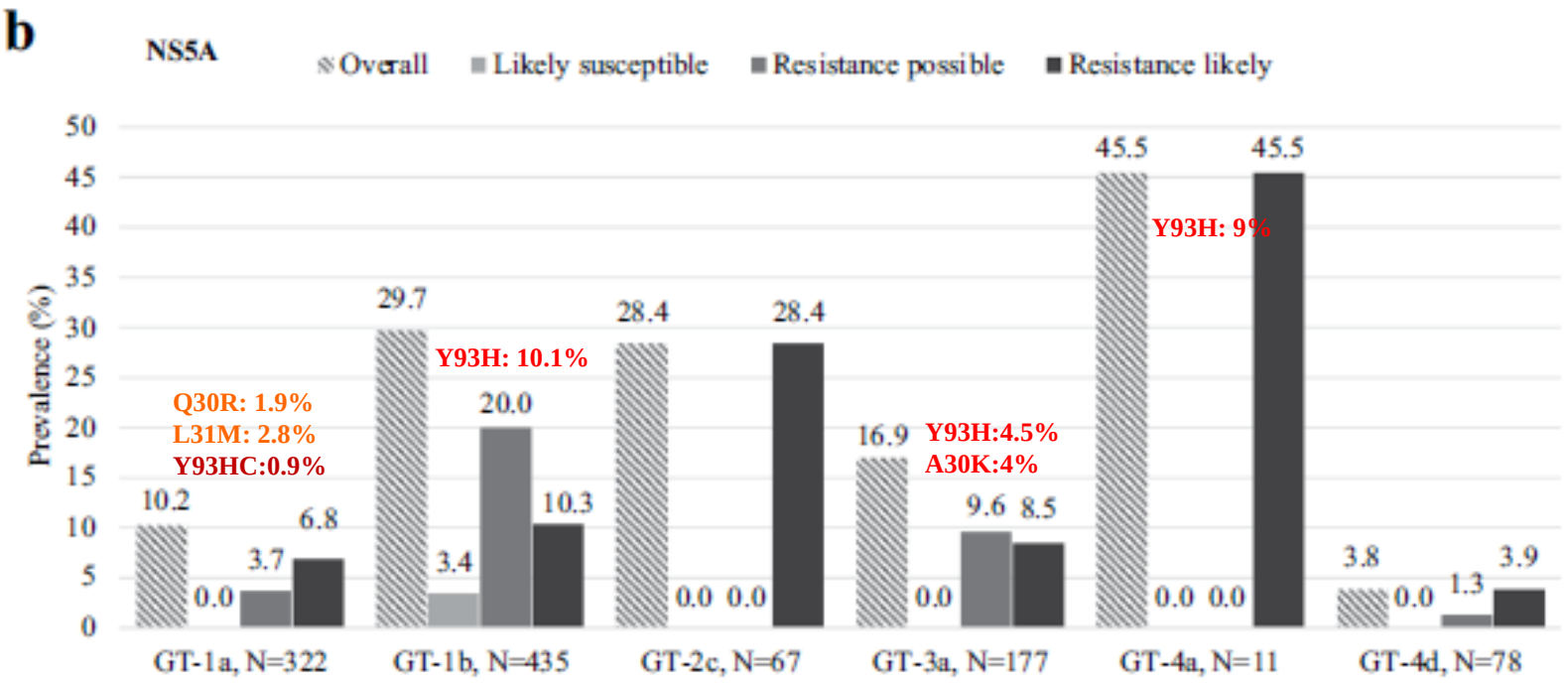
Natural resistance-associated substitutions (RASs) are reported with highly variable prevalence across different HCV genotypes (GTs). Frequency of natural RASs in a large Italian real-life cohort of patients infected with the 4 main HCV-GTs was investigated. NS3, NS5A and NS5B sequences were analysed in 1445 HCV-infected DAA-naïve patients. Sanger-sequencing was performed by home-made protocols on 464 GT1a, 585 GT1b, 92 GT2c, 199 GT3a, 16 GT4a and 99 GT4d samples. Overall, 20.7% (301/1455) of patients showed natural RASs, and the prevalence of multiclass-resistance was 7.3% (29/372 patients analysed). NS3-RASs were particularly common in GT1a and GT1b (45.2-10.8%, respectively), mainly due to 80K presence in GT1a (17%). Almost all GTs showed high prevalence of NS5A-RASs (range: 10.2–45.4%), and especially of 93H (5.1%). NS5A-RASs with fold-change >100x were detected in 6.8% GT1a (30H/R-31M-93C/H), 10.3% GT1b (31V-93H), 28.4% GT2c (28C-31M-93H), 8.5% GT3a (30K-93H), 45.5% GT4a (28M-30R-93H) and 3.8% GT4d (28V-30S-93H). Sofosbuvir RAS 282T was never detected, while the 159F and 316N RASs were found in GT1b (13.4–19.1%, respectively). Natural RASs are common in Italian patients infected with HCV-GTs 1–4. High prevalence of clinically-relevant RASs (such as Y93H) supports the appropriateness of HCV resistance-test to properly guide DAA-based therapy.

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

The Italian experience: different NS5A RASs prevalence according to genotype and subtype in DAA naive patients



Bertoli A. et al., Sci. Rep.,2018

Despite the excellent efficacy of DAA containing regimens, virological failures can occur, often associated with development of resistance and with differences according to the type of regimen and HCV genotype

	GT1a			GT1b			GT2		GT3		GT4		
	NS3	NS5A	NS5B	NS3	NS5A	NS5B	NS5A	NS5B	NS5A	NS5B	NS3	NS5A	NS5B
simeprevir/ sofosbuvir	R155K D168E	n.d.	no RASs	D168V	n.d.	L159F C316N	not applicable		not applicable		Q80R D168E	n.d.	no RASs
daclatasvir/ sofosbuvir	n.d.	Q30H/R L31M	no RASs	n.d.	L31M Y93H	L159F C316N	no patients		Y93H	S282T*	n.d.	L28M	S282T
ledipasvir/ sofosbuvir	n.d.	Q30H/R L31M Y93H	S282T*	n.d.	L31M Y93H	L159F S282T* C316N	not applicable		no RASs	no RASs	n.d.	L28M Y93C/H	S282T
3D/2D	R155K D168V	M28T/V Q30R	S556G	Y56H D168V	Y93H	L159F C316N S556G	not applicable		not applicable		Y56H D168V	L28V Y93H	n.d.
sofosbuvir/ribavirin± pegylated-interferon	n.d.	n.d.	no RASs	n.d.	n.d.	L159F C316N	n.d.	no RASs	n.d.	L159F*	not applicable		

Table 1: Summary of the most frequent treatment-selected RASs according to the HCV genotype and treatment regimen. These characteristic RASs were defined to have a more than 10% increased prevalence after treatment failure compared to DAA-naïve patients (exceptions are marked with asterisks). The color refers the level of resistance conferred by the respective RAS.

Dietz J et al., Gastroenterology 2017

Despite the excellent efficacy of DAA containing regimens, virological failures can occur, often associated with development of resistance and with differences according to the type of regimen and HCV genotype

	GT1a			GT1b			GT2		GT3		GT4		
	NS3	NS5A	NS5B	NS3	NS5A	NS5B	NS5A	NS5B	NS5A	NS5B	NS3	NS5A	NS5B

Understanding more about RASs may help us learn why the patients failed, *and may allow optimization of treatment to other new patients & retreatment choices.*

characteristic RASs were defined to have a more than 10% increased prevalence after treatment failure compared to DAA-naïve patients (exceptions are marked with asterisks). The color refers the level of resistance conferred by the respective RAS.

Dietz J et al., Gastroenterology 2017

Also virological failures to new DAAs occur with resistance

20 out of 1,778 patients (1.1%) treated **Sofosbuvir/Velpatasvir** with for 12 weeks experienced virologic failure: 7 infected with GT1, 12 infected with GT3, and 1 infected with GT4 HCV

Number of patients	GT	NS5A RASs				NS5B NI RASs	
		Baseline (%)	Ref FC VEL at baseline	Virologic failure (%)	Ref FC VEL[a] at virologic failure	Baseline (%)	Virologic failure (%)
n = 2	1a	None	0.8[a]	Y93N (>99%) or Y93N (91.9%)	805[a]	None	None
n = 1	1a	None	NA	Y93H (>99%)	609[b]	None	None
n = 2	1a	None	NA	None	NA	None	None
n = 1	1b	L31M (>99%) Y93H (>99%)	44[b]	L31M (>99%) Y93H (>99%)	ND	V321I (94.1%)	V321I (>99%)
n = 1	1c/1h	Q30R (98.7%) L31M (>99%)	1.4[a]	Q30R (>99%) L31M (88.4%) Y93H (72.3%)	763[a]	None	None
n = 2	3a	Y93H (>99%)	347–1,073[a]	Y93H (>99%)	302–1,221[a]	None	None
n = 1	3a	Y93H (15.2%)	724[b]	Y93H (>99%)	724[b]	None	None
n = 1	3a	A30K (>99%)	30[a], 50[b]	A30K (>99%) Y93H (97.2%)	35154[a]	None	None
n = 8	3a	None	0.2–1.3[a]	Y93H (>99%)	74–1,138[a]	None	None
n = 1	4a	None	ND	None	ND	None	None

Ref FC VEL = VEL half-maximal effective concentration fold change from reference; NA, not applicable.
GT, genotype; HCV, hepatitis C virus; RAS, resistance-associated substitution; SVR12, sustained virologic response at 12 weeks; VEL, velpatasvir.
[a] Susceptibility to velpatasvir was evaluated using patient isolates.
[b] Susceptibility to velpatasvir was evaluated using site-directed mutant and compared to wild-type replicon.

The overall prevalence of Y93H/N across all genotypes was 2.8% (49/1773) at baseline and 84% (16/20) at virologic failure, respectively. Only one patient with a GT1b infection had V321I NS5B NI RAS at baseline and virologic failure. No sofosbuvir NS5B RASs were observed at baseline or virologic failure in these 20 patients

Hezode et al J of Hepatology 2018

Also virological failures to new DAAs occur with resistance

High SVR12 with 8/12-week **Glecaprevir/Pibrentasvir**: Integrated analysis of HCV Genotype 1-6 2041 patients without cirrhosis. In the ITT population, 943/965 (98%) and 1060/1076 (99%) of patients achieved SVR12 when treated for 8 and 12 weeks, respectively

eTable 3. Patients with Virologic Failure: NS3 and NS5A Polymorphisms/Substitutions at Baseline and Time of Failure

			NS3 Variants		NS5A Variants	
Treatment Duration	HCV Subtype	Failure	Baseline	At Failure	Baseline	At Failure
ENDURANCE-1						
8 weeks	1a	Failed to Suppress	None	A156V	None	Q30R + L31M + H58D
SURVEYOR-II						
8 weeks	2a	Relapse	None	None	L31M	L31M
8 weeks	2a	Relapse	None	None	L31M	L31M
ENDURANCE-3						
8 weeks	3a	Relapse	T54S	T54S	None	None
8 weeks	3a	Relapse	None	Q168L	A30K	A30K + Y93H
8 weeks	3a	Relapse	A166S	Y56H, Q168L	A30K	A30K + Y93H
8 weeks	3a	Failed to Suppress	A166S, Q168R	Q80R, A156G	A30K	A30K + Y93H
8 weeks	3a	Relapse	A166S	A166S	None	Y93H
8 weeks	3a	Relapse	None	Y56H	A30K	A30K + Y93H
12 weeks	3a	Relapse	None	Reinfection	None	Reinfection
12 weeks	3a	Breakthrough	Q168R	Y56H+Q168R	A30K/V, Y93H	A30K + Y93H
12 weeks	3a	Relapse	None	None	None	A30G, Y93H
12 weeks	3b	Relapse	None	Q80K	V31M	V31M + Y93H

Detection of baseline polymorphisms and treatment-emergent substitutions was done with next-generation sequencing using a 15% detection threshold. For samples with multiple variants (polymorphisms/substitutions) within a target, if individual variants were detected at ≥90% prevalence , they are considered to be linked and denoted by "+", whereas if one or more of the variants was detected at <90% prevalence, the variants are separated by a comma

Amino acid positions included in analysis of patients with GT1: 36, 43, 54, 55, 56, 80, 155, 156, 168 in NS3; 24, 28, 29, 30, 31, 32, 58, 62, 92, 93 in NS5A

Amino acid positions included in analysis of patients with GT2 or GT3: 36, 43, 54, 55, 56, 80, 155, 156, 166, 168 in NS3; 24, 28, 29, 30, 31, 32, 58, 92, 93 in NS5A

Puoti et al., J of Hepatol 2018

Patient: 47 years, male, with GT3 started Gle/Pib (16 weeks) with high viral load (5,787,000 IU/ml), F3, 266 IU/ml at 10d, 90 IU/ml at 21d, 1739 IU/ml at 5w, 46395 IU/ml at 7w.

geno2pheno®

HCV resistance prediction from genotype (version 1.0)

Sample with 1739 IU/ml

II. Sequence information

NS5A codons covered	1 - 163
NS5A region (w.r.t. D17763)	T7D, A17S, A21T, A30K, R48GR, A62S, A75AV, T79A, Y93H, S103A, A147P
NS5A region (w.r.t. H77)	S3D, I12V, E14S, K24S, L27I, Q30K, I34L, V37I, R41K, R44K, R48GR, I52V, H54S, H58P, E62S, T71S, I74L, V75AV, T79A, R81A, S85H, A92E, Y93H, C98S, L101C, K107T, F108R, S114A, E116N, E117S, I121V, V130I, S131T, M133A, T135E, N137E, I144V, S146A, L153

II. Sequence info

NS3 codons covered	17 - 181
NS3 region (w.r.t. D17763)	

...ted one of these

The patient had at baseline the NS5A RAS A30K

Did the p... mutations pre...

El...		susceptible	none
Le...		not licensed for subtype	not available
Om...	Paritaprevir	not licensed for subtype	not available
Pibr...	Simeprevir	not licensed for subtype	not available
Velpa...	Telaprevir	not licensed for subtype	not available
	Voxilaprevir	substitution on scored position	156G,170I

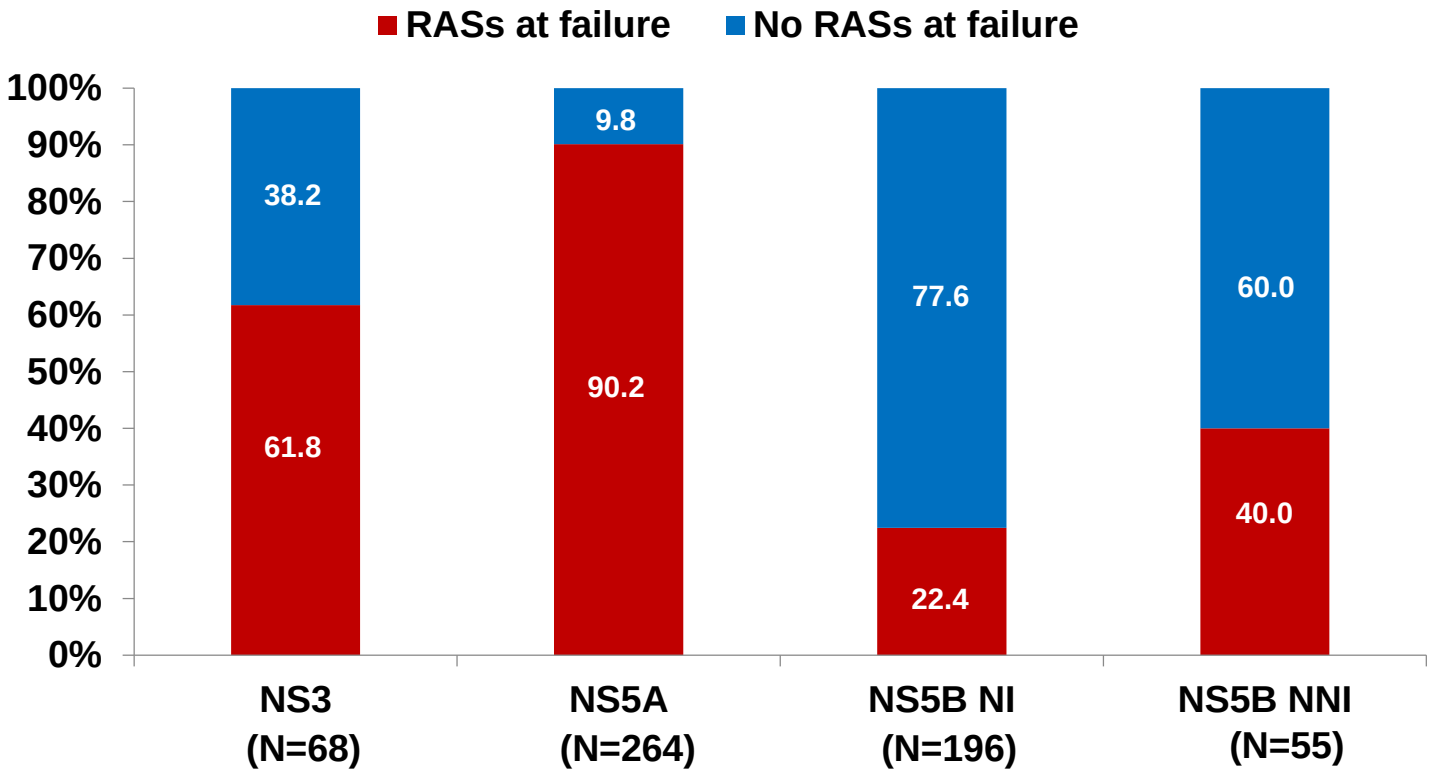
Q/R...	D, E, K, R G, K*	D: 95 E: 2 K: 1 * R: 2
Y/T93	C, H, N H, N H *	C: 1 H: 7, 1, 2-3* N: 7, <2
30+93	A30K+Y93H	70 *
	Q30H+Y93H	17
	Q30R+Y93H	260
	Q30R+Y93N	131

A156	G, T, V T, V G	G: 1654 * T: 1400, 630 V:1800

Sorbo MC , et al Drug Resistance Update 2018

RASs prevalence was found in all genes tested: NS5A very frequent (90.2%), NS3 frequent (61.8%), NS5B less common (22.4% NI and 40.0% NNI)

RASs prevalence at failure was high in almost all HCV genotypes/subtypes



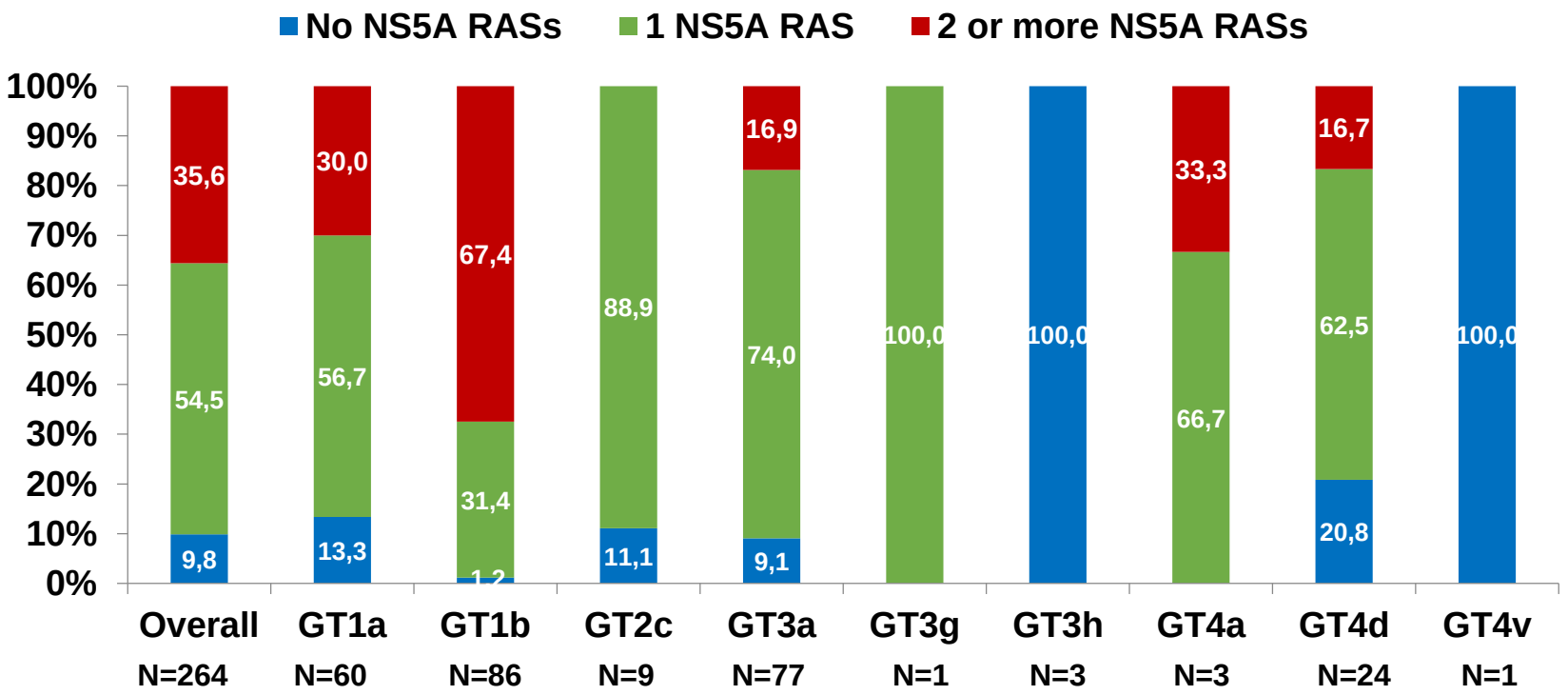
264 HCV-infected patients failed a currently recommended INF-free NS5A containing regimen

NI, Nucleotide inhibitor; NNI Non-Nucleoside Inhibitor

UPDATE of Vironet C from Di Maio VC et al. J Hepatol. 2017
Di Maio VC et al European Drug Resistance Workshop 2018

94/264 (35.6%) of NS5A-failing patients presented ≥2 NS5A-RASs

9.8% (26/264) of DAA failing patients didn't show NS5A RASs at failure

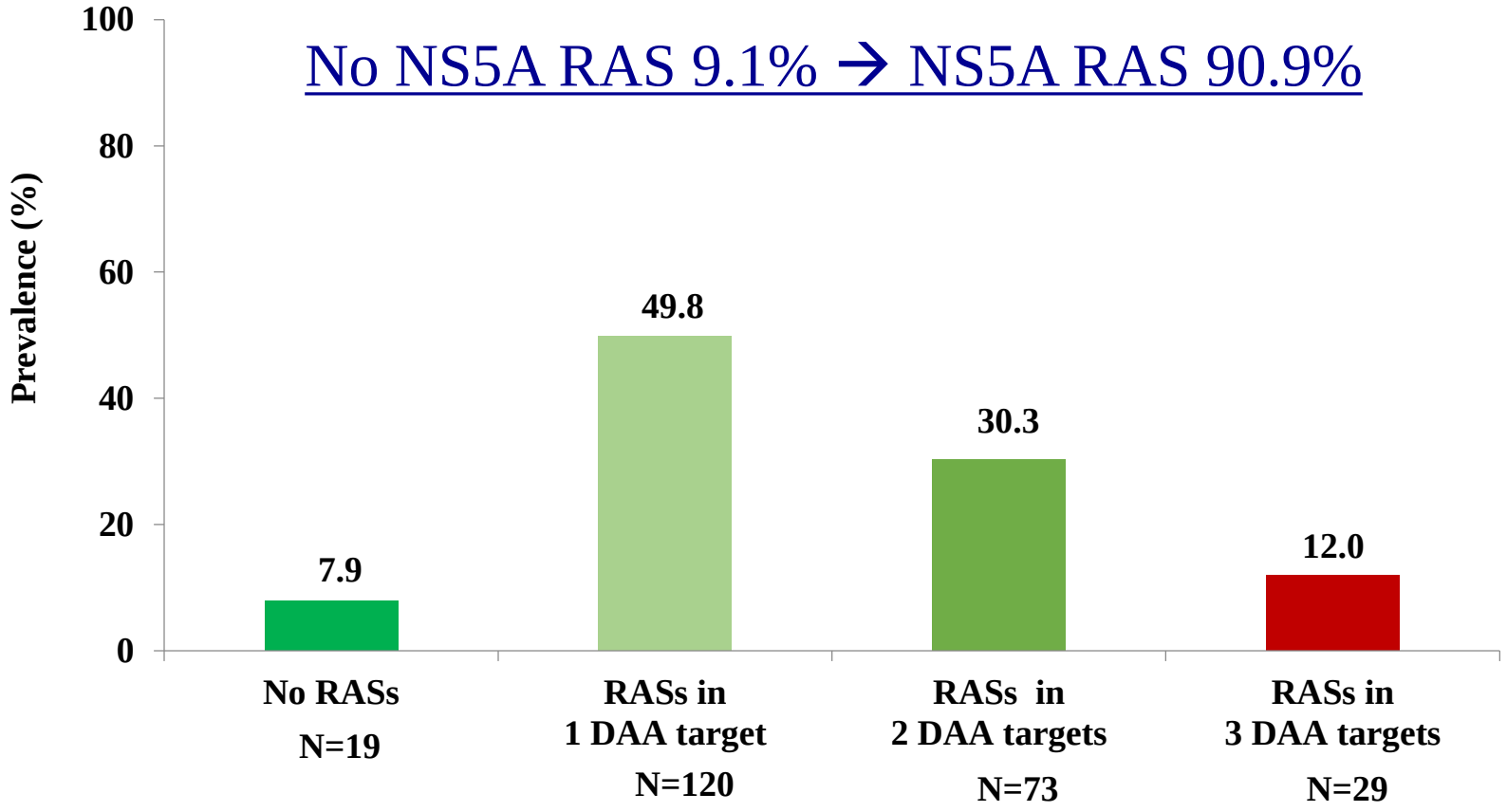


*One GT3h infected patient who experienced a virological failure to daclatasvir+sofosbuvir regimen showed the major SOF RAS S282T in NS5B gene.

UPDATE of Vironet C from Di Maio VC et al. J Hepatol. 2017
Di Maio VC et al European Drug Resistance Workshop 2018

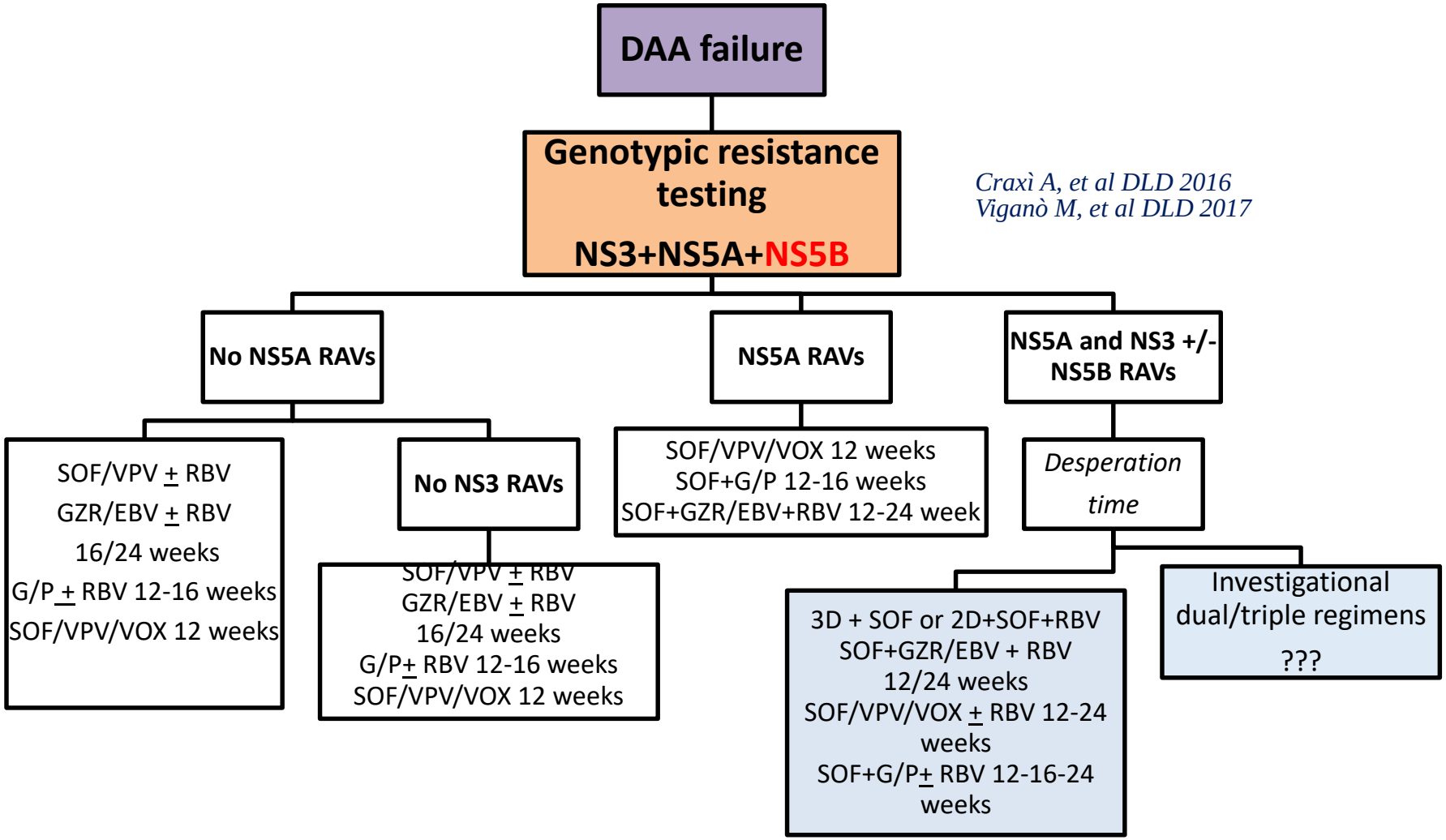
42.3% HCV-infected patients that failed a currently recommended INF-free NS5A containing regimen showed RASs on ≥2 DAA-targets at failure

All patients were treated with ≥2 DAA classes



UPDATE of Vironet C from Di Maio VC et al. J Hepatol. 2017
Di Maio VC et al European Drug Resistance Workshop 2018

Retreatment may require «unconventional» approaches with multiple DAAs

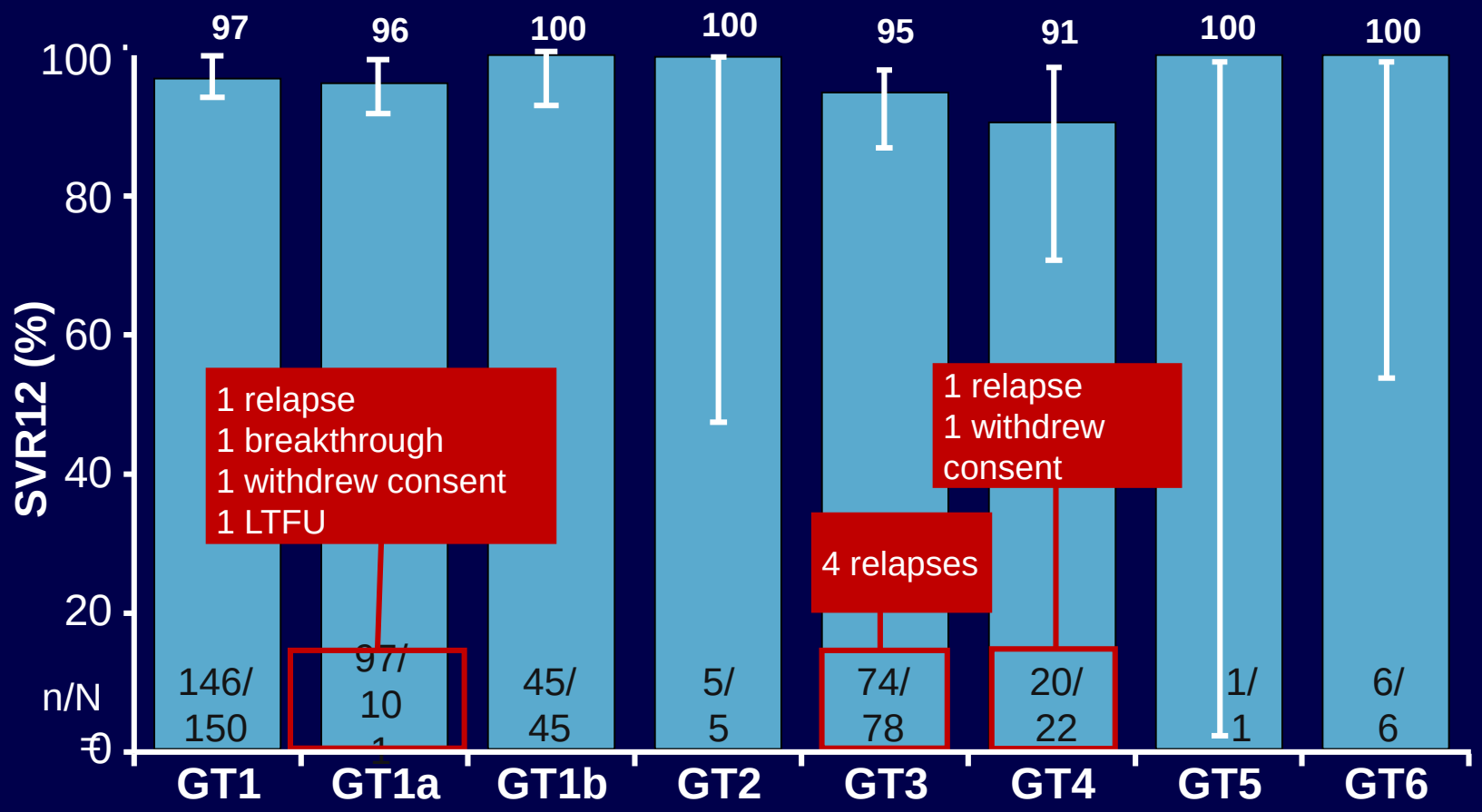


Craxì A, et al DLD 2016
Viganò M, et al DLD 2017

Modified by Wyles D, AASLD 2015

POLARIS-1: SVR12 by Genotype With 12-Wk SOF/VEL/VOX in NS5A Inhibitor–Experienced Pts

- Only 1 GT4 pt developed a treatment-emergent RAS (NS5A Y93H)

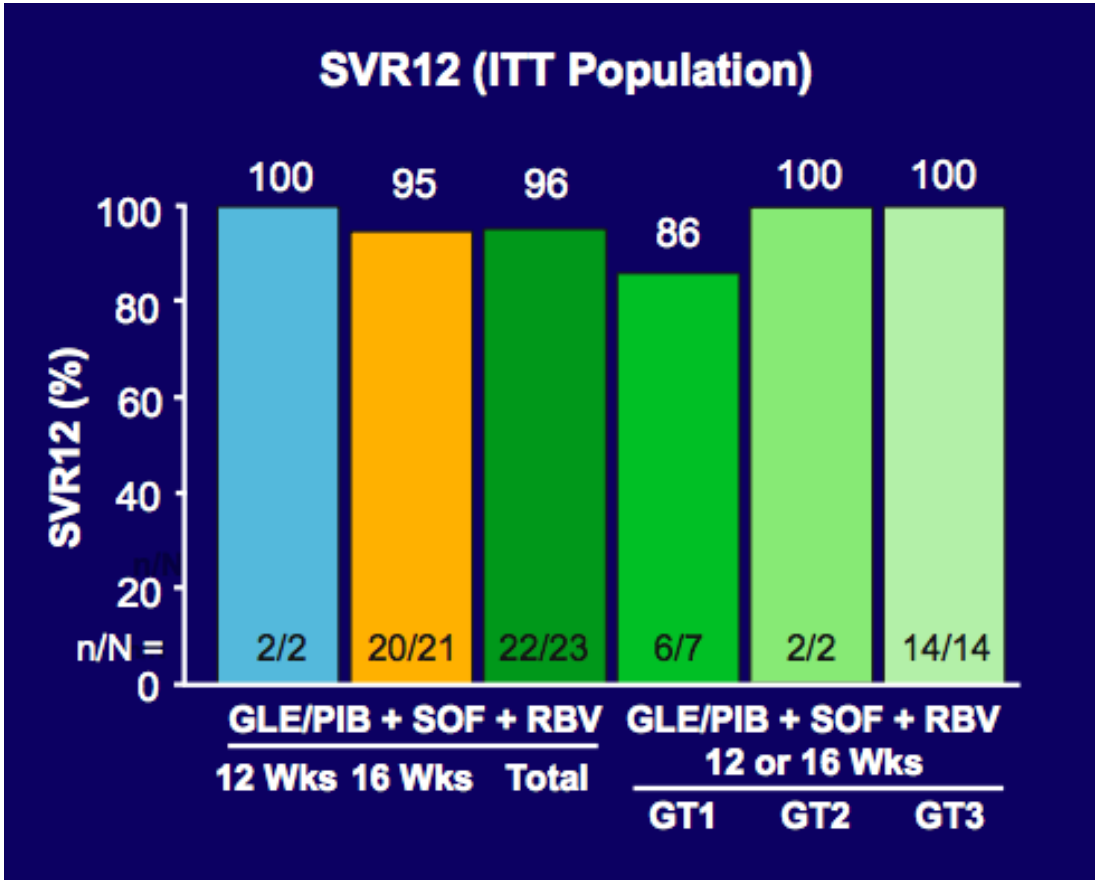


Bourlière M, et al. N Engl J Med. 2017;376:2134-2146. Bourlière M, et al. AASLD 2016. Abstract 194.

Slide credit: clinicaloptions.com

MAGELLAN-3: GLE/PIB + SOF + RBV for 12-16 weeks for Retreatment After Failure of GLE/PIB

(N = 23)



12 w: Non-cirrhotic patients with GT1,2,4,5,6 HCV infection ± HIV coinfection with VF on/after GLE/PIB ± **no previous NS5AI or PI** (N = 2)

16 w: Cirrhotic and non-cirrhotic patients with GT1-6 HCV infection ± HIV coinfection with VF on/after GLE/PIB ± **previous NS5AI or PI** (N = 21)

VF occurred in 1 patient in 16-wk arm

GT1a HCV infection, cirrhosis, previous LDV/SOF, NS5A RAS (Q30K + Y93H), and no NS3 RAS at MAGELLAN-3 BL (but at previous G/P failure A156V)

Baseline RAS:

NS5A RAS detected in 18 (78%) of 23 patients

- 12-wk arm: 2/2
- 16-wk arm: 16/21

NS3 + NS5A RAS detected in 5/23 patients, all in 16-wk arm

Wyles D, et al. EASL 2018

Retreatment of DAA Failures

- HCV resistance testing useful to guide retreatment
- Recommendations for patients who experienced DAA regimen (PI and/or NS5AI) failure: management should be in context of multidisciplinary team including experienced treaters and virologists

Failure of DAA (PI and/or NS5AI)-Containing Regimen	Retreatment Recommendation
± Compensated cirrhosis	SOF/VEL/VOX for 12 wks
± Compensated cirrhosis with predictors of lower response*	GLE/PIB + SOF for 12 wks†
Very difficult to cure: NS5A RASs after 2 failures of PI and/or NS5AI-containing regimens	SOF/VEL/VOX or GLE/PIB + SOF: + RBV for 12 wks, no RBV for 16-24 wks, or + RBV for 16-24 wks†
Decompensated cirrhosis	SOF/VEL + RBV for 24 wks†

*Advanced liver disease, multiple courses of DAA-based treatment, complex NS5A RAS profile.
†Based on individual decision.

EASL recommendations on the treatment of hepatitis C 2018. J Hepatol 2018

Summary & Conclusions

HCV - a curable disease

We can cure HCV. SVR 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 reduce HCV infections and therefore to reduce the HCC HCV-related.

However, only a small proportion of infected persons are likely to have access to new therapies in most countries!!!!!

Conclusions

SVR rates are very high with new IFN-free regimens (in both mono and co-infected HIV populations)…**2-5-10% virologic failures = 1.2-17 million patients with antiviral resistance worldwide**…Patients with advanced liver diseases are more difficult to treat. Newer DAAs might not be universally available.

Prior treatment (DAA-naive patient):
HCV-RNA load, HCV genotyping and HCV resistance testing can support personalized-treatment (adjustment for duration, RBV-use? and choise of regimen) leading to near 100% SVR. Important for particular patients (e.g. GT3) *vs* all patients?

After failure, prior to retreatment (DAA-experienced patient):
- It is necessary to verify all the possible causes of virologic failure, including: incorrect genotype, poor compliance, suboptimal treatment, and potential reinfection.
- HCV resistance testing prior to retreatment is helpful to make a decision if reliable resistance testing is available. The resistance test should be performed in all 3 genes NS3+NS5A+NS5B in all infected patients independently of HCV genotype, and of the failure regimen. Retreatment can be guided by probabilities of response according to the resistance profile observed in the context of an experienced multidisciplinary team (complexity: virus, host, clinical aspects, previous treatment outcome, DAA)
- HCV sequencing can be based on Sanger population method and should also confirm the previous genotype and subtype assignment.

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