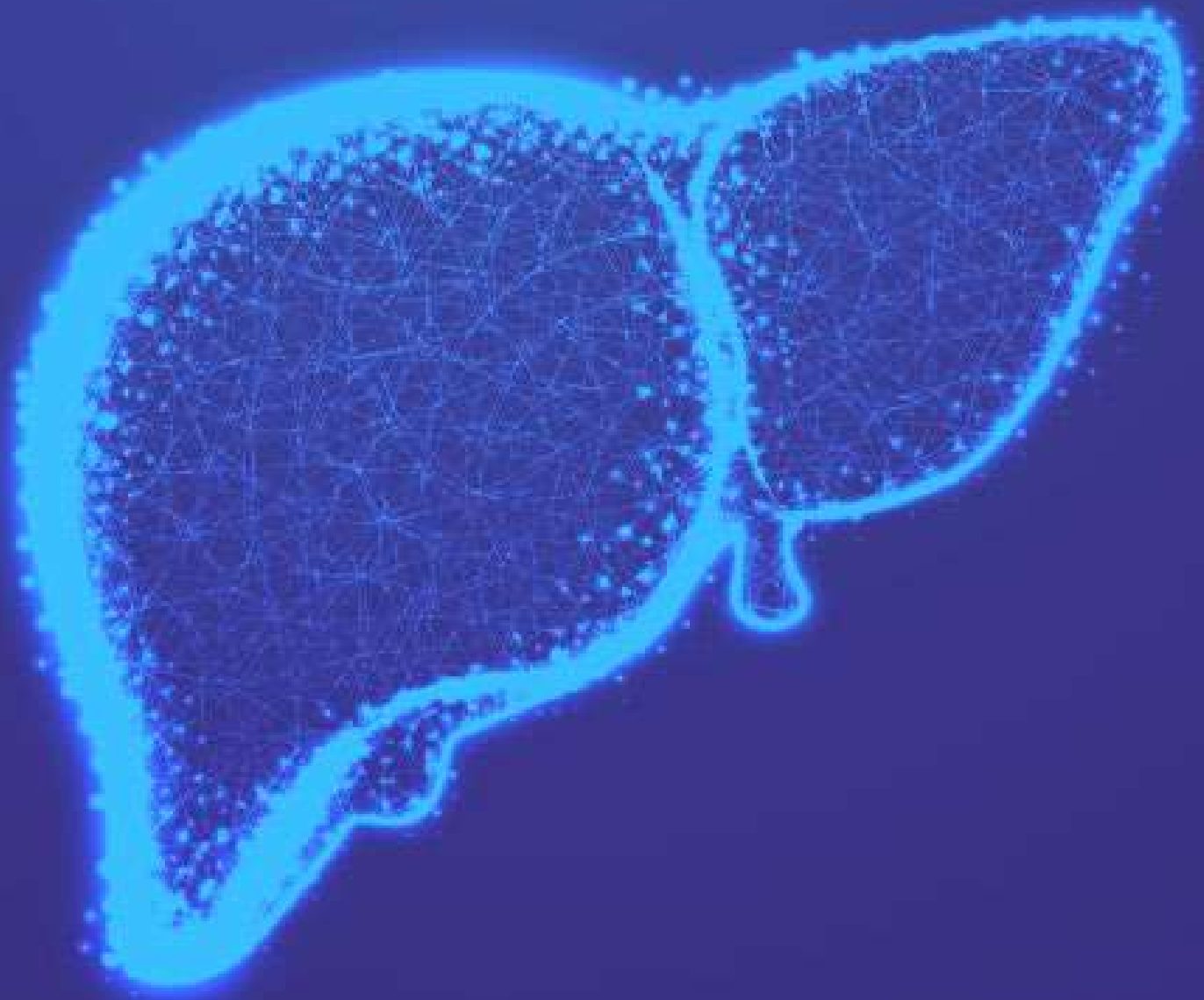
HCV IERI, OGGI... E DOMANI?

Reggio Emilia, 10 Marzo 2023
Sala Conferenze Laboratorio Aperto



Dal non-A non-B alle farmaco-resistenze: storia di un virus

Francesca Ceccherini Silberstein

Università degli Studi di Roma Tor Vergata

Disclosure: Advisory/Consulting/Conferences/Grants: Gilead, MSD, ViiV, Theratechnologies

Chronic viral hepatitis: an uncertain beginning

Diseases of the Liver and Biliary System

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FOURTH EDITION

The ultimate proof of the progression of virus hepatitis to cirrhosis must await the development of specific tests for the virus infection. The sequence must be exceedingly rare. This allows reassurance of most patients who, after hepatitis, believe they are developing cirrhosis. In a study of 367 patients who had had hepatitis 4–6 years before, hepatic functional abnormalities were no greater than in a control group of healthy young males [92]. A follow-up of 1293 cases of hepatitis occurring in British Forces in the Middle East 7 years before showed that apparently no one had subsequently developed cirrhosis [19]. Follow-up of 304 persons suffering hepatitis in the Delhi epidemic of 1955/56 has shown no clinical evidence of persistence of liver damage. In those who showed minor biochemical abnormalities liver biopsies were within normal limits [11]. However, following the epidemic in Denmark in the mid-1940s, an increased incidence of cirrhosis, particularly in women, has been noted [7].

By Courtesy of Prof A. Craxì

Chronic viral hepatitis: an uncertain beginning

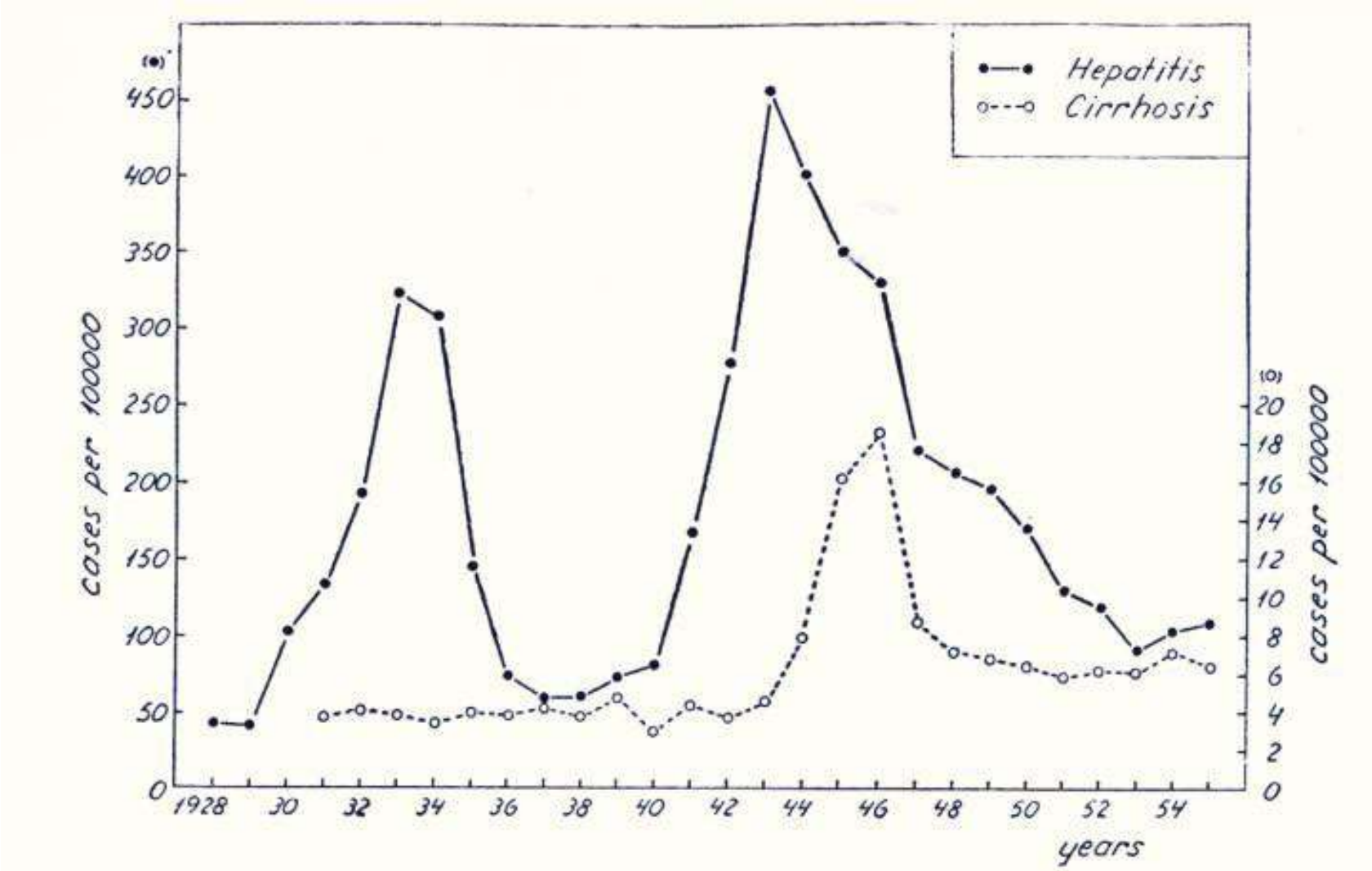
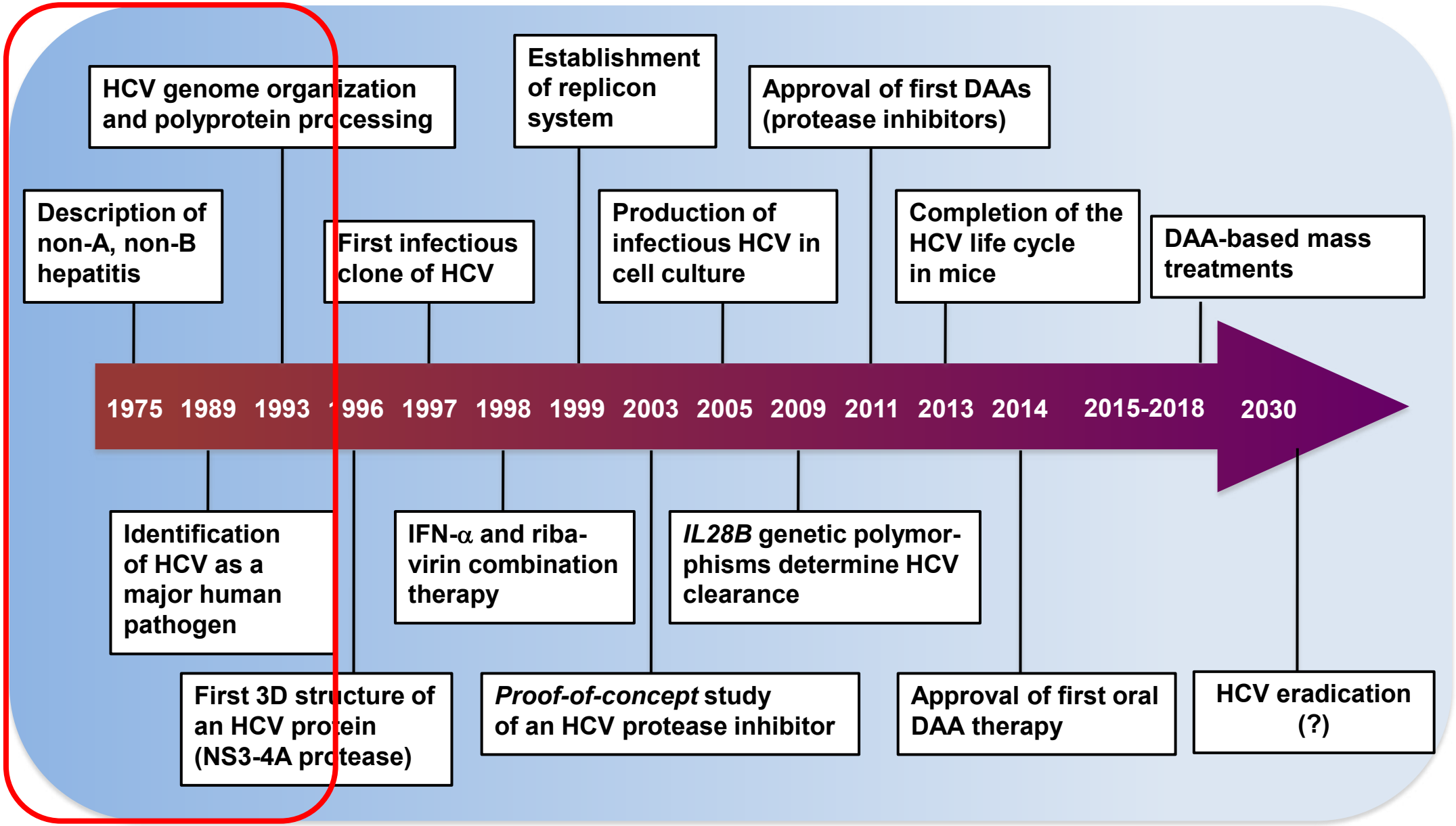


FIGURE 5. Frequency of cirrhosis of the liver as cause of death as compared with frequency of hepatitis.

From: Bjorneboe M, in: Hepatitis Frontiers. Eds Hartman FW & al. J & C Churchill, 1957:565-9

By Courtesy of Prof A. Craxì

Milestones in HCV research and cure



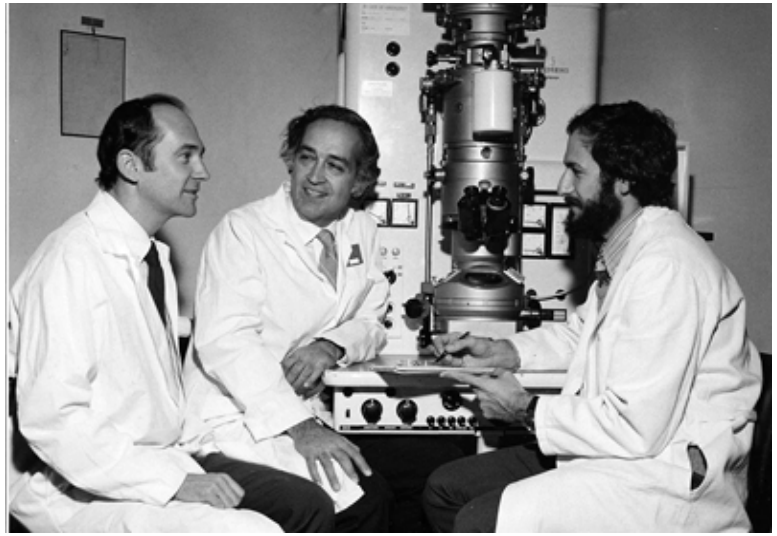
Modified from Moradpour D *et al*. Nat Rev Microbiol 2007;5:453-463.

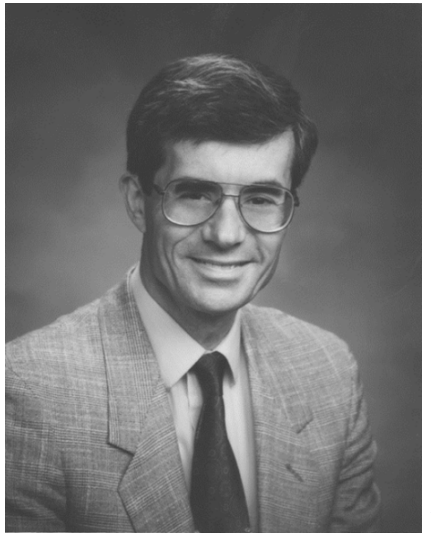
The First Description of Non A non B Hepatitis in Blood Transfusion Recipients

Feinstone SM, Kapikian AZ, Purcell RH, Alter HJ, Holland PV: **Transfusion-associated hepatitis not due to viral hepatitis type A or B**. *N Engl J Med* 1975, **292**(15):767-770.

> *It was soon recognized that the exclusion of HBV antibody-positive blood donors prevented only 20% of post-transfusion associated hepatitis. Thus, the remaining 80% of cases appeared to be unrelated to HBV infection. This new form of on "non-B" hepatitis became increasingly prevalent and it differed in the clinical manifestations it caused. While HBV-associated hepatitis had a long incubation period and often presented with severe acute symptoms, the "non-B" serum hepatitis had shorter incubation period and milder symptoms during the acute phase*

Feinstone SM, Kapikian AZ, Purcell RH: **Hepatitis A: detection by immune electron microscopy of a viruslike antigen associated with acute illness**. *Science* 1973, **182**(4116):1026-1028.
Alter HJ, Holland PV, Morrow AG, Purcell RH, Feinstone SM, Moritsugu Y: **Clinical and serological analysis of transfusion-associated hepatitis**. *Lancet* 1975, **2**(7940):838-841.
Alter HJ, Holland PV, Purcell RH: **The emerging pattern of post-transfusion hepatitis**. *Am J Med Sci* 1975, **270**(2):329-334.
Alter HJ, Purcell RH, Holland PV, Popper H: **Transmissible agent in non-A, non-B hepatitis**. *Lancet* 1978, **1**(8062):459-463.





By Courtesy of Prof A. Craxì

HCV discovery: one of the most significant biomedical breakthroughs in the last 30 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

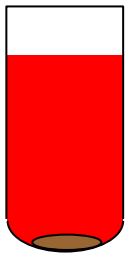
21 APRIL 1989 SCIENCE, VOL. 244

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….

The Discovery of the Hepatitis C Virus and Development of a Serological Assay



Ultracentrifuge
NANBH-Infectious
Chimpanzee Plasma

Pellet

Extract total RNA+DNA

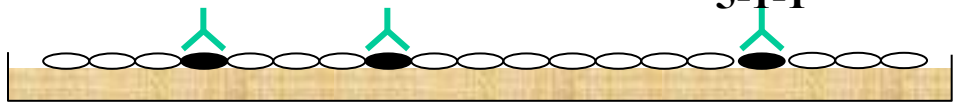
NANBH
Patients

Serum Antibodies

Incubate

False positives

Clone
5-1-1



Bacterial cDNA libraries in "expression" vector λgt11

DETERMINE PROPERTIES OF CLONE 5-1-1

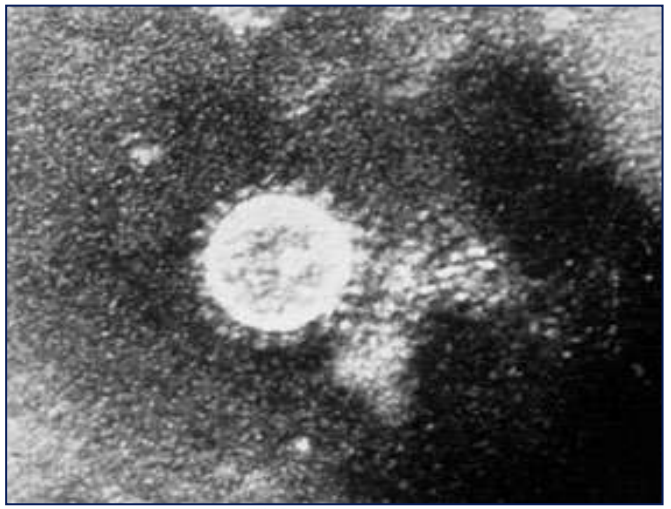
- *Extra*-chromosomal
- Derived from RNA (~9600 nt) found *only* in NANBH samples
- Encodes protein that binds antibodies found *only* in NANBH infections

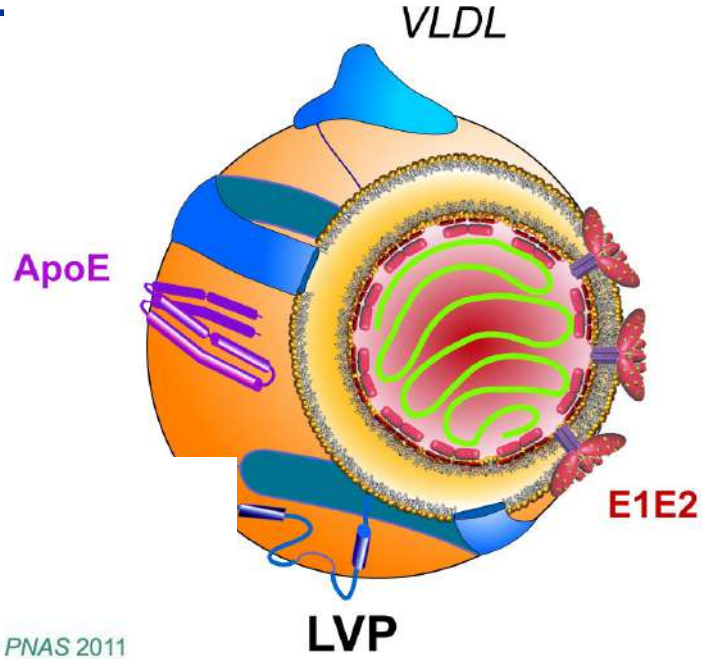
IDENTIFICATION OF HEPATITIS C VIRUS (HCV)

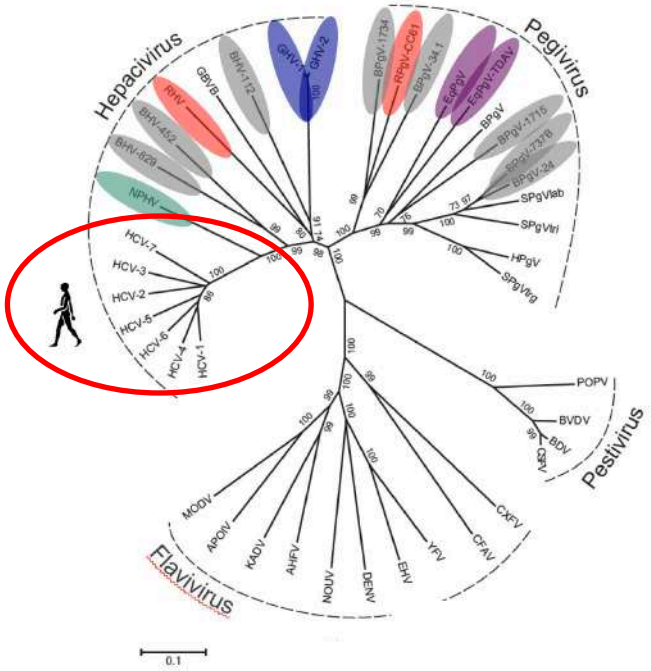
By Courtesy of Prof A. Craxì

The Hepatitis C Virus

- + SS RNA 9.6 Kb
- *Flaviviridae* family
- *Hepacivirus* genus
- High error rate during replication leads to the formation of quasispecies







Kapoor *PNAS* 2011
Burbelo *J Virol* 2012

Chandriani *PNAS* 2013
Kapoor *J Virol 2013*

Kapoor *mBio* 2013
Drexler *PLoS Pathog* 2013

Quan *PNAS* 2013

Lauck *J Virol* 2013

By Courtesy of Prof A. Craxì

https://www.nobelprize.org/prizes/medicine/2020/advanced-information/

The Nobel Prize in Physiology or Medicine 2020

Harvey J. Alter
Michael Houghton
Charles M. Rice

Share this

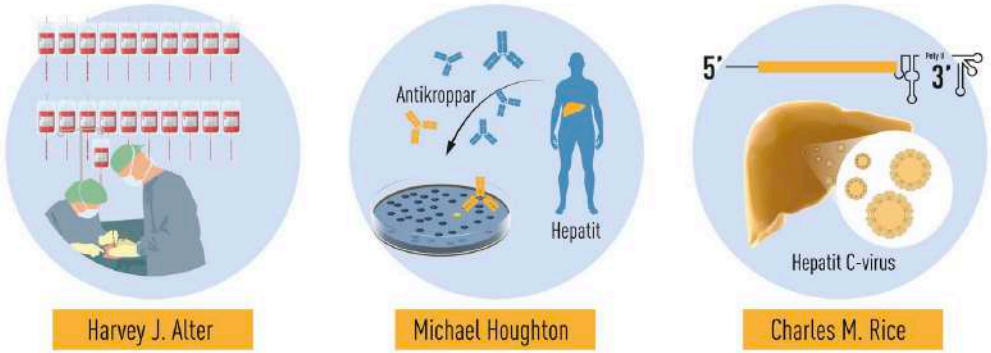


Figure 6. The pioneering discoveries of the Laureates.

Advanced information

Scientific background:
The discovery of Hepatitis C virus (pdf)

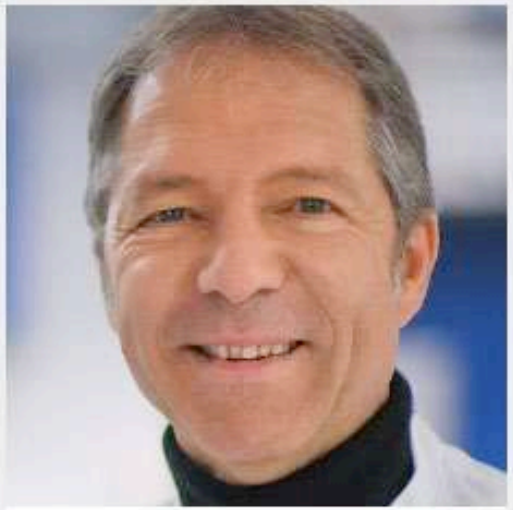
Nobelförsamlingen
The Nobel Assembly at Karolinska Institutet

The discovery of Hepatitis C virus

*The 2020 Nobel Prize in Physiology or Medicine is awarded to **Harvey J. Alter**, **Michael Houghton** and **Charles M. Rice** for the discovery of Hepatitis C virus. Hepatitis, from the Greek names for liver and inflammation, is a disease characterized by poor appetite, vomiting, fatigue and jaundice – yellow discoloration of the skin and eyes. Chronic hepatitis leads to liver damage, which may progress to cirrhosis and liver cancer. Viral infection is the leading cause of hepatitis, with some forms persisting without symptoms for many years before life-threatening complications develop. Until the 1960's, exposure to blood from infected individuals was a major health hazard, with up to 30% risk of chronic hepatitis following surgery or multiple blood transfusions. This risk was only partially reduced by the discovery of the Hepatitis B virus (HBV) and the eventual elimination of HBV-contaminated blood through testing. A more insidious form of hepatitis, characterized by very mild symptoms in the acute phase and a high risk of progression to chronic liver damage and cancer, remained. The work of **Alter**, **Houghton** and **Rice** characterized this form of hepatitis to be a distinct clinical entity, caused by an RNA virus of the Flavivirus family, now known as Hepatitis C virus (HCV). This pioneering work has paved the way for the development of screening methods that have dramatically reduced the risk of acquiring hepatitis from contaminated blood and has led to the development of effective antiviral drugs that have improved the lives of millions of people.*

2016 Lasker~DeBakey Clinical Medical Research Award

Hepatitis C replicon system and drug development



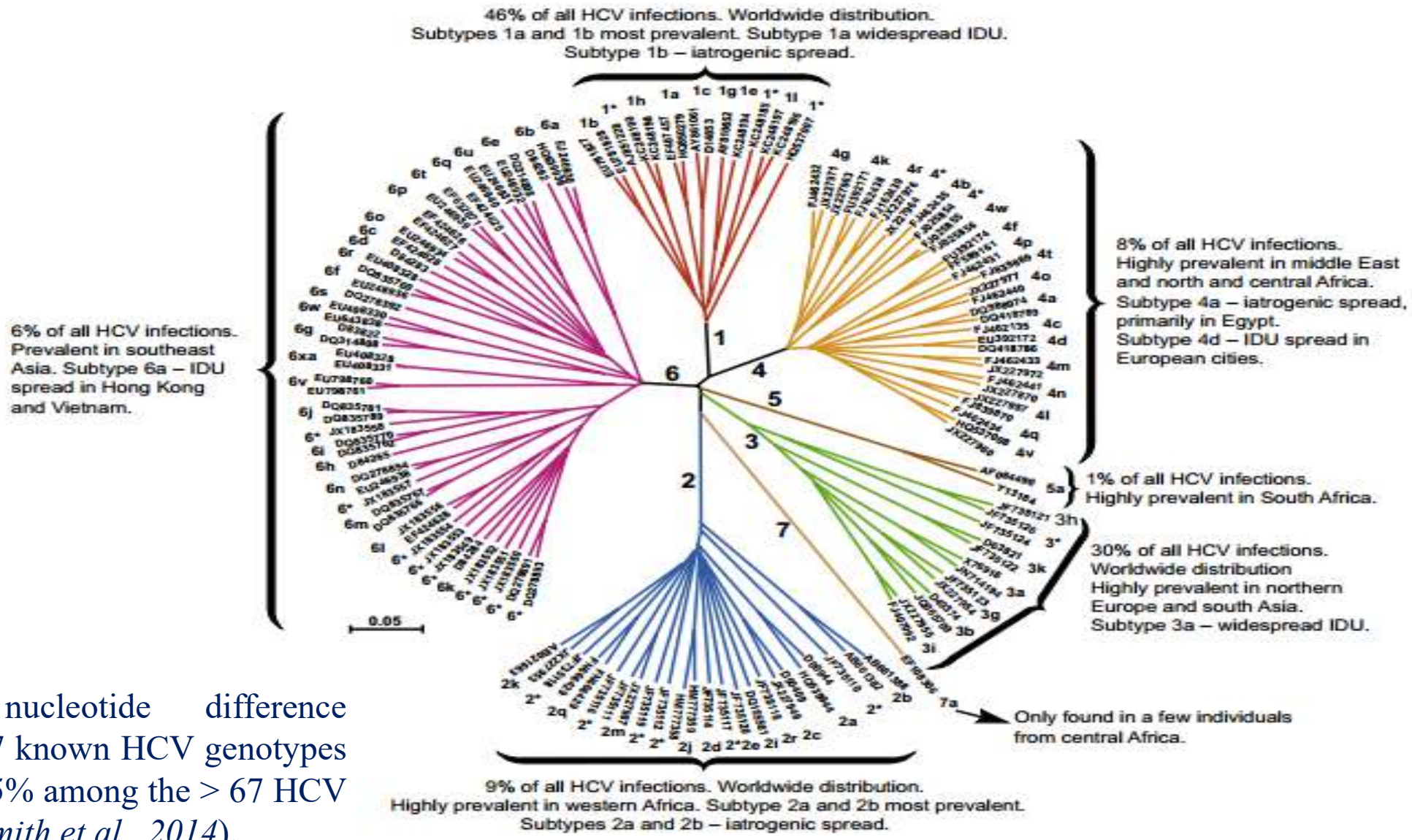
Ralf F.W. Bartenschlager
Heidelberg University

Charles M. Rice
Rockefeller University

Michael J. Sofia
Arbutus Biopharma

For development of a system to study the replication of the virus that causes hepatitis C and for use of this system to revolutionize the treatment of this chronic, often lethal disease.

HCV variants: 7 main genotypes and at least 67 subtypes

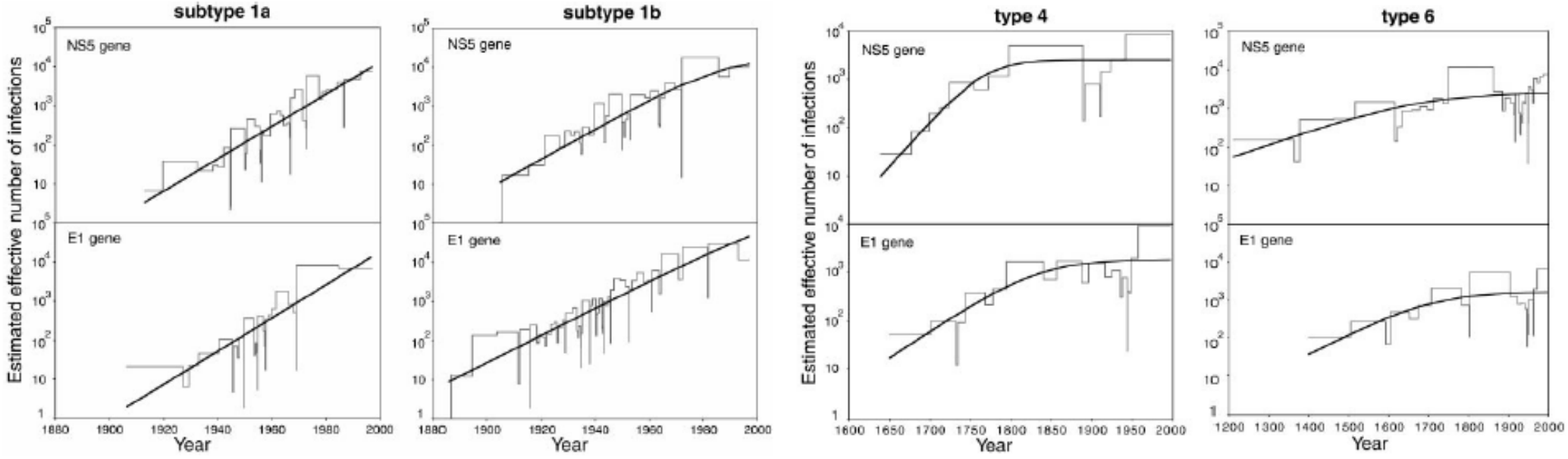


31%–33 nucleotide difference among the 7 known HCV genotypes and 20%–25% among the > 67 HCV subtypes (*Smith et al., 2014*).

Jens Bukh J Hepatol, 2016

HCV: an old foe

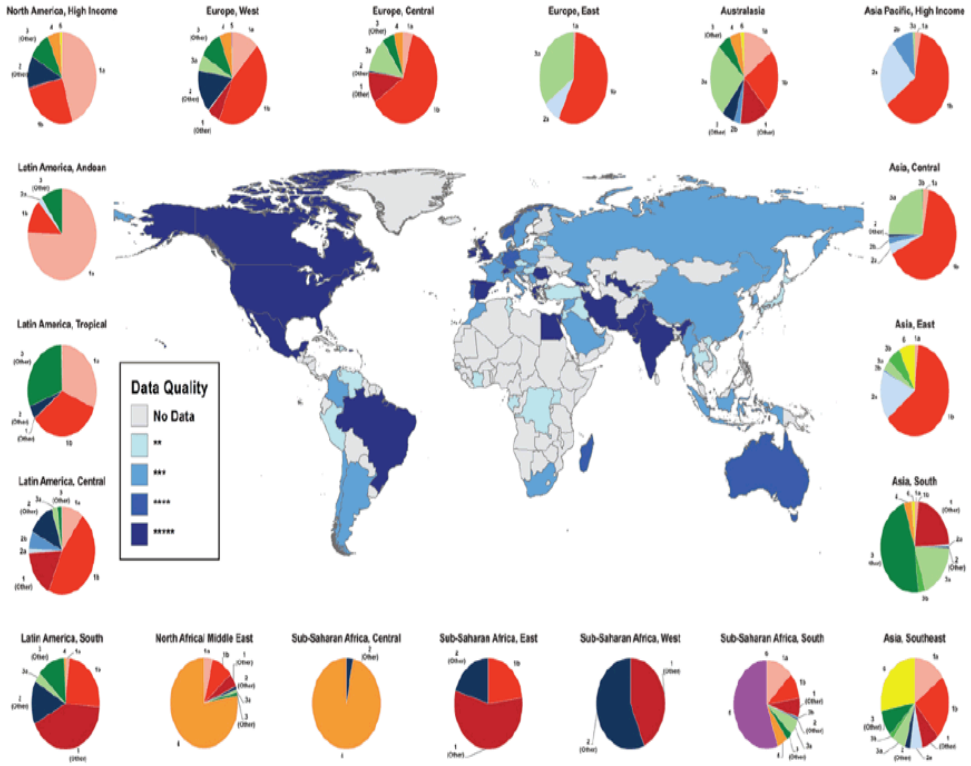
- The origin of the primate Flaviviridae could be as ancient as the differentiation of primate species some 35 million years ago.
- HCV could have been coevolving with human populations during their migration out of Africa within the past 100,000 to 150,000 years, **but the current HCV genotypes appeared 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**.
- HCV started its epidemic spread in 1900, and expanded globally after World War II



Pybus et al Science 2001

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

Key Facts

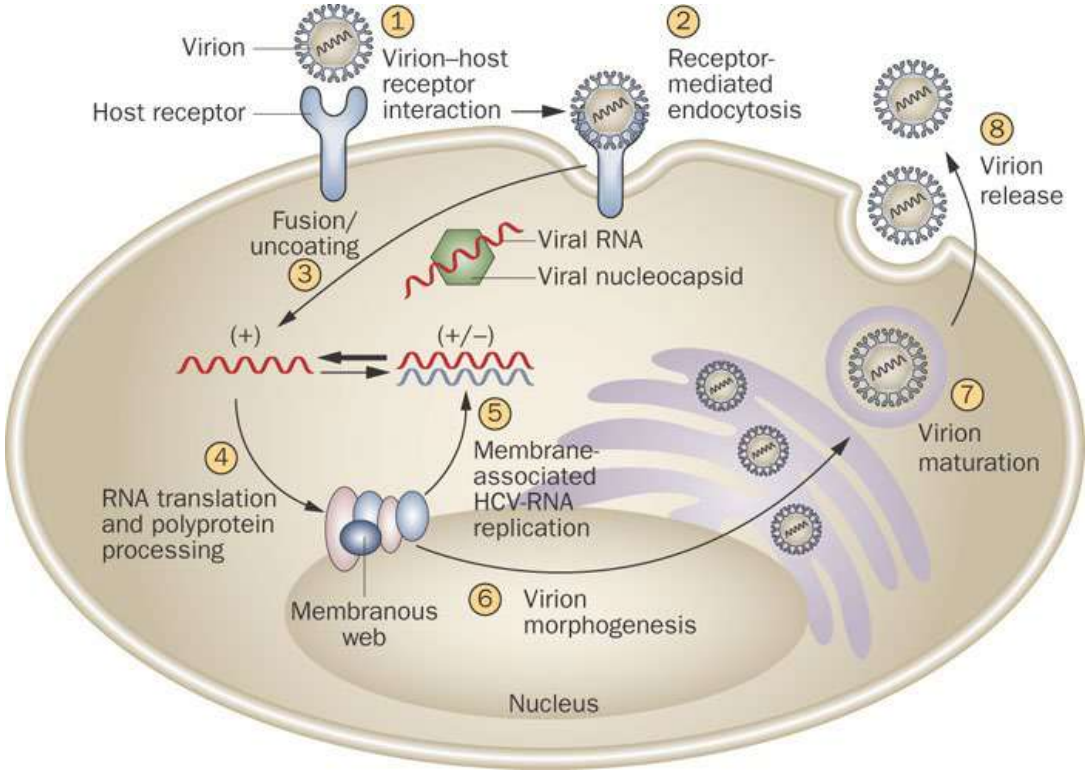
The World Health Organization (WHO) estimates that during 2019

- **296 million people worldwide are living with hepatitis B**
- **58 million people worldwide are living with hepatitis C**
- **1.5 million people were newly infected with chronic HBV**
- **1.5 million people were newly infected with chronic HCV**

Both hepatitis B and hepatitis C can lead to lifelong infection. WHO estimates that 1.1 million deaths occurred in 2019 due to these infections and their effects including liver cancer, cirrhosis, and other conditions caused by chronic viral hepatitis

World Health Organization. Global progress report on HIV, viral hepatitis and sexually transmitted infections, 2021. Available at: https://www.who.int/publications/i/item/9789240027077external icon.

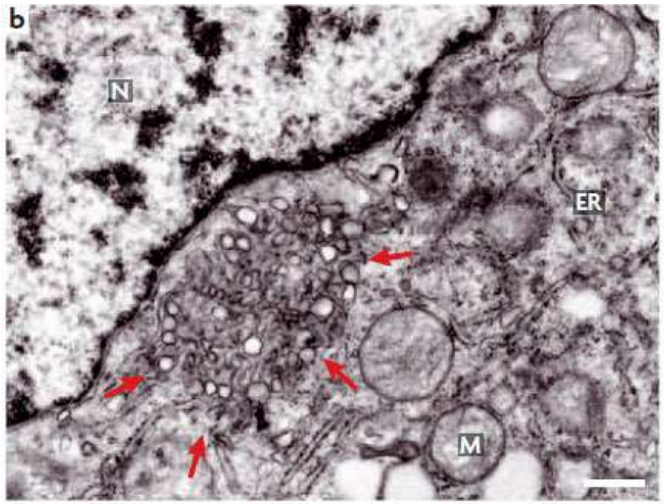
The long-term persistence of HCV infection is unique among RNA viruses that replicate without a DNA form



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 demonstrated**

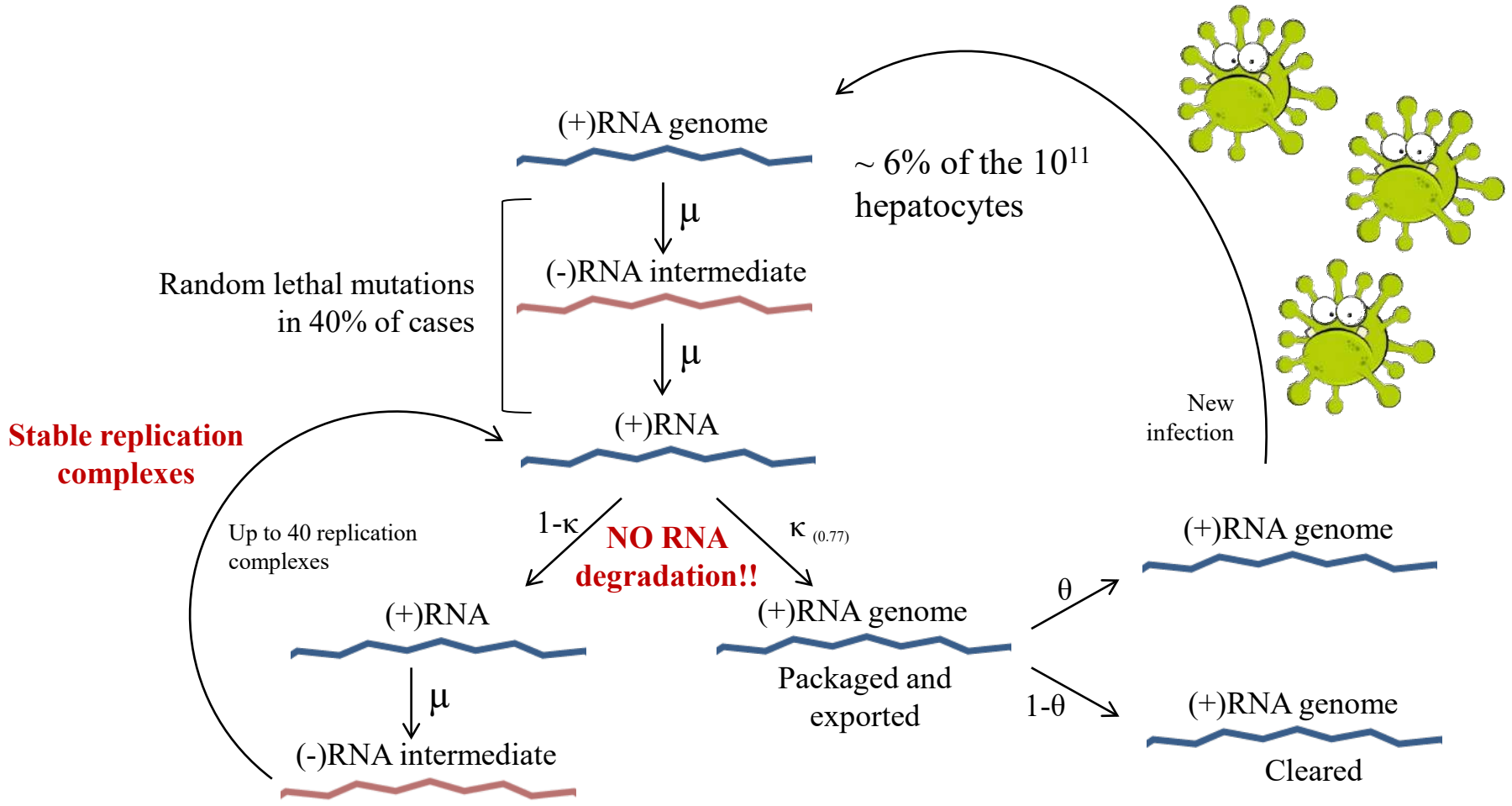
- **HCV replication occurs only in cytoplasm**

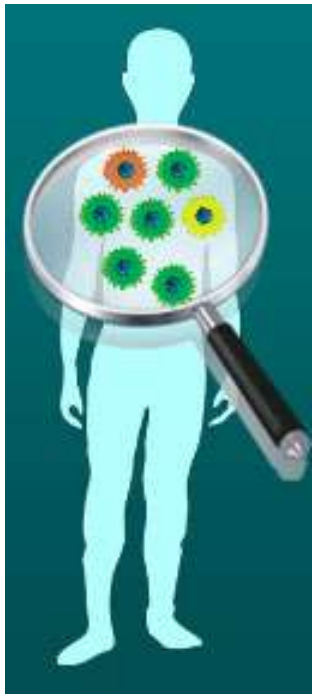


Moradpour D et al., Nature 2007

… This makes HCV curable!!!!

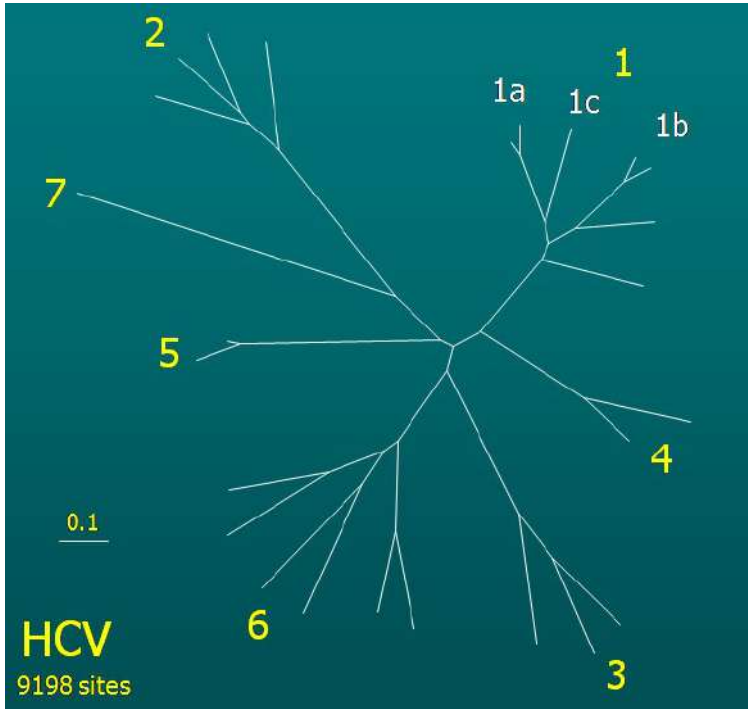
HCV Genome Variability

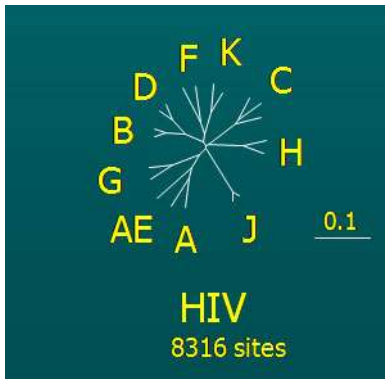


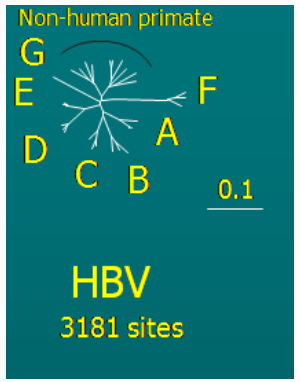


$\mu = 2.5 \cdot 10^{-5}$/nucleotide/replication cycle

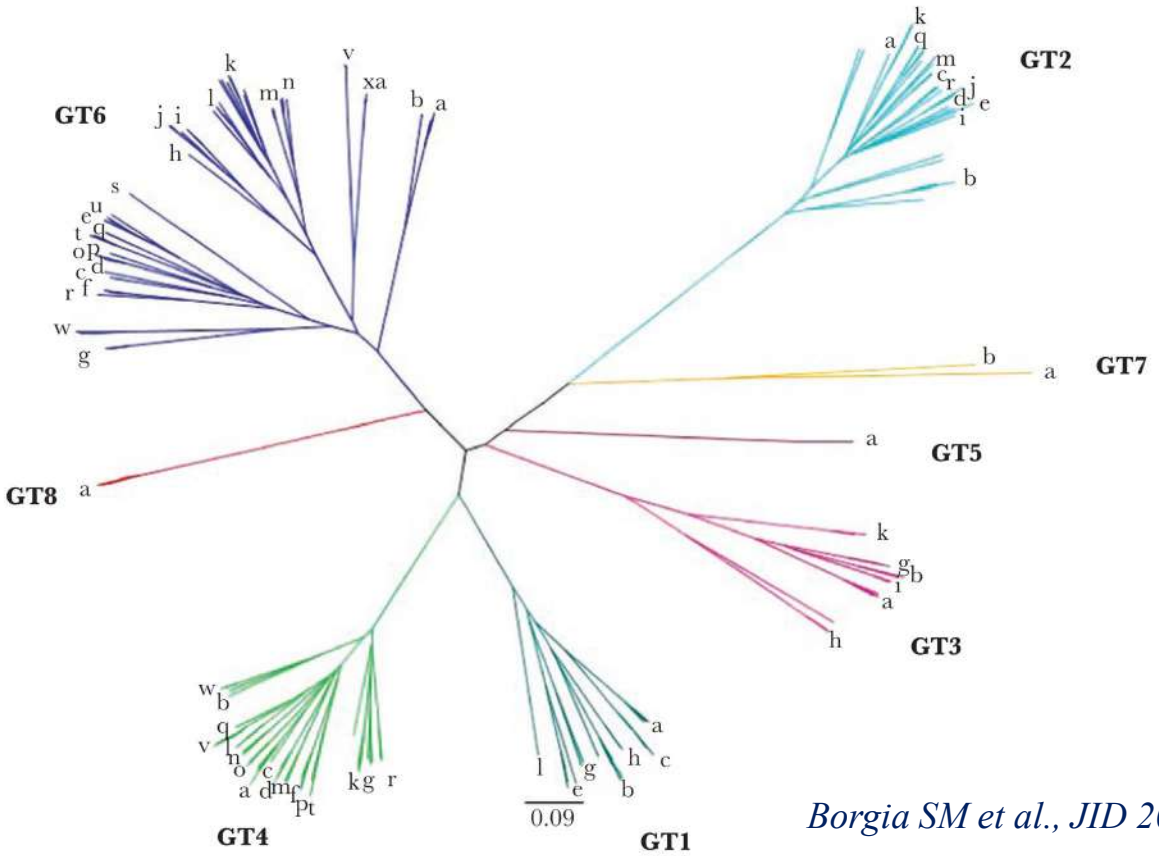
HCV genetic variability is higher than HIV's and HBV's







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



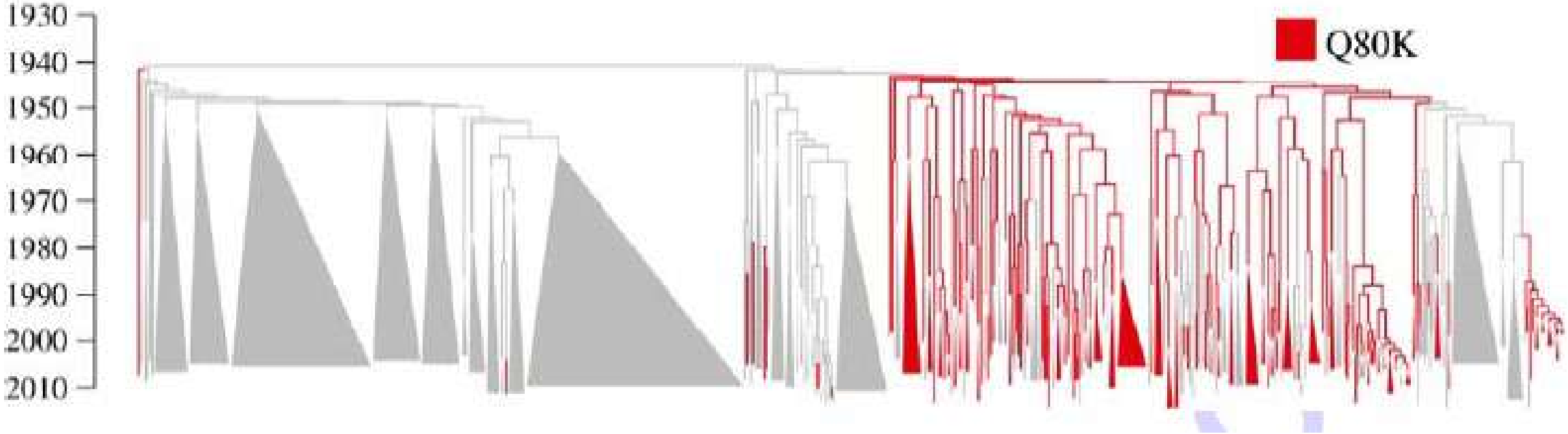
Borgia SM et al., JID 2018

31%–33% nucleotide difference among the 7 known HCV genotypes and 20%–25% among the nearly 67 HCV subtypes

Smith et al., 2014

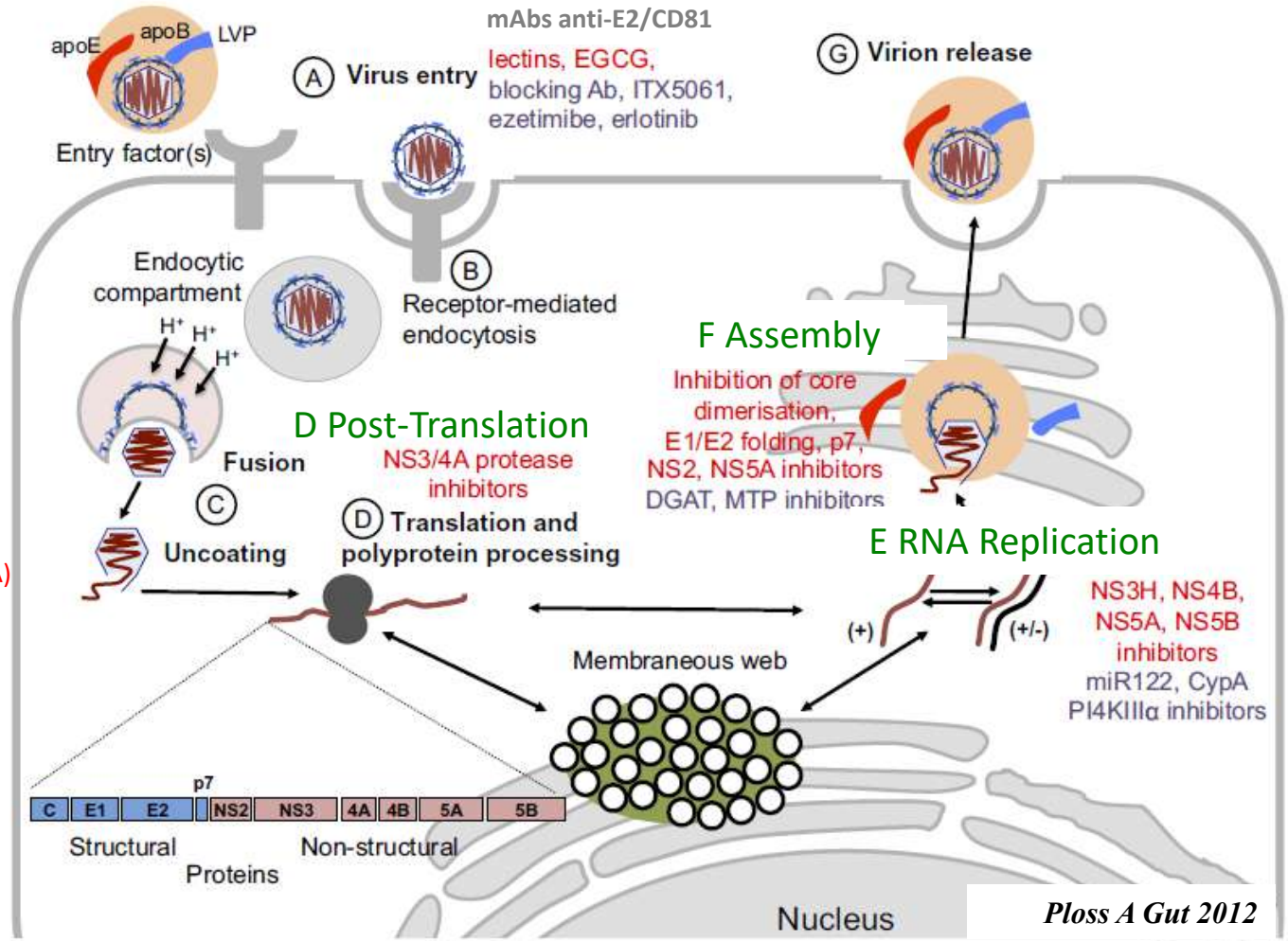
Some RASs can be highly transmissible

96% of HCV strains carrying Q80K descend from a single lineage in which a Q80K substitution occurred in the 1940's in the USA



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

Drugs active on viral enzymes
Drugs active on host cell enzymes



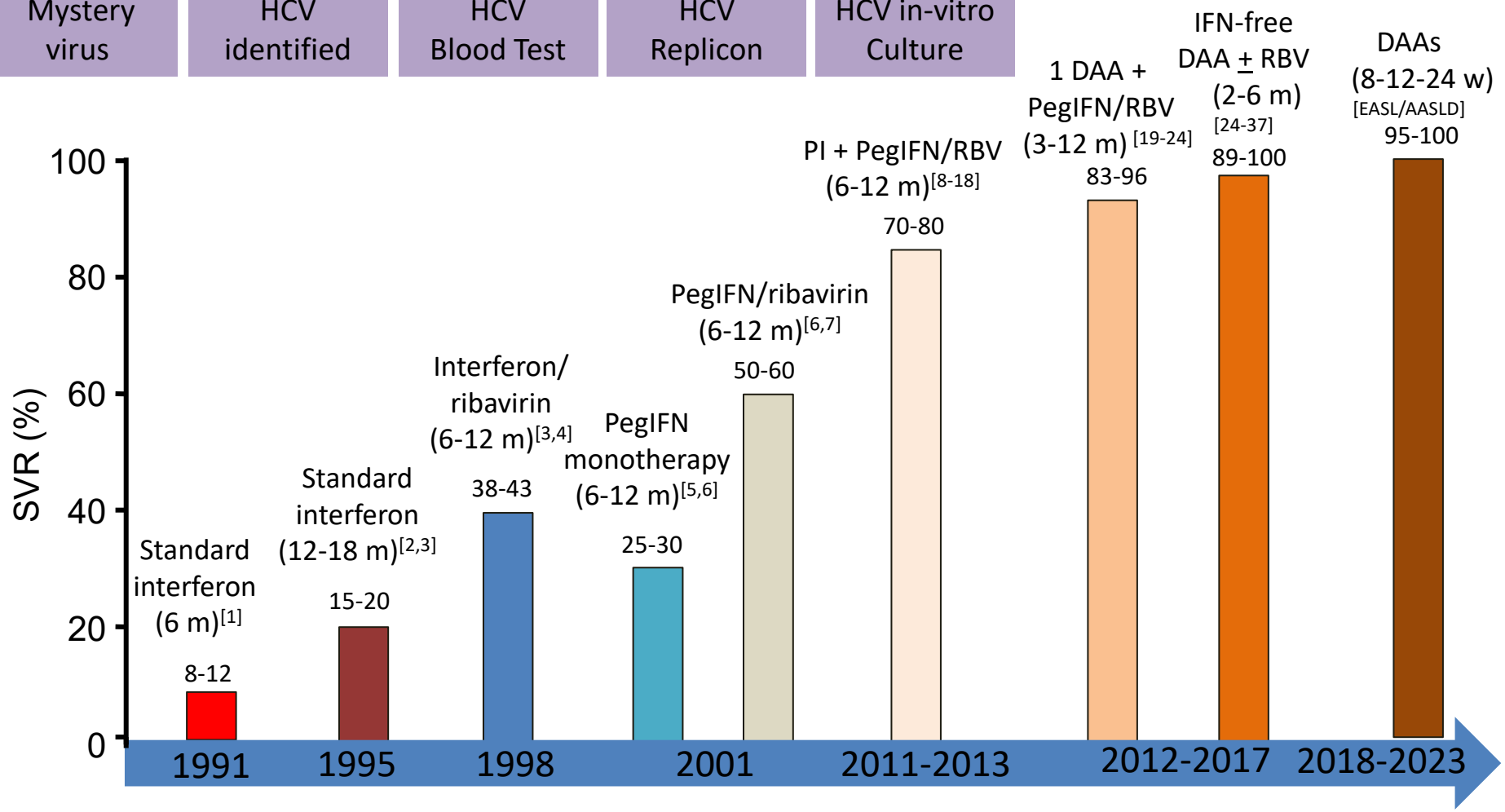
D Translation
miRNA
ISIS 14803 (antisense)
AVI- 4066 (antisense)
Heptazyme (ribozyme)
VGX-410C (small molecules
IRES inhibitor) TT 033 (sIRNA)
eIF2a phosphorilation inhibitors: Nitazoxanide

Ploss A Gut 2012

From August 2011, Telaprevir and Boceprevir, 2 linear protease inhibitors, have been approved by both FDA and EMA

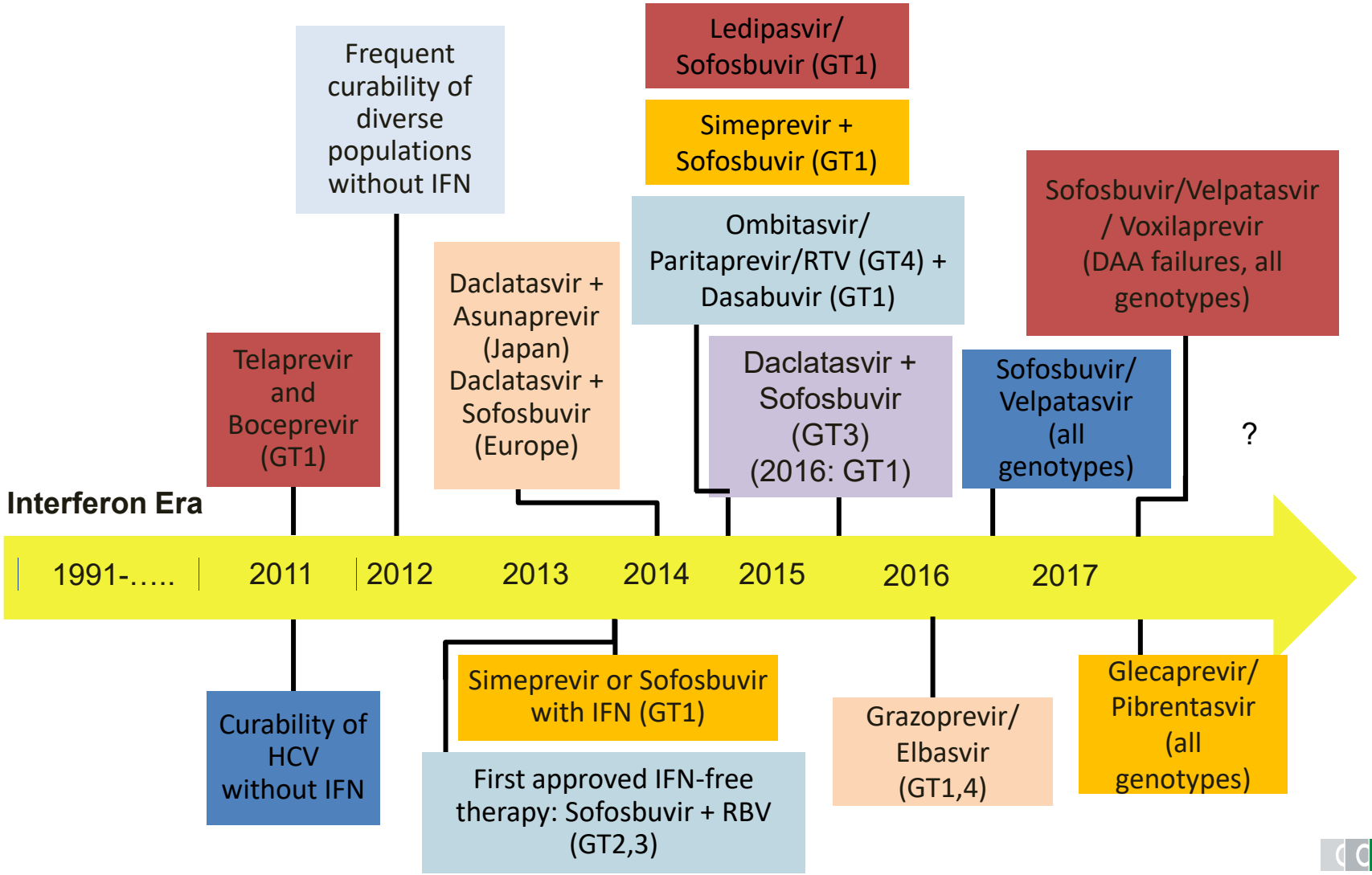
The standard of care for HCV patients has greatly improved

1974	1989	1992	1999	2005
Mystery virus	HCV identified	HCV Blood Test	HCV Replicon	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.

The Evolution of HCV Therapy



References in slidenotes.

Slide credit: clinicaloptions.com

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

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: >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

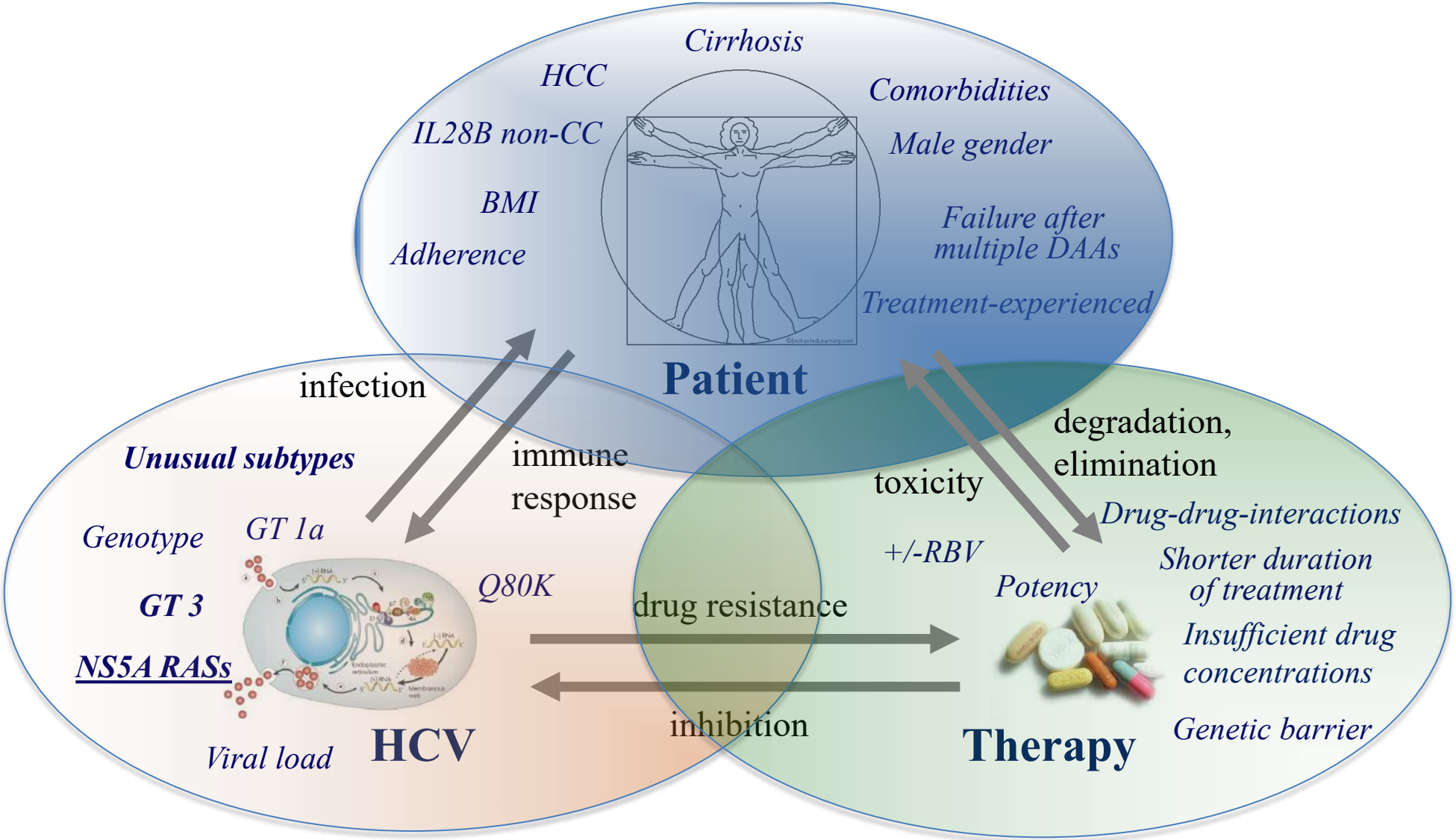
Disease Eradication *vs* Elimination *vs* Control

……From individual health to community health perspective……

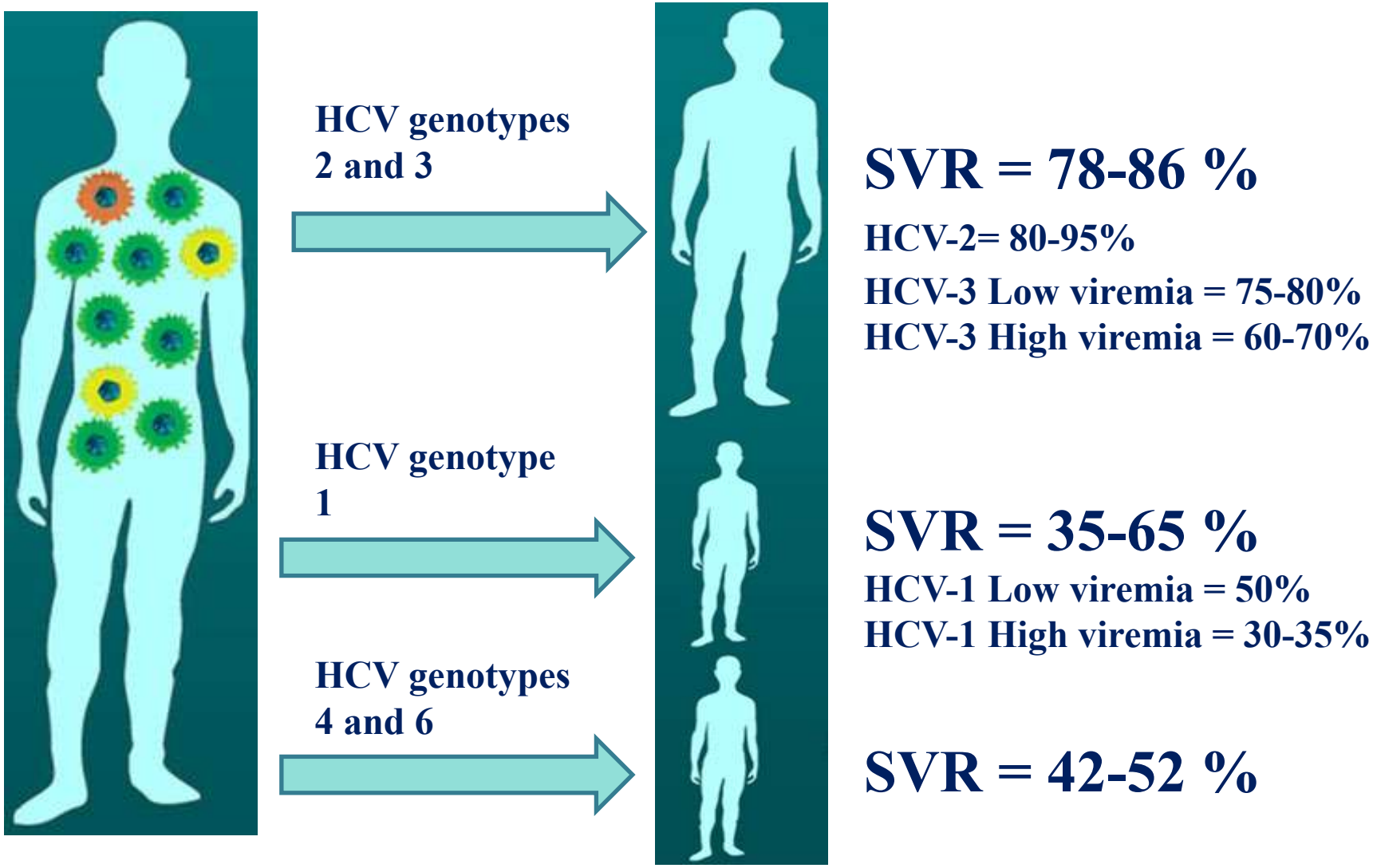
- **Control:** reduction in the incidence, prevalence, morbidity, or mortality of an infectious disease to a locally acceptable levels; continued intervention measures required
- **Elimination:** reduction to zero of incidence in a defined geographical area as a result of deliberate efforts; continued 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.

Many factors contribute to viral response to DAA-treatment



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



SVR = 78-86 %

HCV-2= 80-95%

HCV-3 Low viremia = 75-80%
HCV-3 High viremia = 60-70%

SVR = 35-65 %

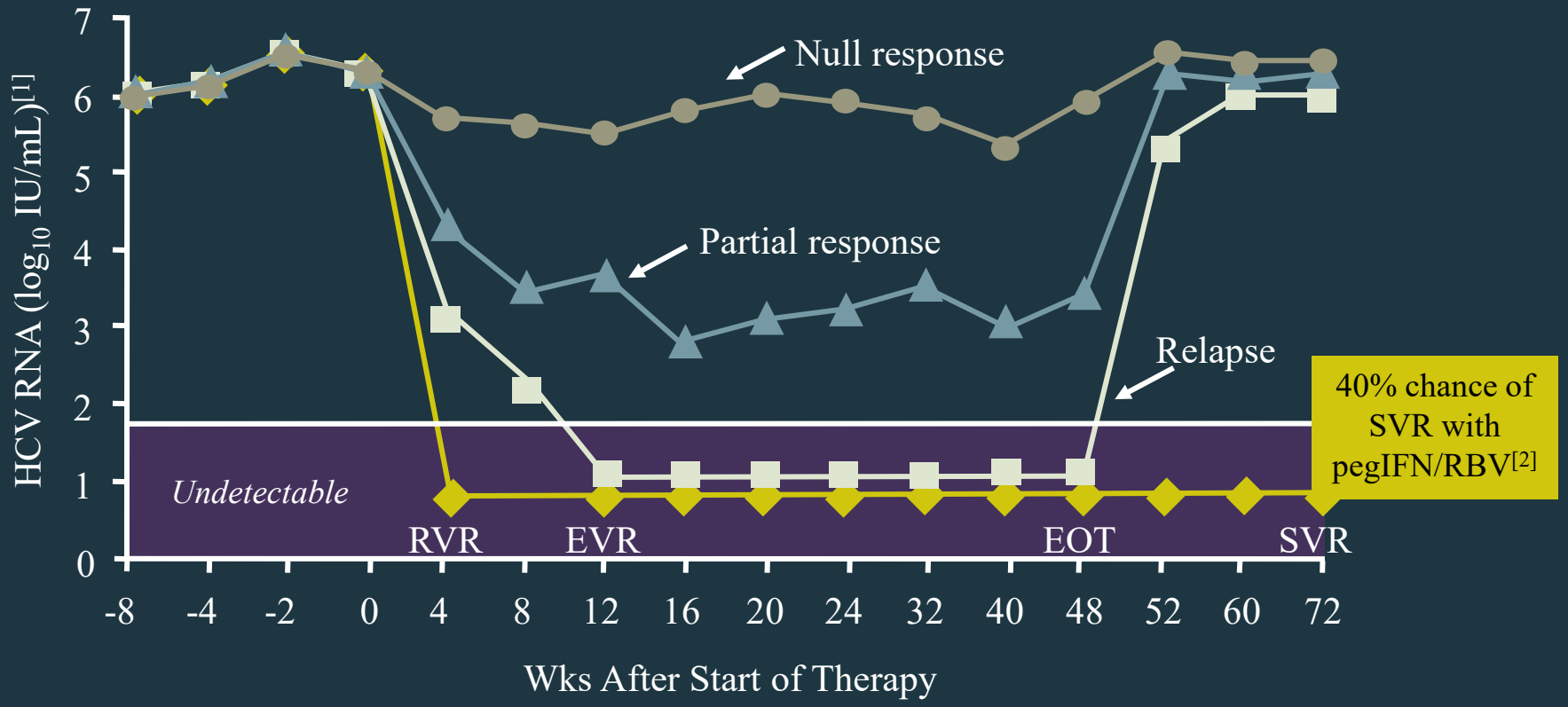
HCV-1 Low viremia = 50%
HCV-1 High viremia = 30-35%

SVR = 42-52 %

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

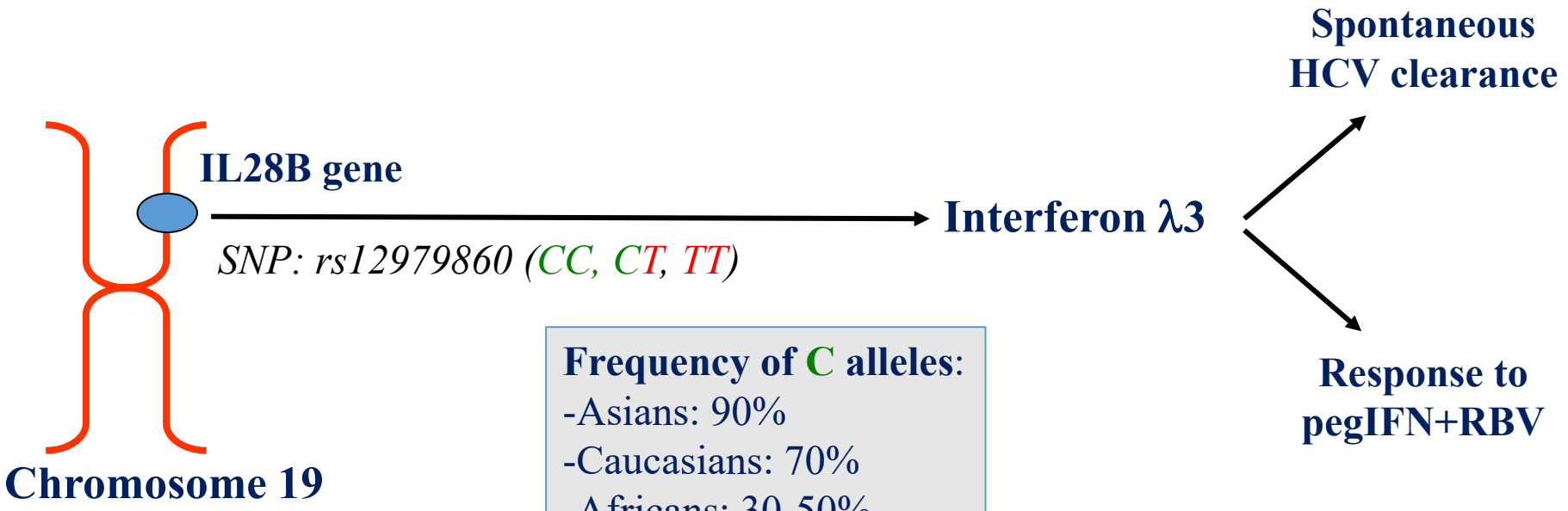
The underlying functional mechanisms for lower SVR rates of the different HCV genotypes were unknown

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.

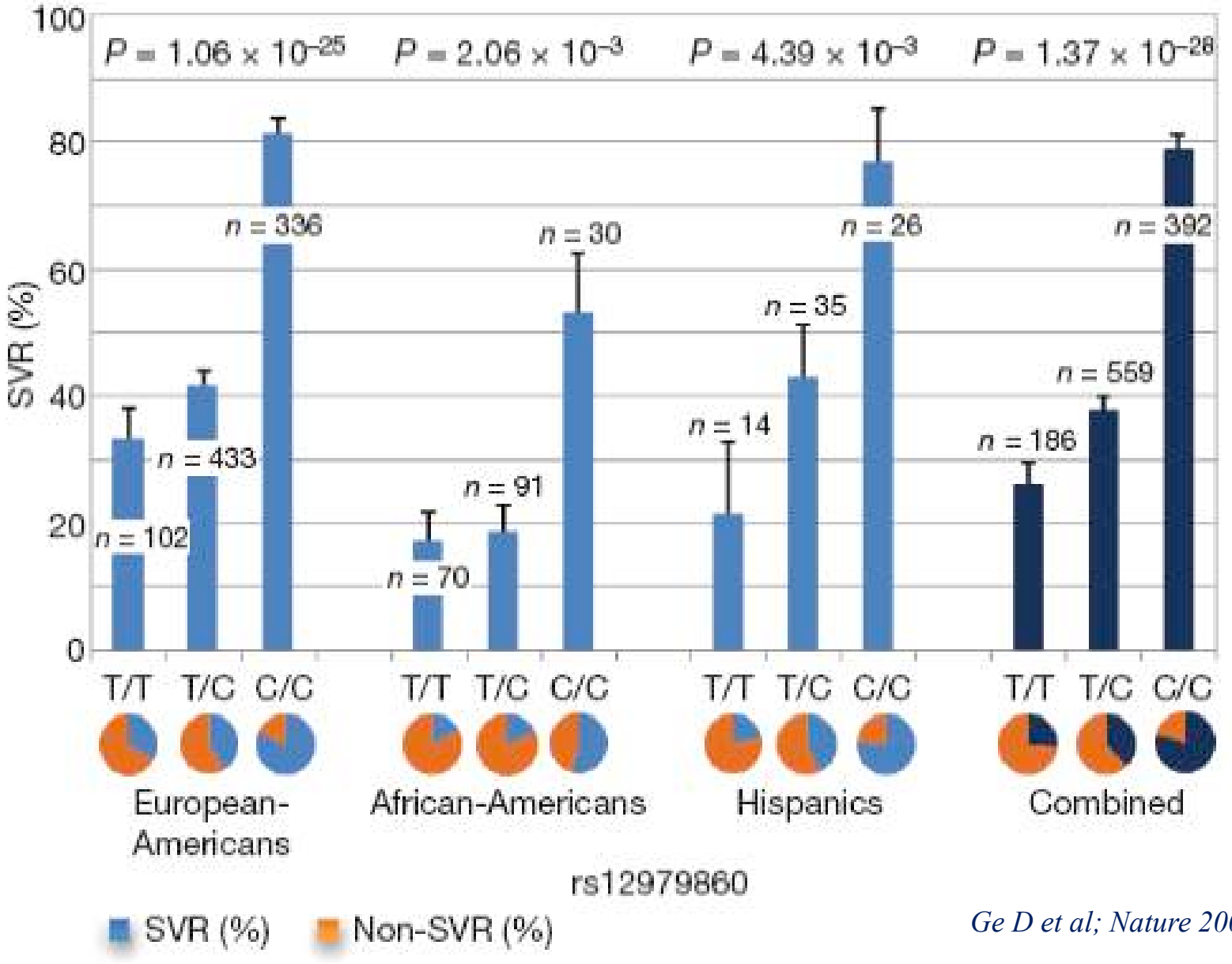
IL28B polymorphisms & hepatitis C outcome



Genetic polymorphism near the IL28B gene, encoding interferon λ3 is associated with an approximately two-three fold change in response to treatment, both among patients of European ancestry (P51.06310225) **and African-Americans.** *Ge et al. Nature 2009*

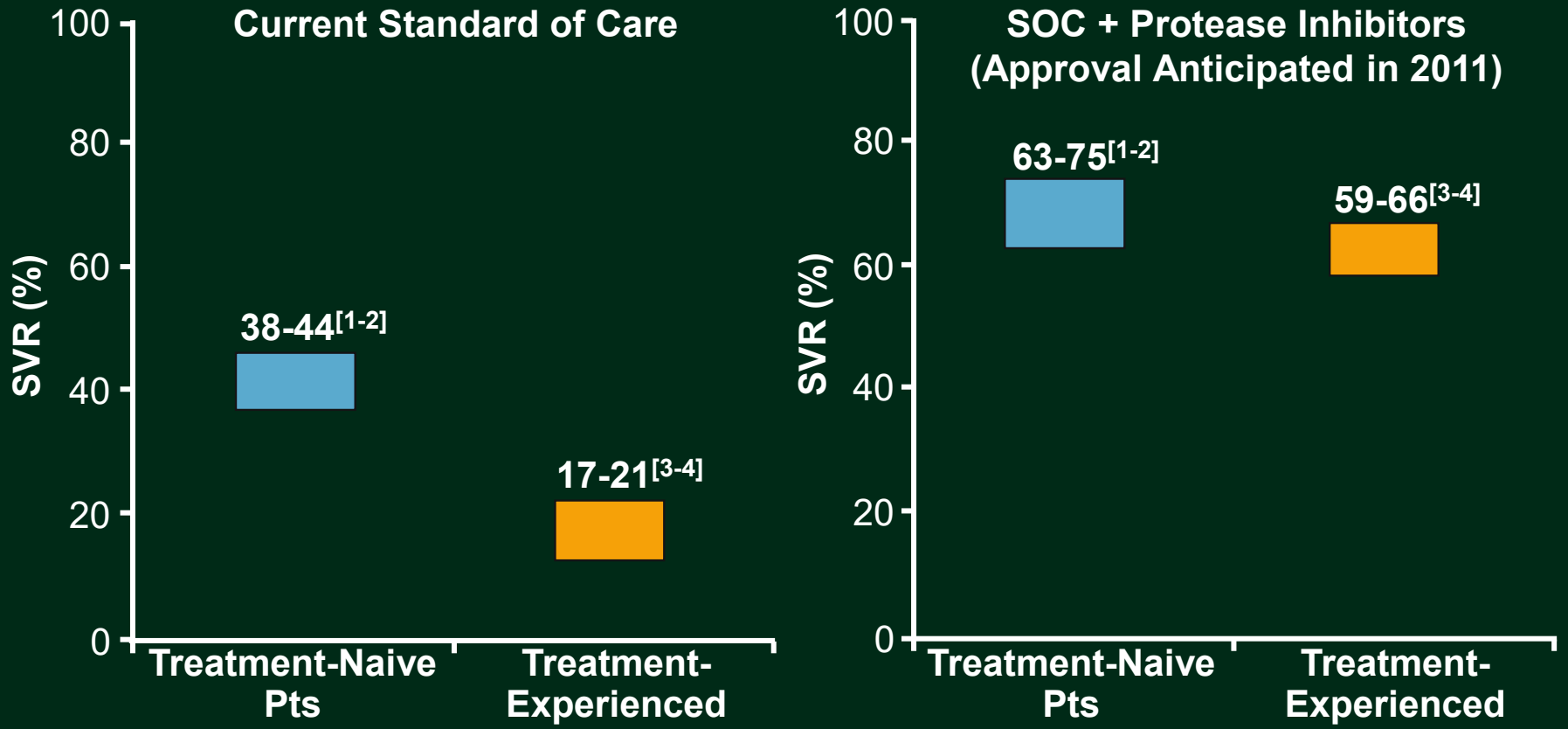
Ge et al. Nature 2009; 461: 399-401.
Thomas et al. Nature 2009; 461: 798-802.
Suppiah et al. Nature Gen 2009; 41: 1100-4.
Tanaka et al. Nature Gen 2009; 41: 1105-9.

Percentage of SVR by genotypes of rs12979860



Ge D et al; Nature 2009

SVR Rates With BOC and TPV in GT1 Treatment-Naive and -Experienced Pts

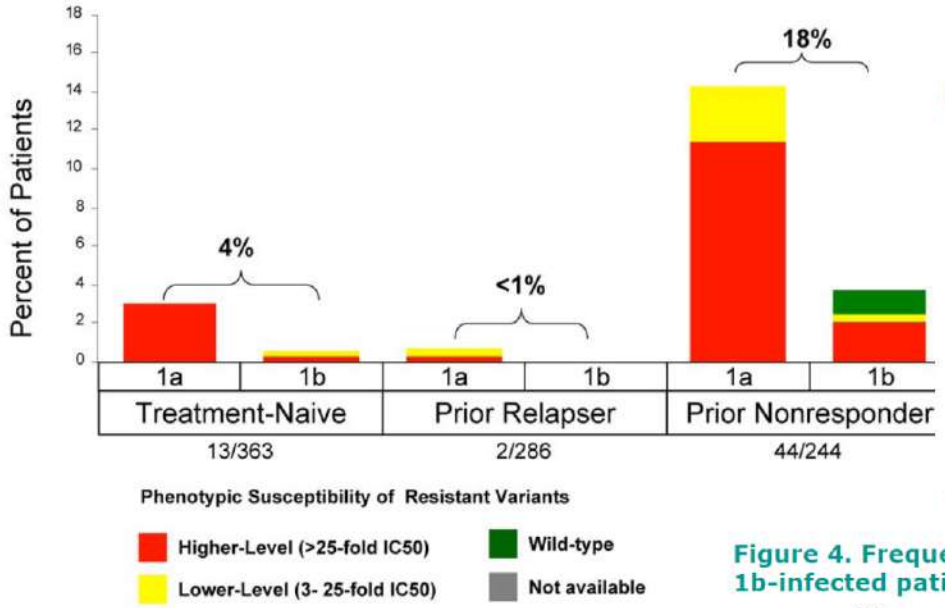


1. Poordad F, et al. AASLD 2010. Abstract LB-4. 2. Jacobson IM, et al. AASLD 2010. Abstract 211. 3. Bacon BR, et al. AASLD 2010. Abstract 216. 4. Foster GR, et al. APASL 2011. Abstract 1529.

Critical factors determining response to pegIFN/RBV ± DAA

- **Host factors**
  - Ethnicity and race (i. e. host genetic background)
  - Older age
  - Obesity and insulin resistance
- **Virus factors**
  - Viral load
  - Genetic variability
  - Viral kinetics within the first few weeks of treatment
- **Stage of liver disease**
- **Treatment history and response**
- **Treatment factors (current)**
  - Adherence
  - Side effects
  - Pharmacokinetic problems: poor drug absorption, inadequate dosing or drug-drug interaction

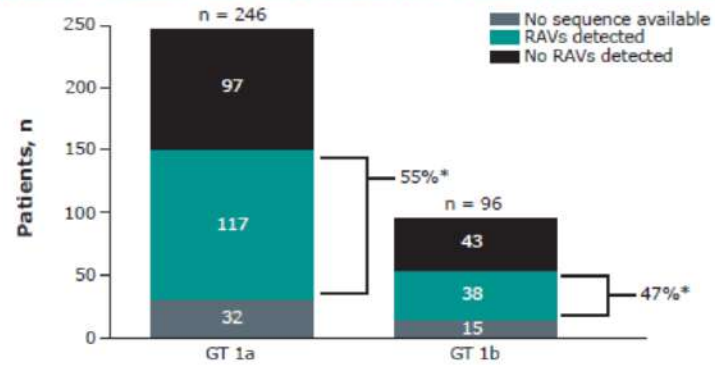
Virologic failure during telaprevir or boceprevir treatment was more common in patients with genotype 1a



Kieffer, PLoS ONE 2012

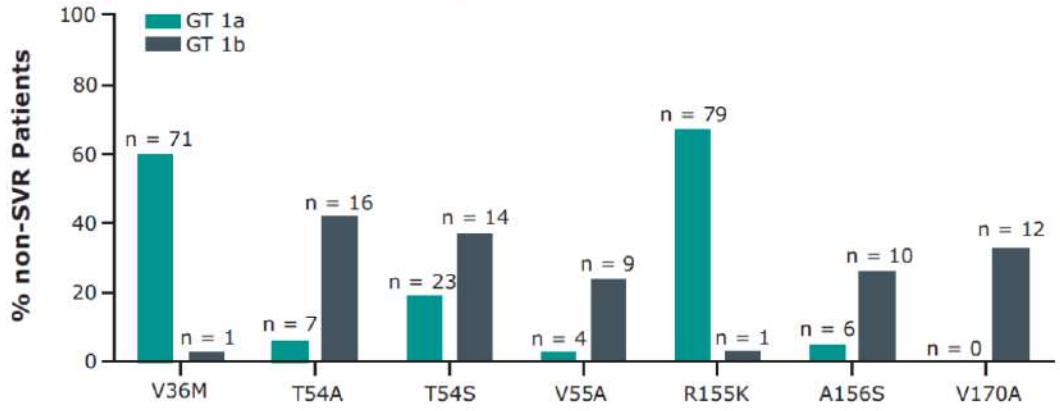
RAVs overall occurred more frequently in HCV GT 1a than GT 1b for patients who did not achieve SVR

Figure 3. Patients with HCV GT 1a showed a higher rate of RAVs than GT 1b.



*RAVs expressed as percentage of patients with sequence data available.

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



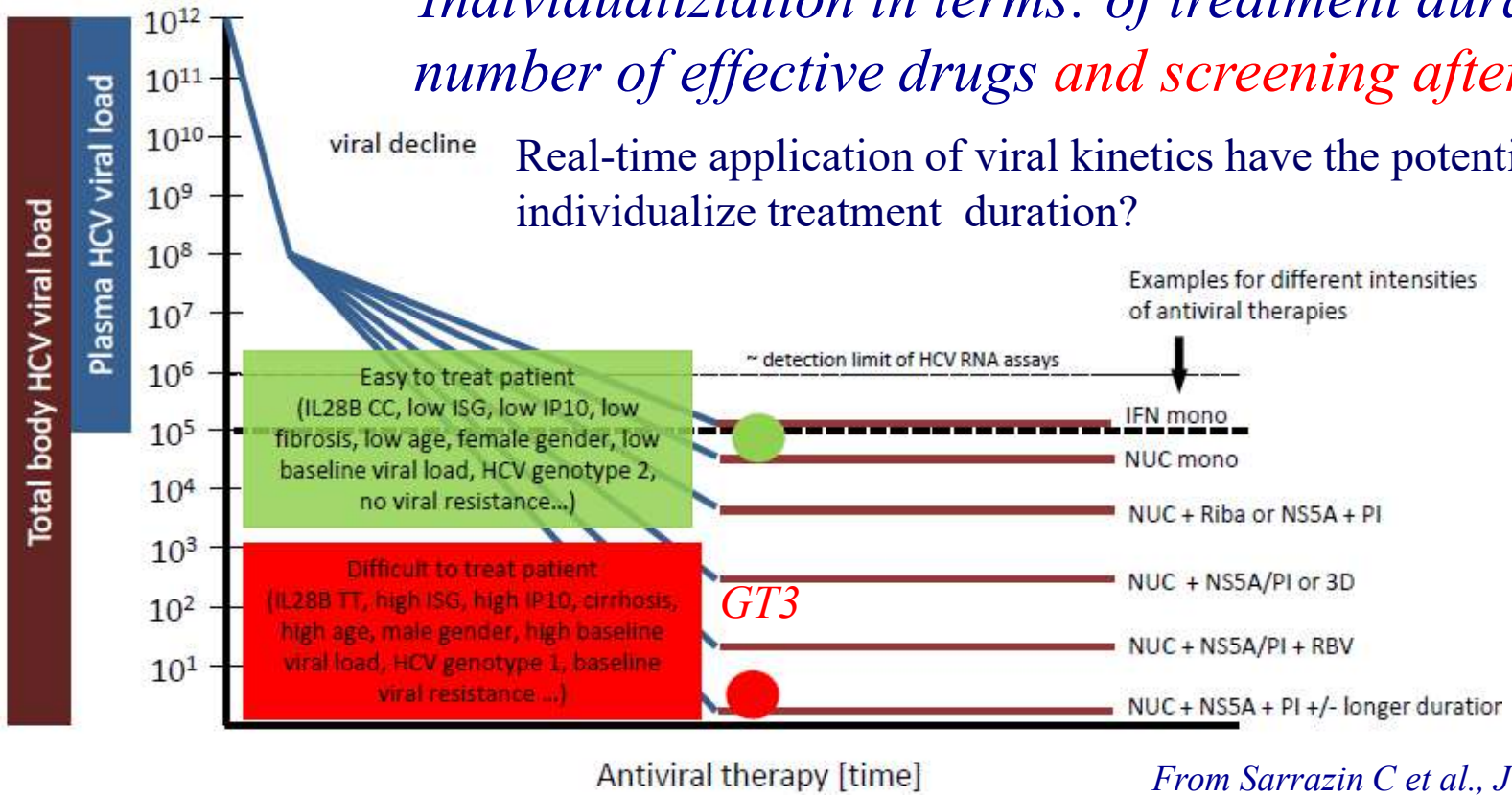
…… and with different emergency of resistance variants

Ogert RA et al., AASLD 2011

Treatment should be individualized: before starting the DAA treatment and particularly before re-treatment

Individualiziation in terms: of treatment duration, number of effective drugs and screening after SVR

Real-time application of viral kinetics have the potential to individualize treatment duration?



From Sarrazin C et al., J Hepatol 2016

HIV *versus* HCV

Resistance testing during / after a failure of DAA regimen can help physicians for the best choice of retreatment

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

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

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

➢***Resistance testing at failure is standard of care***

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

➢ ***Baseline resistance testing is not standard of care*** *(except GT1a or GT3 specific drugs)*

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

➢***Resistance testing at failure is recommended /suggested***

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

1a vs 1b

First-line regimen

1% cutoff
No RAS, SVR:

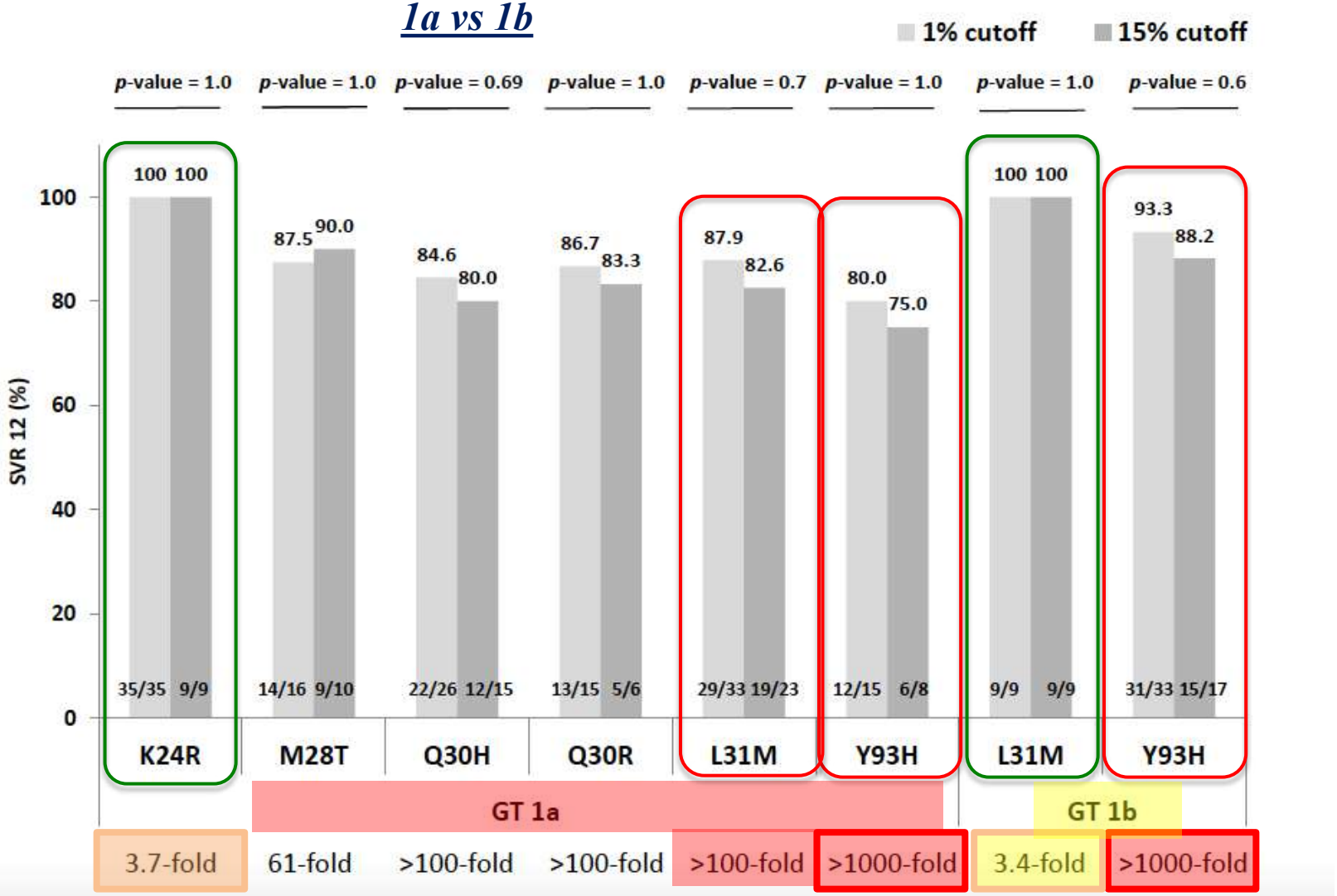
GT1a: 98.3%
1306/1329 pts

GT1b: 98.6%
1741/1770 pts

15% cutoff
No RAS, SVR:

GT1a: 98%
1416/1445 pts

GT1b: 98.7%
1880/1915 pts



No difference between 1% and 15% cutoff

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

Impact of baseline NS5A RAVs with >100 fold-resistance can be reduced by changing the regimen (longer duration of treatment and inclusion of RBV) in HCV-1 patients treated with Ledipasvir/Sofosbuvir

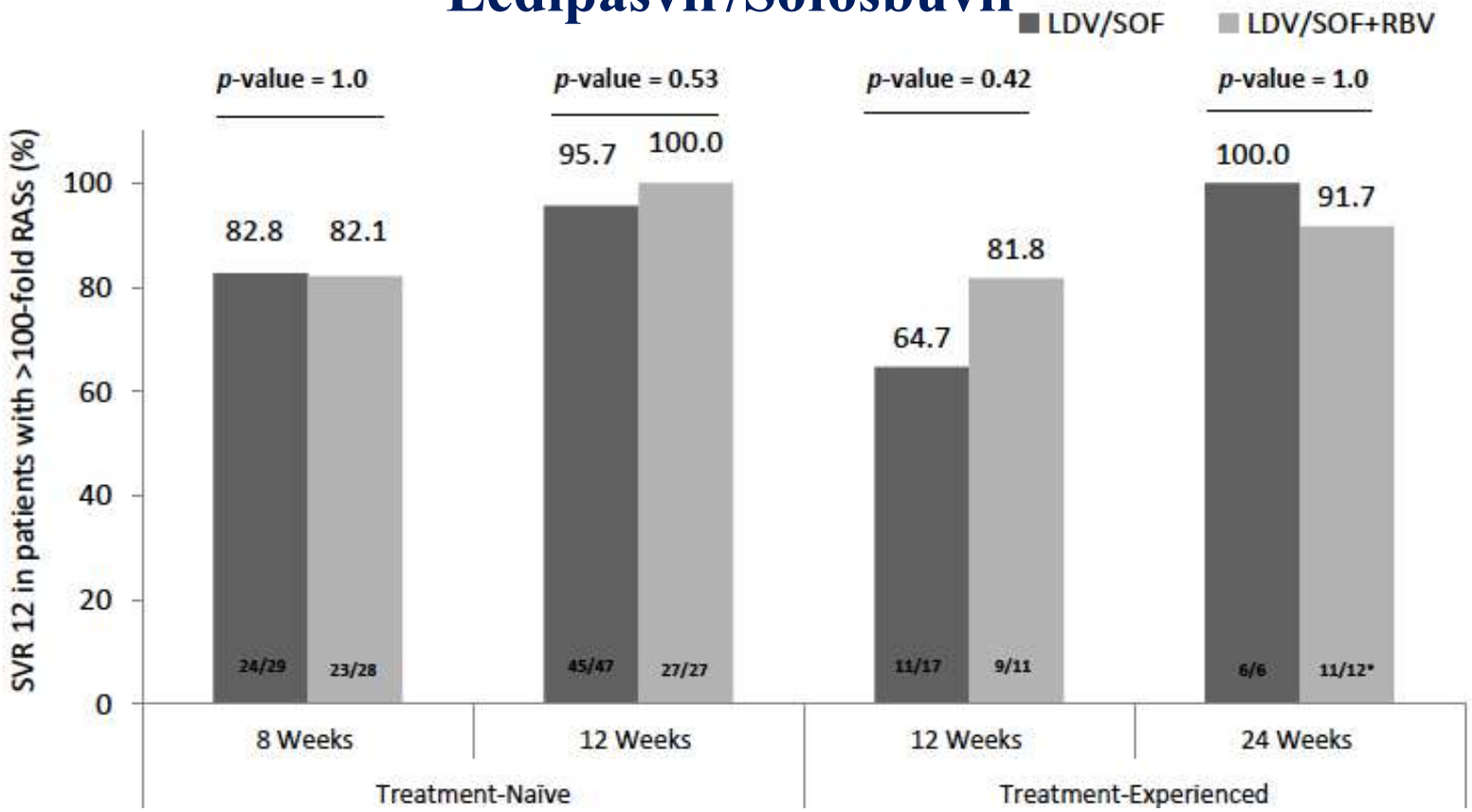
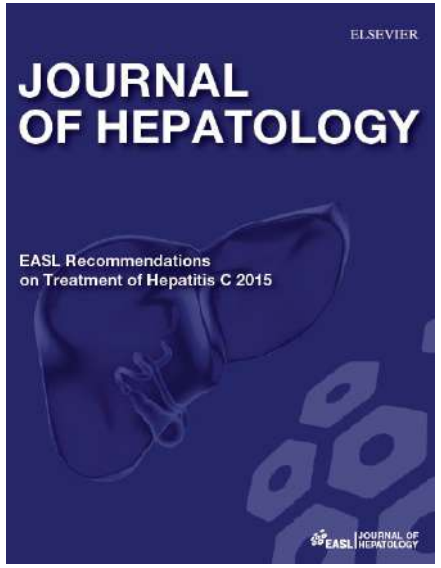


Figure 2. SVR12 by Level of NS5A RASs in those Treated with Ledipasvir/Sofosbuvir.
Patient baseline sequences generated by population and deep sequencing were pooled (using 1 percent cutoff for deep sequencing and population sequencing with a substitution detection of ~15 percent). (c) SVR12 for patients with NS5A RASs with >100-fold-resistance to ledipasvir in treatment-naïve patients treated for 8 or 12 weeks and treatment-experienced patients treated for 12 or 24 weeks with and without ribavirin. * One patient experienced breakthrough due to documented noncompliance during the dosing period. LDV, ledipasvir. SOF, sofosbuvir. RBV, ribavirin

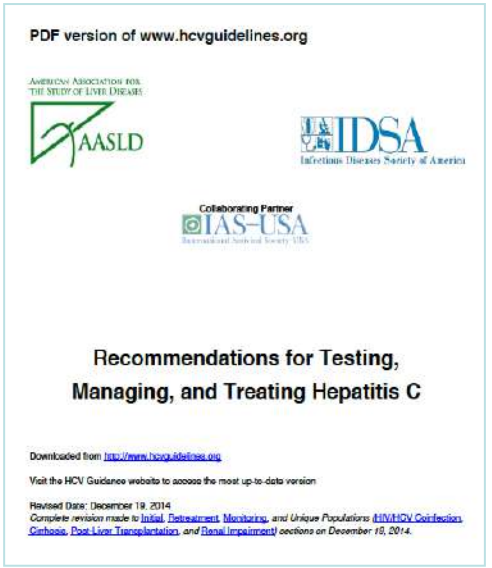
Sarrazin et al Gastroenterology 2016

Virological issues in the DAAs Era

After treatment failure: useful / recommended the resistance test?



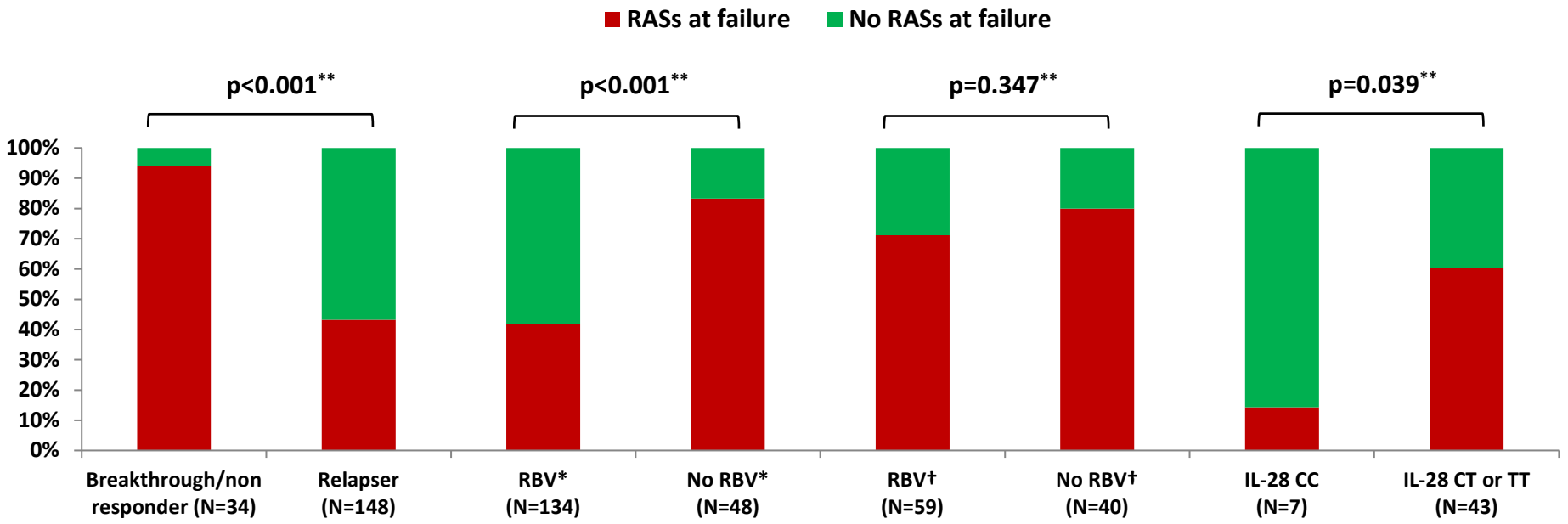
[…] Currently, there is no data to firmly support retreatment recommendations, which must be based on indirect evidence (HCV genotype, known resistance profiles of the administered drugs, number of drugs used, use of ribavirin, treatment duration). Whether assessing the sequence of the target HCV genes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



For patients with cirrhosis or other patients who require retreatment urgently, testing for RAVs that confer decreased susceptibility to NS3 protease inhibitors (eg, Q80K) and to NS5A inhibitors should be performed using commercially available assays prior to selecting the next HCV treatment regimen.

Overall, 96/182 patients (52.7%) showed at least one RAS related to the DAA-regimen at failure

RASs prevalence was significantly higher in breakthrough/non responders 32/34 (94.1%) than in relapsers 64/148 (43.2%), in patients who did not receive ribavirin (RBV) and in patients with unfavorable IL-28 CT/TT genotype compared to patients with favorable IL-28 CC genotype



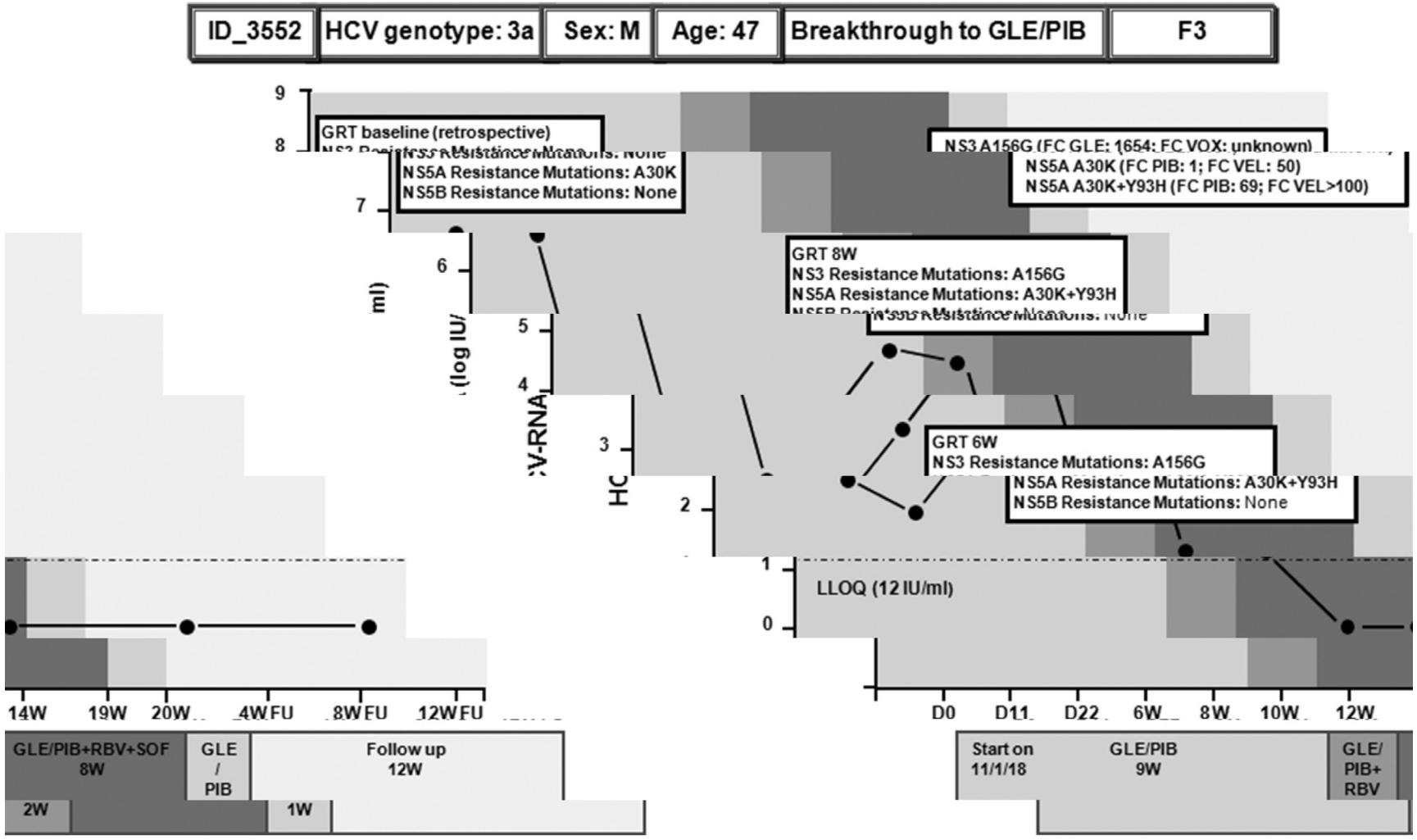
(**) p value was calculated by Chi2 test.

(*) In this group were considered all the patient treated with a RBV containing regimen (including also all suboptimal SOF+RBV containing regimen).

(†) In this group were considered only patients treated with a recommended regimen.

Di Maio VC et al., EASL 2016; update will be presented at AASLD 2016
Published in J Hepatol. 2017

Successful ongoing retreatment with G/P+sofosbuvir+ribavirin guided by resistance test in a patient with HCV genotype 3 who failed G/P with both NS3 and NS5A resistance



ent. D, day; FC, fold change; FU, follow up; GLE/PIB, glecaprevir/pibrentasvir; GRT, genotypic resistance
fosbuvir; VEL, velpatasvir; VOX, voxilaprevir; W, week. *FC in HCV-3 according to Ceccherini-Silberstein F, Cento V,
Curr Opin Virol. 2018; 32:115-27.

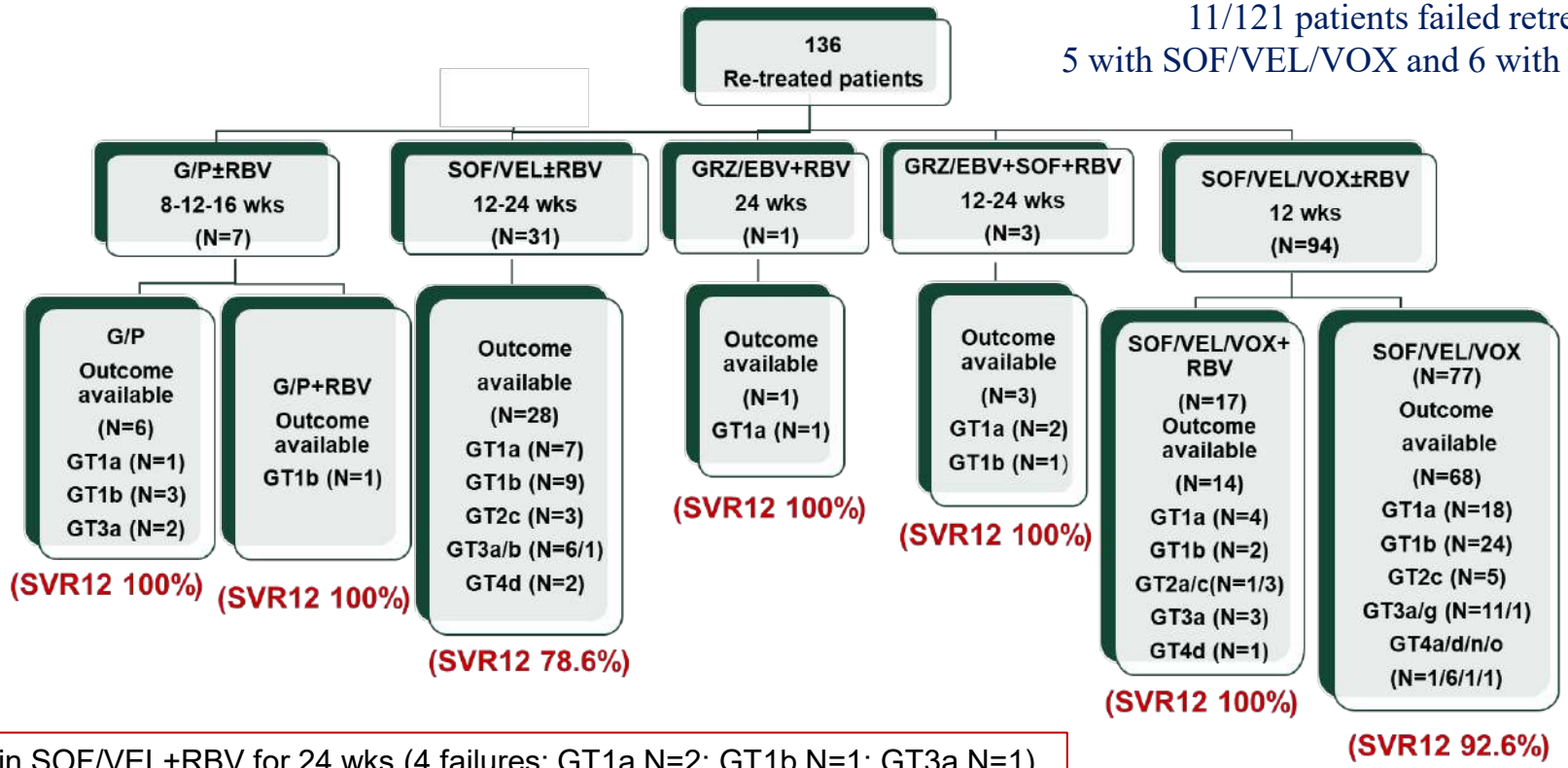
Fig. 1. Clinical, virological and treatment history of the HCV-3a infected pati
testing; LLOQ, lower limit of quantification; RBV, ribavirin; SOF, so
Di Maio VC, Perno CF, Craxì A. Viral resistance in HCV infection.

Aragri M et al., CMI 2020

Overall SVR12 in 110/121 (90.9%) patients completing post retreatment follow-up

- 86% (48/56) SVR in cirrhotic patients
- different SVR according to GT: 85% GT1a, 92% GT1b, 92% GT2, 95% GT3, 91% GT4

11/121 patients failed retreatment:
5 with SOF/VEL/VOX and 6 with SOF/VEL±RBV



SVR12: 69.2% (9/13) in SOF/VEL+RBV for 24 wks (4 failures: GT1a N=2; GT1b N=1; GT3a N=1)
SVR12: 71.4% (5/7) in SOF/VEL for 12 wks (2 failures: GT1b N=1; GT2c N=1)

Ceccherini-Silberstein, et al AASLD 2019

Considerations in DAA Treatment Failure

- **Re-infection as a cause of recurrent viremia?**
- **Was initial therapy sub-optimal?**
  - Drug combination
  - Duration
  - RBV use
- **Indications of other problems on treatment?**
  - Adherence?
  - Significant drug interactions?
- **Are there other baseline host/disease factors that may have contributed?**
  - Cirrhosis, especially decompensation
  - IL28B, age, treatment experience, high viral load, baseline RASs, unusual subtype

HCV genotyping and subtyping still important for the choice and duration of anti-HCV drugs

Recommendations

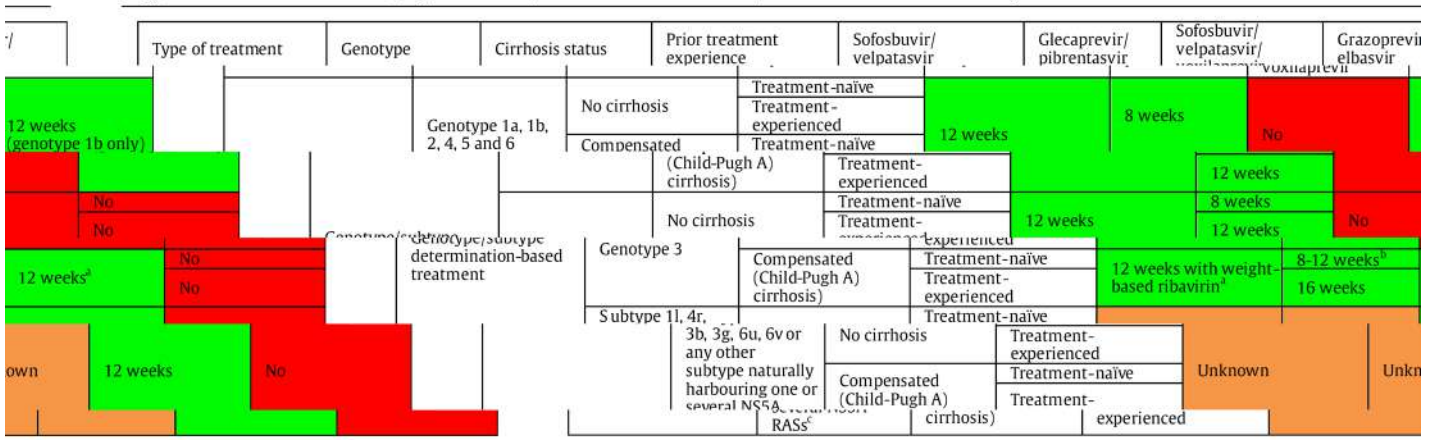
- Treatment with pangenotypic regimens, including sofosbuvir/velpatasvir or glecaprevir/pibrentasvir, can be initiated without knowledge of the genotype and subtype with a high probability of success **(A1)**.
- It is still useful to determine the HCV genotype and subtype where such determination is available and does not limit access to care, to identify patients who may benefit from treatment tailoring **(A1)**.
- Migrants from countries where distinct, less treatment-susceptible HCV subtypes are known to be prevalent may benefit from determination of genotype and subtype by means of population or deep sequencing of the NS5B or another coding region followed by phylogenetic analysis, to identify HCV subtypes inherently resistant to NS5A inhibitors (such as subtypes 1l, 4r, 3b, 3g, 6u, 6v and other undetermined subtypes) in order to avoid treatment failure **(B1)**.
- In geographical areas or settings where HCV subtypes inherently resistant to NS5A inhibitors (such as subtypes 1l, 4r, 3b, 3g, 6u, 6v and other undetermined subtypes) are present, the HCV genotype and subtype should be determined whenever possible by means of population or deep sequencing of the NS5B or another coding region followed by phylogenetic analysis (but population or deep sequencing methods are not available for patients in most low- and middle-income countries where these HCV subtypes are present) **(B2)**.

Table 6A. Recommendations for simplified, genotyping/subtyping-free treatment of HCV-monoinfected or HCV-HIV coinfected adult (≥18 years) and adolescent (12–17 years) patients with chronic hepatitis C without cirrhosis or with compensated (Child-Pugh A) cirrhosis, including treatment-naïve patients (defined as patients who have never been treated for their HCV infection) and treatment-experienced patients (defined as patients who were previously treated with pegylated IFN-α and ribavirin; pegylated IFN-α, ribavirin and sofosbuvir; or sofosbuvir and ribavirin).

Type of treatment	Genotype	Cirrhosis status	Prior treatment experience	Sofosbuvir/ velpatasvir	Glecaprevir/ pibrentasvir	Sofosbuvir/ velpatasvir/ voxilaprevir	Grazoprevir/ elbasvir
Simplified treatment, no genotype/subtype determination[a]	All genotypes	No cirrhosis	Treatment-naïve	12 weeks	8 weeks	No	No
			Treatment-experienced				
		Compensated (Child-Pugh A) cirrhosis)	Treatment-naïve		12 weeks		
			Treatment-experienced				

IFN, interferon.
[a]Whenever HCV genotype and subtype determination is not available, not affordable and/or limits access to care.

Table 6B. Recommendations for genotype/subtype-based treatment of HCV-monoinfected or HCV-HIV coinfected adult (≥18 years) and adolescent (12-17 years) patients with chronic hepatitis C without cirrhosis or with compensated (Child-Pugh A) cirrhosis, including treatment-naïve patients (defined as patients who have never been treated for their HCV infection) and treatment-experienced patients (defined as patients who were previously treated with pegylated IFN-α and ribavirin; pegylated IFN-α, ribavirin and sofosbuvir; or sofosbuvir and ribavirin).



IFN, interferon; RASs, resistance-associated substitutions.

eated with sofosbuvir/velpatasvir plus ribavirin or with sofosbuvir/velpatasvir alone.
eatment with glecaprevir/pibrentasvir can be shortened to 8 weeks, but

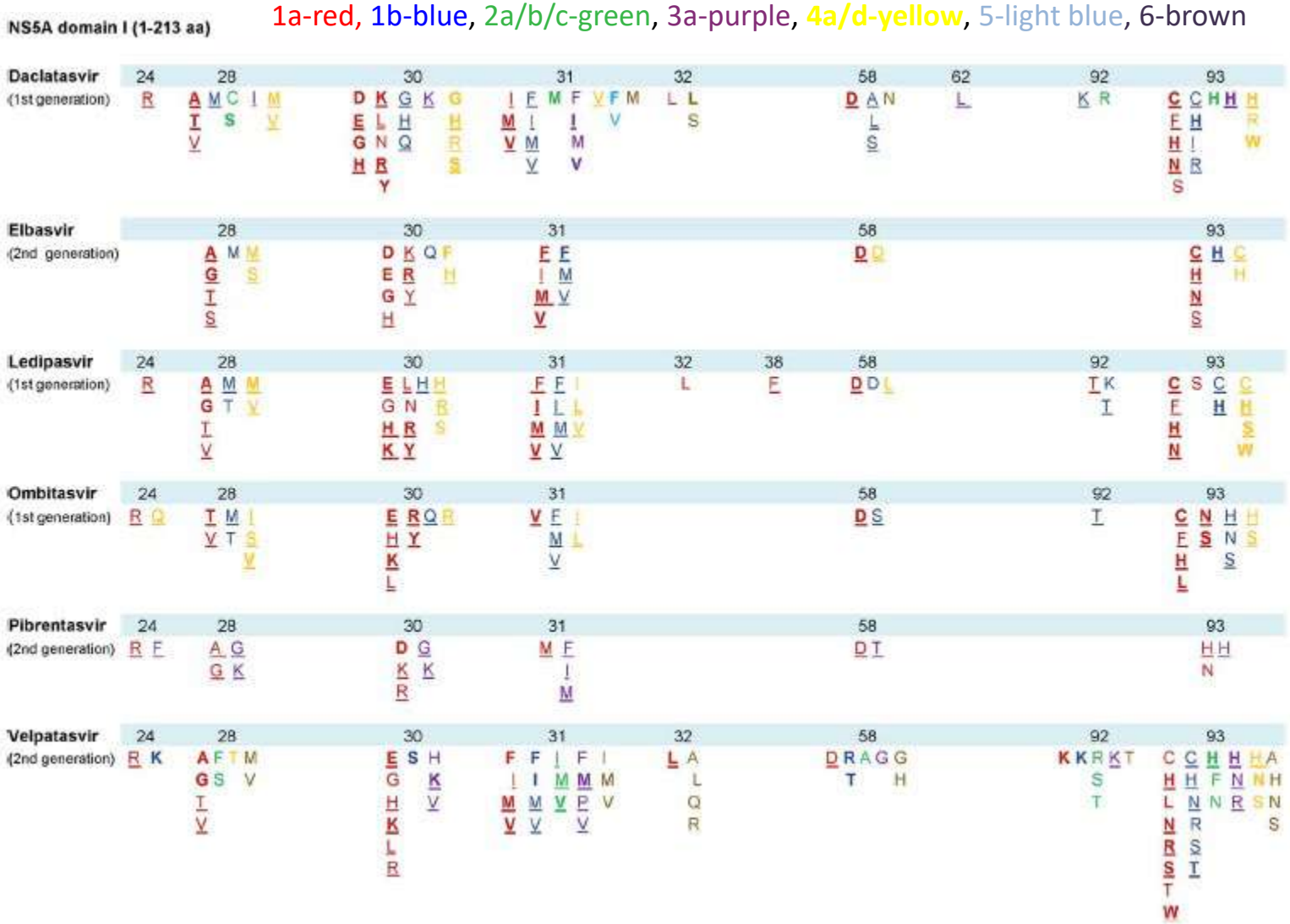
cing or deep sequencing (cutoff 15%).

[a]If resistance testing is performed, only patients with the NS5A Y93H RAS at baseline should be tr
patasvir/voxilaprevir, whereas patients without the Y93H RAS should be treated with sofosbuvir/ve
[b]In treatment-naïve patients infected with genotype 3 with compensated (Child-Pugh A) cirrhosis, tr
[c]As determined by sequence analysis of the NS5A region by means of population sequen

EASL Recommendations on Treatment of Hepatitis C 2020

Not all 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 ε 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

Different impact according to genotype and subtype…..

HEPATOLOGY
HEPATOLOGY, VOL. 00, NO. 00, 2018

Resistance Analysis of Genotype 3 Hepatitis C Virus Indicates Subtypes Inherently Resistant to Nonstructural Protein 5A Inhibitors

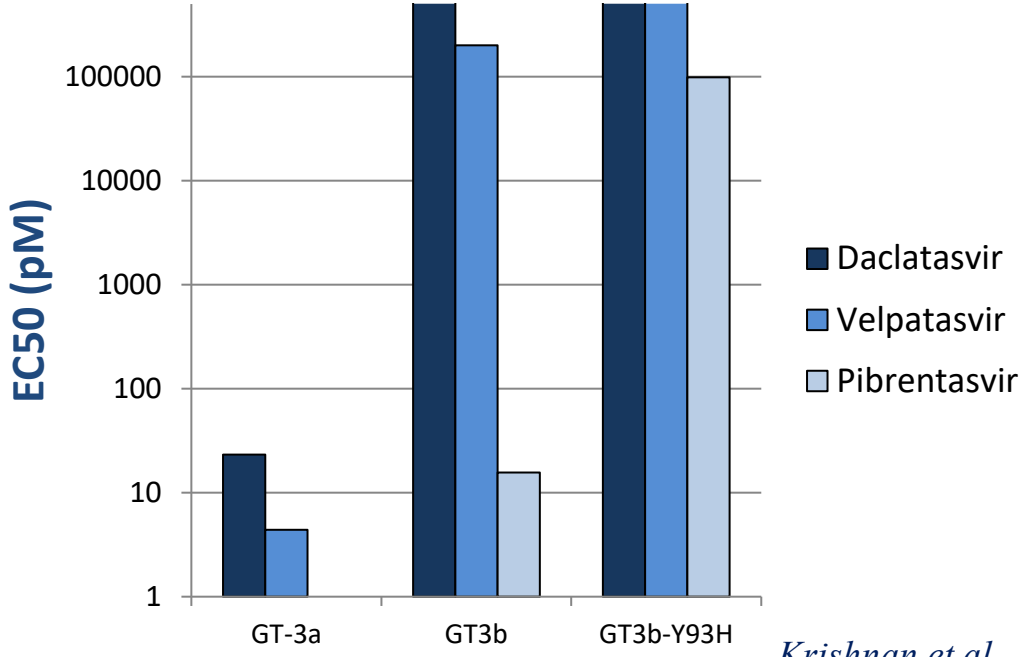
David Smith,[1*] Andrea Magri,[1,2*] David Bonsall,[1,3] Camilla L.C. Ip,[1,3] Amy Trebes,[3] Anthony Brown,[1] Palo Piazza,[3] Rory Bowden,[3] Dung Nguyen,[1] M. Azim Ansari,[1,2*] Peter Simmonds,[1*] and Eleanor Barnes,[1*] STOP-HCV Consortium

Paired RASs (A30K + L31M and A30K + Y93H) were identified in 18 patients (9 of each pair); these combinations were shown to be highly resistant to daclatasvir, velpatasvir, elbasvir, and pibrentasvir. The A30K + L31M combination was found in all gt3b and gt3g samples. *Conclusion:* Our study reveals high frequencies of RASs to nonstructural protein 5A inhibitors in gt3 HCV; the paired A30K + L31M substitutions occur in all patients with gt3b and gt3g virus, and *in vitro* analysis suggests that these subtypes may be inherently resistant to all approved nonstructural protein 5A inhibitors for gt3 HCV. (HEPATOLOGY 2018; 00:000-000).

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	12-145	749-1750	2154-3733	45-169
Elbasvir	220-600	12-67	-	157	7.5
Ledipasvir	1677-3309	1319-1807	-	30[b]	1000
Ombitasvir	41383	77	4710	6728	20-100
Pibrentasvir	7	0.6	-	2.5	-
Velpatasvir	609	3	46	724	3

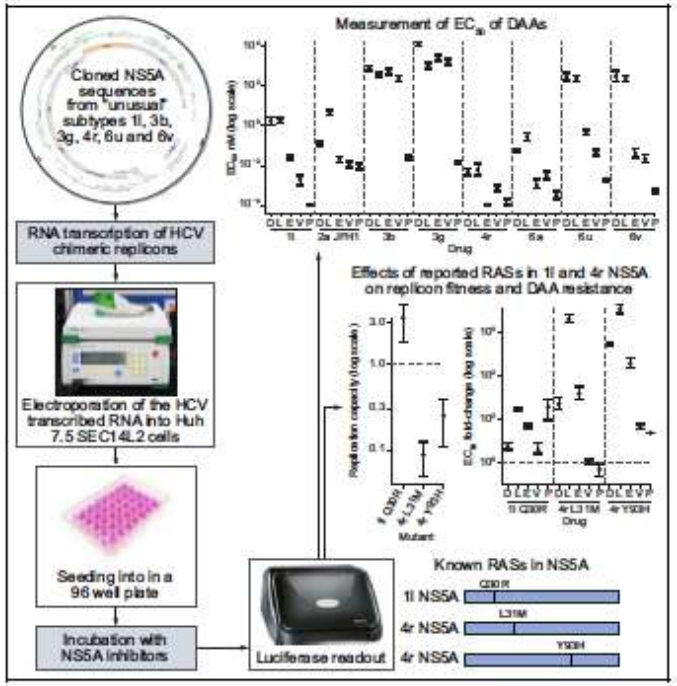
Sorbo MC , et al Drug Resistance Update 2018



Krishnan et al. AAC 2018

Efficacy of NS5A inhibitors against unusual and potentially difficult-to-treat HCV subtypes commonly found in sub-Saharan Africa and South East Asia

Graphical abstract



Authors

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Lay summary

Little is known about the efficacy of NS5A inhibitors against some "unusual" hepatitis C virus (HCV) subtypes including 1l, 3b, 3g, 4r, 6u and 6v. In this study, we manufactured HCV replicons which express the NS5A protein from the unusual HCV subtypes 1l, 3b, 3g, 4r, 6u, 6v. We then tested the effect of the NS5A inhibitors daclatasvir, elbasvir, ledipasvir, pibrentasvir and velpatasvir on blocking replication, using these replicons. We show that these replicons are resistant at some level to all NS5A inhibitors other than pibrentasvir.

Highlights

- The "unusual" HCV subtypes 1l, 3b, 3g, 4r, 6u and 6v show variable levels of resistance to all NS5A inhibitors *in vitro*.
- Pibrentasvir shows the greatest level of activity against all the unusual subtypes.
- HCV subtypes 3b and 3g are resistant to all NS5A inhibitors other than pibrentasvir in *in vitro* testing.
- Daclatasvir and ledipasvir are ineffective against subtypes 6u and 6v in *in vitro* testing.
- The presence of the resistance-associated substitutions Q30R in 1l, and L31M or Y93H in 4r confers resistance to ledipasvir.

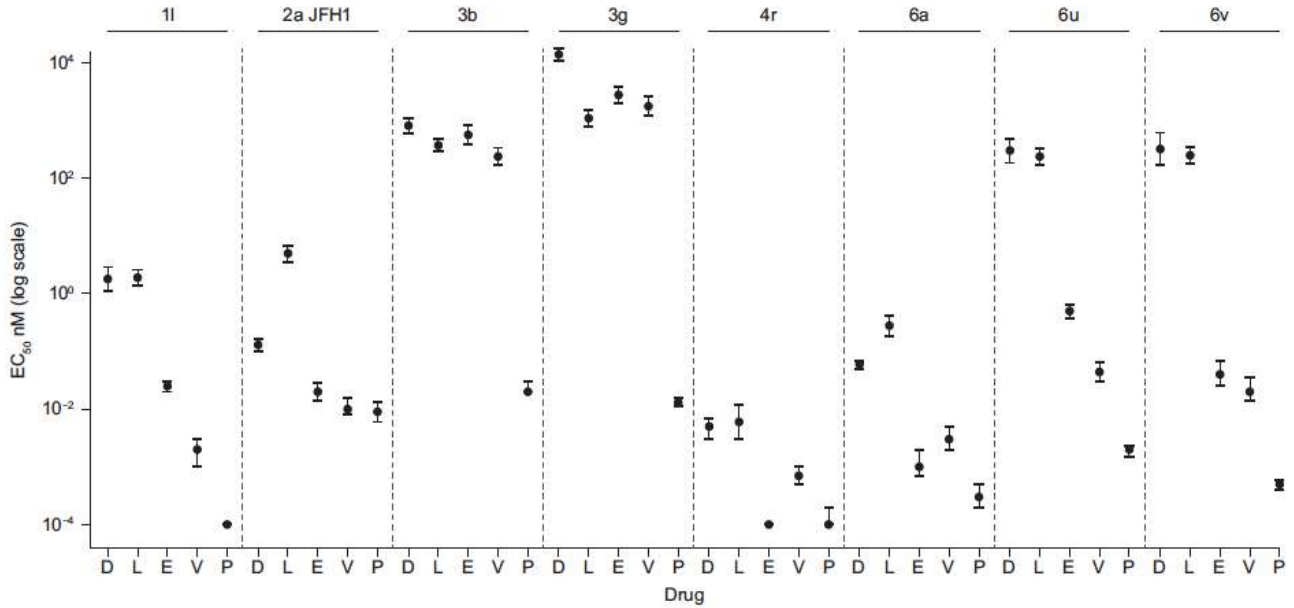

Fig. 1. Efficacy of NS5A inhibitors against the tested subtypes. The chart showed the EC_{50} values with 95% CIs. D, Daclatasvir; L, Ledipasvir; E, Elbasvir; V, Velpatasvir; P, Pibrentasvir.

These findings support the new recommendations from EASL

Genotype	Cirrhosis status	Prior treatment experience	Sofosbuvir/ velpatasvir	Glecaprevir/ pibrentasvir	Sofosbuvir/ velpatasvir/ voxilaprevir	Grazoprevir/ elbasvir
Genotype 3	No cirrhosis	Treatment-naïve	12 weeks	8 weeks	No	No
		Treatment-experienced		12 weeks		No
	Compensated (Child-Pugh A) cirrhosis)	Treatment-naïve	12 weeks with weight-based ribavirin[a]	8-12 weeks[b]	12 weeks[a]	No
		Treatment-experienced		16 weeks		No
Subtype 1l, 4r, 3b, 3g, 6u, 6v or any other subtype naturally harbouring one or several NS5A RASs[c]	No cirrhosis	Treatment-naïve	Unknown	Unknown	12 weeks	No
		Treatment-experienced				
	Compensated (Child-Pugh A) cirrhosis)	Treatment-naïve				
		Treatment-experienced				

Nguyen D et al., J. Hepatol. 2020, 73, 794–799

EASL Recommendations on Treatment of Hepatitis C 2020

Suboptimal SVR rates in African patients with atypical genotype 1 subtypes: Implications for global elimination of hepatitis C

Background & Aims: HCV subtypes which are unusual in Europe are more prevalent in the African region, but little is known of their response to direct-acting antivirals (DAAs). These include non-1a/1b/ non-subtypeable genotype 1 (G1) or non-4a/4d (G4). In this report we aimed to describe the genotype distribution and treatment outcome in a south London cohortof African patients.

Methods: We identified all patients born in Africa who attendedour clinic from 2010-2018. Information on HCV genotype, treatment regimen and outcome were obtained. Non-subtypeable samples were analysed using Glasgow NimbleGen nextgeneration sequencing (NGS). Phylogenetic analysis was carried out by generating an uncorrected nucleotide p-distance tree from the complete coding regions of our sequences.

Results: Of 91 African patients, 47 (52%) were infected with an unusual subtype. Fourteen novel, as yet undesignated subtypes (G1*), were identified by NGS. Three individuals were infected with the same subtype, now designated as subtype 1p. Baseline sequences were available for 22 patients; 18/22 (82%) had baseline NS5A resistance-associated substitutions (RASs). Sustained virological response (SVR) was achieved in 56/63 (89%) overall, yet only in 21/28 (75%) of those with unusual G1 subtypes, with failure in 3/16 G1*, 1/2 G1p and 3/3 in G1l. Six treatment failures occurred with sofosbuvir/ledipasvir compared to 1 failure on a PI-based regimen. The SVR rate for all other genotypes and subtypes was 35/35 (100%).

Conclusions: Most individuals in an unselected cohort of African patients were infected with an unusual genotype, including novel subtype 1p. The SVR rate of those with unusual G1 subtypes was 75%, raising concern about expansion of DAAs across Africa. Depending on the regimen used, higher failure rates in African cohorts could jeopardise HCV elimination.

Childs K et al., J Hepatol 2019

HEPATOLOGY

AASLD
AMERICAN ASSOCIATION FOR THE STUDY OF LIVER DISEASES

HEPATOLOGY, VOL. 69, NO. 2, 2019

Frequent Antiviral Treatment Failures in Patients Infected With Hepatitis C Virus Genotype 4, Subtype 4r

Slim Fourati,[1,2] Christophe Rodriguez,[1,2] Christophe Hézode,[2,3] Alexandre Soulier,[1,2] Isaac Ruiz,[2,3] Lila Poiteau,[1,2] Stéphane Chevaliez,[1,2] and Jean-Michel Pawlotsky[1,2]

Hepatitis C virus (HCV) genotype 4 is highly heterogeneous. HCV subtype 4r has been suggested to be less responsive to direct-acting antiviral (DAA) drug treatment than other genotype 4 subtypes. Among 537 DAA-treated patients who experienced a virological failure (VF) in France between 2015 and 2018, 121 (22.5%) were infected with genotype 4 and 27 of them (22.3%) with subtype 4r; subtype 4r was thus over-represented as compared to its prevalence in the French general population. Population sequencing of the nonstructural protein (NS) 3, NS5A, and NS5B genes was performed in all subtype 4r patients at treatment failure and in 6 at baseline, whereas full-length HCV genome sequencing was performed in two baseline and three treatment failure samples by means of an original shotgun metagenomics method based on deep sequencing. At treatment failure, all subtype 4r patients harbored two to three dominant NS5A resistance-associated substitutions (RASs), including at least L28A/C/I/M/V and L30R. Among 13 patients exposed to sofosbuvir and an NS5A inhibitor (daclatasvir, ledipasvir, or velpatasvir), 5 (38.5%) also harbored NS5B S282C/T RASs at treatment failure. An additional patient harbored S282C/T RASs at treatment failure by deep sequencing. Prevalence of S282C/T RASs at treatment failure was significantly higher in patients infected with genotype 4r than with other genotypes, including other subtypes of genotype 4. *Conclusion:* The lower rates of sustained virological response in patients infected with subtype 4r are related to the frequent preexistence at treatment baseline and subsequent selection by DAA treatment of both NS5A and NS5B S282 RASs. Our study suggests that these patients should be identified and receive a triple DAA combination regimen as first-line treatment. (HEPATOLOGY 2019;69:513-523).

Total of samples (N=3876) analyzed between 2011-2020 stratified according to gene and treatment status (N=3554 patients)

NS3 sequences (N=3684), NS5A sequences (N=2975), NS5B sequences (N=2706)

Year	NS3		NS5A		NS5B	
	DAA Naive	Failure	DAA Naive	Failure	DAA Naive	Failure
2011	34	6	0	0	1	0
2012	100	86	2	0	2	0
2013	128	103	2	1	4	5
2014	122	100	83	19	84	22
2015	680	269	575	251	379	223
2016	373	326	360	332	321	324
2017	363	203	360	206	357	205
2018	217	203	210	208	214	200
2019	155	120	147	122	150	120
2020	68	28	67	30	68	27
Total	**2240**	**1444**	**1806**	**1169**	**1580**	**1126**

Last update: March 2021

Number of failures to a DAA recommended regimen collected in the DB VIRONET C

January 2019

DAA Regimen	DAA Response			Total
	Relapser	Breakthrough	Non responder	
G/P	9	0	1	10
VEL/SOF+/-RBV	27	0	2	29
GRZ/EBV+/-RBV	24	2	2	28
SOF/VEL/VOX	0	0	1	1

March 2021

DAA Regimen	DAA Response			Total
	Relapser	Breakthrough	Non responder	
G/P	57	1	5	63
VEL/SOF+/-RBV	98	1	8	107
GRZ/EBV+/-RBV	97	4	5	106
SOF/VEL/VOX	6	0	4	10

Research Article
Viral Hepatitis

JOURNAL OF HEPATOLOGY

Failure on voxilaprevir, velpatasvir, sofosbuvir and efficacy of rescue therapy

Julia Dietz[1,2,†], Velia Chiara Di Maio[3,†], Adolfo de Salazar[4,†], Dolores Merino[5], Johannes Vermehren[1,2], Stefania Paolucci[6], Andreas E. Kremer[7], Magdalena Lara[8], Maria Rodriguez Pardo[9], Heinz Zoller[10], Elisabetta Degasperi[11], Kai-Henrik Peiffer[1,2], Laura Sighinolfi[12], Francisco Téllez[13], Christiana Graf[1,2], Valeria Ghisetti[14], Jonas Schreiber[15], Elisa Fernández-Fuertes[16], Lucio Boglione[17], Leopoldo Muñoz-Medina[18], Rudolf Stauber[19], William Gennari[20], Blanca Figueruela[21], Jesús Santos[22], Pietro Lampertico[11], Stefan Zeuzem[1,2], Francesca Ceccherini-Silberstein[3,a,†], Federico García[4,b,†], Christoph Sarrazin[1,2,23,*,c,†]

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Background & Aims: There are limited data on patients with chronic HCV infection in whom combination voxilaprevir (VOX), velpatasvir (VEL), sofosbuvir (SOF) retreatment fails. Thus, we aimed to assess treatment failure and rescue treatment options in these patients.

Methods: Samples from 40 patients with HCV genotypes (GT) 1-4 in whom VOX/VEL/SOF retreatment failed were collected within the European Resistance Study Group. Population-based resistance analyses were conducted and clinical parameters and retreatment efficacies were evaluated retrospectively in 22 patients.

Results: Most VOX/VEL/SOF failure patients were infected with HCV GT3a (n = 18, 45%) or GT1a (n = 11, 28%) and had cirrhosis (n = 28, 70%). Previous treatments included an NS3-inhibitor (30%), an NS5A-inhibitor (100%) and SOF (85%). Baseline RAS data from a subgroup of patients before VOX/VEL/SOF retreatment (78%) showed few NS3 RASs apart from Q80K in GT1a (40%), typical NS5A RAS patterns in most patients (74%) and no S282T in NS5B. Sequencing after VOX/VEL/SOF failure was available in 98% of patients and showed only minor changes for NS3 and NS5A RASs. In 22 patients, rescue treatment was initiated with glecaprevir, pibrentasvir alone (n = 2) or with SOF±ribavirin (n = 15), VOX/VEL/SOF±ribavirin (n = 4) or VEL/SOF and ribavirin (n = 1) for 12 to 24 weeks. Sustained virologic response was achieved in 17/21 (81%) patients with a final treatment outcome. Of these, 2 GT3a-infected patients had virologic failure after rescue treatment with VEL/SOF or glecaprevir/pibrentasvir+SOF+ribavirin, and 2 patients with cirrhosis died during treatment or before reaching SVR12.

Conclusions: VOX/VEL/SOF failure was mainly observed in HCV GT3- and GT1a-infected patients with cirrhosis and was not associated with specific RAS patterns within NS3, NS5A or NS5B target regions. Rescue treatment with multiple targeted therapies was effective in most patients.

Lay summary: The advent of direct-acting antivirals has enabled the effective cure of chronic hepatitis C in most patients. However, treatment failure occurs in some patients, who are often retreated with a combination regimen called VOX/VEL/SOF, which is associated with very high rates of cure. However, VOX/VEL/SOF retreatment also fails in some patients. Herein, we analysed samples from patients in whom VOX/VEL/SOF retreatment failed and we assessed the efficacy of different rescue

Keywords: Hepatitis C virus; HCV; Resistance-associated substitutions; Voxilaprevir/velpatasvir/sofosbuvir; Direct-acting antivirals; DAA; Rescue therapy.
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[b] On behalf of the Spanish GEHEP-004 study group.
[c] On behalf of the German HCV study group.
https://doi.org/10.1016/j.jhep.2020.11.017

Table 1. Clinical characteristics of VOX/VEL/SOF failure patients (n = 40).

Male gender	**30 (75)**
Mean age (years)	59 (31-76)
Sampling country	
Germany	13 (33)
Italy	10 (25)
Spain	12 (30)
Austria	3 (8)
Switzerland	1 (2)
Belgium	1 (2)
HCV genotype:	
Genotype 1a	11 (28)
Genotype 1b	9 (23)
Genotype 3a	18 (45)
Genotype 3b	1 (2)
Genotype 4d	1 (2)
HIV coinfection	6 (15)
Past HBV infection*	6 (15)
Fibrosis stage (in patients w/o cirrhosis)	
F0/F1	2/7** (29)
F2	3/7** (42)
F3	2/7** (29)
Cirrhosis	28 (70)
Child grade A	20/23** (87)
Child grade B	3/23** (13)
Child grade C	-
Pre-treatment history	
P/R ± BOC/TVR	15 (38)
DAA treatment history	
One DAA treatment	31 (78)
Two DAA treatments	5 (12)
Three DAA treatments	4 (10)
Pre-treatment regimens	
Any SOF-based regimen	34 (85)
Any PI-based regimen	12 (30)
NS5Ai-based regimen	40 (100)
HCC	11 (28)
Liver Transplantation	6 (15)
SOF/VEL/VOX retreatment	
RBV added	-
12 weeks duration	39 (98)
Discontinuation week 4	1 (3)
Virologic Treatment Failure	39 (98)

Most VOX/VEL/SOF failure patients were infected with HCV GT3a (n = 18, 45%) or GT1a (n = 11, 28%) and had cirrhosis (n = 28, 70%).
Previous treatments included an NS3-inhibitor (30%), an NS5A-inhibitor (100%) and SOF (85%).

Sequencing before and after VOX/VEL/SOF failure showed only minor changes for NS3 and NS5A RASs.

VOX/VEL/SOF failure was mainly observed in HCV GT3- and GT1a-infected patients with cirrhosis and was not associated with specific RAS patterns within NS3, NS5A or NS5B target regions.

Rescue treatment with multiple targeted therapies was effective in most patients.

Dietz J et al., J of Hepatology 2021

Table 3. RASs after VOX/VEL/SOF failure and retreatment outcome (n = 22).

		RASs at VOX/VEL/SOF failure				Rescue treatment			
PAT.	HCV GT	NS3	NS5A	NS5B	Comorbidities	Initiation (month/year)	Regimen	Duration (weeks)	Response
5	1a	Q80K	L31M	No RASs	Cirrhosis Child A, HIV coinfection	11/18	G/P+SOF	12	SVR12
9	1a	No RASs	M28V	No RASs	Cirrhosis Child B, LTX, HCC	03/19	G/P+SOF	16	Death
34	3a	Q168R	Y93H	No RASs	Cirrhosis Child A, HCC	12/19	G/P+SOF	12	SVR12
20	1b	Y56H, D168V	L31V, Y93H	L159F, C316N	3 previous DAA treatments	09/19	G/P+SOF+RBV	24	SVR12
24	3a	No RASs	Y93H	No RASs	Cirrhosis, LTX, HCC	02/19	G/P+SOF+RBV	12	SVR12
28	3a	Q168K	Y93H	No RASs	Cirrhosis Child A, portal hypertension, diabetes	01/19	G/P+SOF+RBV	24	SVR12
33	3a	No RASs	Y93H	No RASs	Cirrhosis Child A	11/19	G/P+SOF	12	SVR12
27	3a	No RASs	Y93H	No RASs	Cirrhosis Child A, LTX	12/19	G/P+SOF	12	SVR4, death
22	3a	No RASs	Y93H	No RASs	Cirrhosis, HCC[#]	01/19	G/P+SOF+RBV	16	Relapse[*]
35	3a	Q168K	Y93H	A150V, V321A	Fibrosis F3	09/19	G/P+SOF+RBV	16	SVR12
2	1a	No RASs	Q30H, L31V, Y93H	No RASs	Cirrhosis, Child A	01/20	G/P+SOF	24	SVR12
4	1a	No RASs	Q30R, L31M	No RASs	Cirrhosis, Child A, LTX, HCC	12/19	G/P+SOF+RBV	16	SVR12
38	3a	No RASs	A30K, Y93H	No RASs	Cirrhosis	05/20	G/P+SOF+RBV	16	FU pending
40	4d	D168V, A156S	M31V, Y93H	No RASs	Obesity	03/20	G/P+SOF	24	SVR12
1	1a	Q80K	No RASs	No RASs	-	04/20	G/P+SOF+RBV	12	SVR12
8	1a	Q80K	No RASs	No RASs	HIV coinfection	02/20	G/P+RBV	12	SVR12
15	1b	No RASs	Y93H	No RASs	Cirrhosis, Child A, Diabetes	12/18	G/P	12	SVR12
13	1b	No RASs	L31I	No RASs	LTX, chronic kidney disease	06/19	VOX/VEL/SOF	24	SVR12
23	3a	Q168R	A30K, Y93H	No RASs	-	01/19	VOX/VEL/SOF+RBV	24	SVR12
26	3a	No RASs	No RASs	No RASs	Cirrhosis Child A, HCC, Obesity, HIV coinfection	09/19	VOX/VEL/SOF	24	SVR12
29	3a	No RASs	No RASs	No RASs	Cirrhosis Child A, HIV coinfection	03/20	VOX/VEL/SOF	24	SVR12
32	3a	No RASs	Y93H	No RASs	Cirrhosis, Child B	03/19	VEL/SOF+RBV	24	Relapse[**]

Detailed information on the patients can be found in Table S4.
FU, follow-up; G/P, glecaprevir/pibrentasvir; GT, genotype; HCC, hepatocellular carcinoma; LTX, liver transplantation; RASs, resistance-associated substitutions; RNA neg. EOT, HCV RNA negativity at end-of-treatment; SOF, sofosbuvir; SVR, sustained virologic response; VEL, velpatasvir; VOX, voxilaprevir.
[#]HCC was diagnosed after start of rescue treatment.[c]
[*]RASs detected after G/P+SOF failure: NS3: no RASs; NS5A: A30K, L31F, Y93H; NS5B: no RASs.
[**]RASs detected after VEL/SOF+RBV relapse: NS3: no RASs; NS5A: Y93H; NS5B: no RASs.
[c]To test whether the SVR rates were significantly different in patients with *versus* without HCC, the Wilcoxon Mann-Whitney U test was used.

In 22 patients, rescue treatment was initiated with glecaprevir, pibrentasvir alone (n = 2) or with SOF±ribavirin (n = 15), VOX/VEL/SOF±ribavirin (n = 4) or VEL/SOF and ribavirin (n = 1) for 12 to 24 weeks. **Sustained virologic response was achieved in 17/21 (81%) patients with a final treatment outcome**.

2 GT3a-infected patients had virologic failure after rescue treatment with VEL/SOF or glecaprevir/ pibrentasvir+SOF+ribavirin, and 2 patients with cirrhosis died during treatment or before reaching SVR12.

Dietz J et al., J of Hepatology 2021

Long-term persistence of HCV resistance-associated substitutions after DAA treatment failure

Authors

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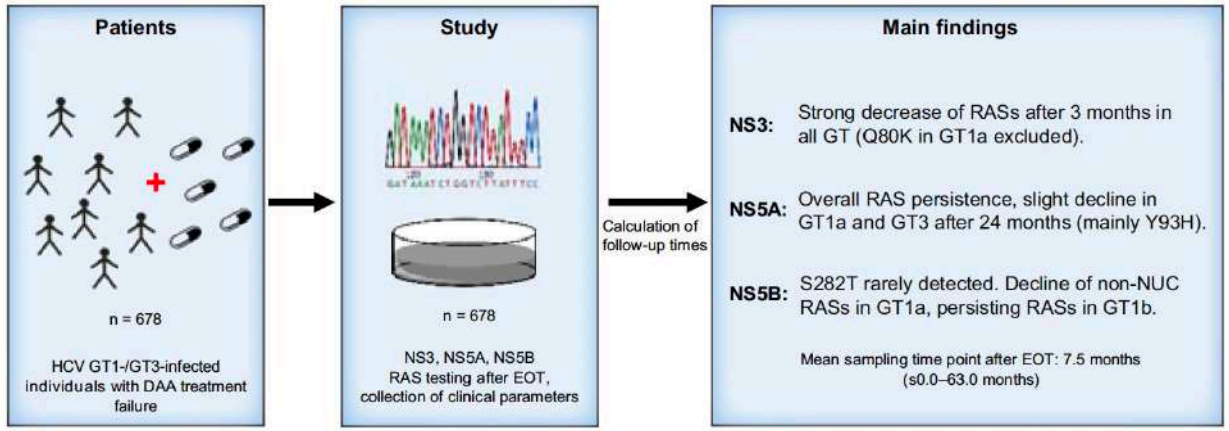
Graphical abstract



Highlights

- Real-world study of the long-term persistence of NS3, NS5A, and NS5B RASs in 678 individuals with GT1 or GT3 infection after DAA failure.
- Sequencing showed a rapid decrease of NS3 RASs after FU month 3 and almost no SOF-resistant RASs.
- NS5A RASs persisted for more than 2 years, with a tendency to decrease in GT1a and GT3 owing to the loss of Y93H.
- Patterns of RAS persistence could have implications for retreatment with first-generation DAAs and for global HCV elimination goals.

Impact and implications

There are little data on the long-term persistence of HCV resistance-associated substitutions (RASs) after DAA treatment failure, and RASs could have an impact on the efficacy of a rescue treatment. Especially in countries with limited availability of VOX/VEL/SOF or G/P/SOF, different patterns of RAS persistence could have implications for retreatment with first-generation DAAs and for global HCV elimination goals. The different patterns of RAS persistence identified in this study can be used to derive general rules regarding the persistence of RASs after DAA failure that could be applied by physicians in less developed countries to plan individualized HCV retreatment.

They found that low-to medium-level RASs persisted, whereas high-level resistant RASs disappeared over time.
Different patterns of RAS persistence according to HCV subtype could have implications for retreatment with first-generation DAAs and for global HCV elimination goals.

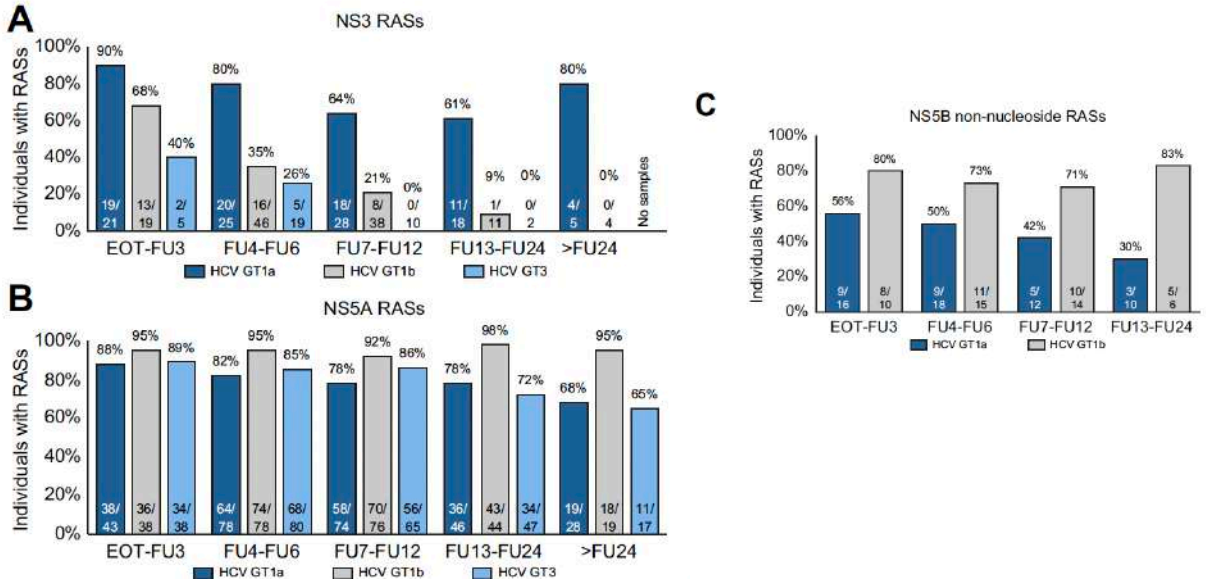
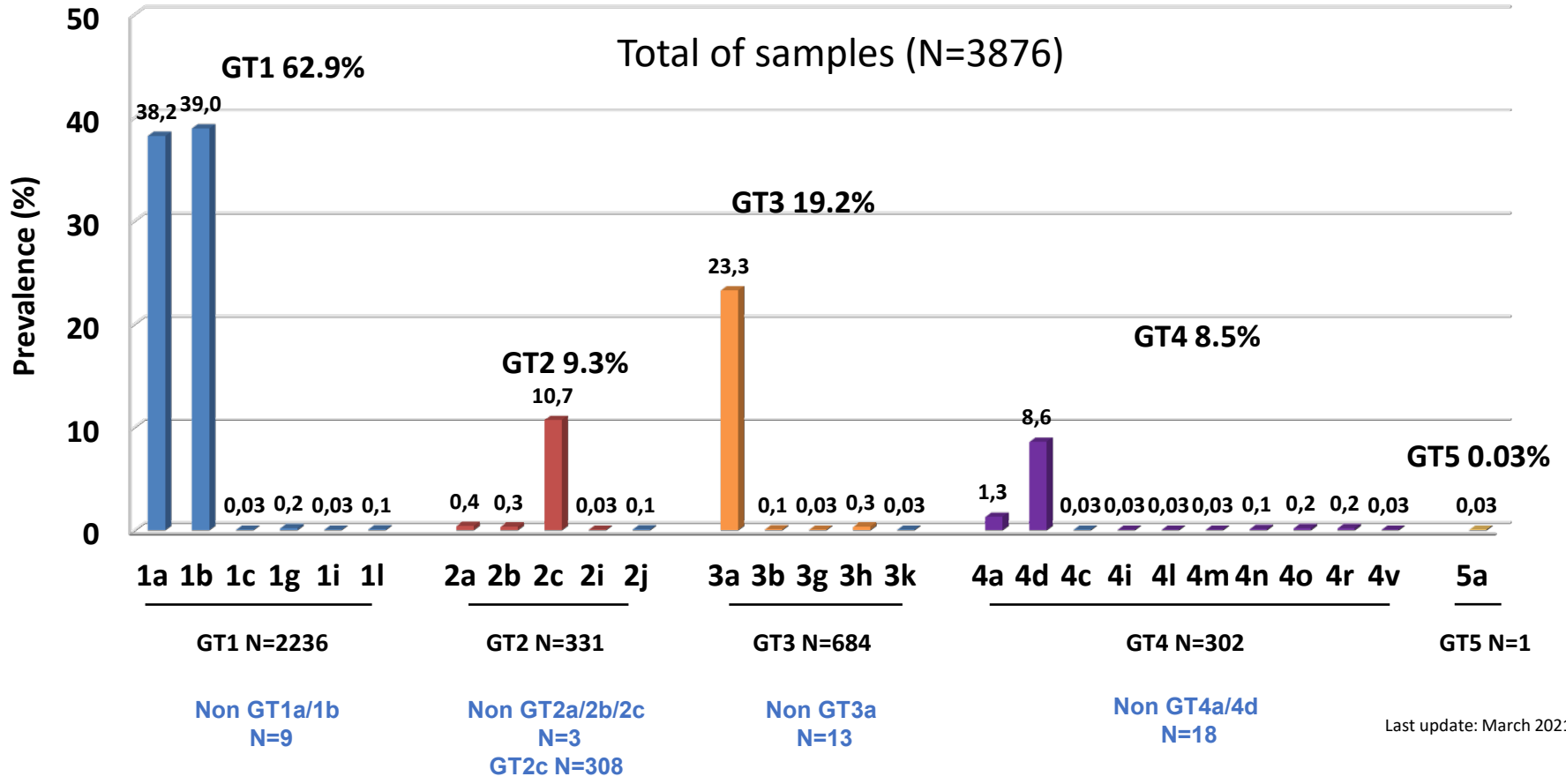


Fig. 2. Overall rate of RASs in individuals with HCV GT1 and GT3. Individuals with RASs in (A) NS3, (B) NS5A, and (C) NS5B. The numbers given correspond to the available NS3, NS5A or NS5B sequences. Treatment regimens: NS3 RASs: EBR/GZR (GT1), PrOD(GT1), SMV/SOF (GT1), G/P (GT3). NS5A: LDV/SOF (GT1), DCV/SOF (GT1, GT3), EBR/GZR (GT1), PrOD(GT1), VEL/SOF (GT1, GT3), G/P (GT3). NS5B: PrOD. DCV, daclatasvir; EBR, elbasvir; EOT, end of treatment; FU, follow-up; G/P, glecaprevir/pibrentasvir; GT, genotype; GZR, grazoprevir; LDV, ledipasvir; PrOD, paritaprevir/ritonavir/ombitasvir with dasabuvir; RAS, resistance-associated substitution; SMV, simeprevir; SOF, sofosbuvir; VEL, velpatasvir.

Dietz J, et al J Hepatol. 2023

HCV genotypes/subtypes distribution within the Italian Resistance Database VIRONET C

9.9% patients (N=351) were infected with “unusual” HCV subtypes (including the GT2c)



Last update: March 2021

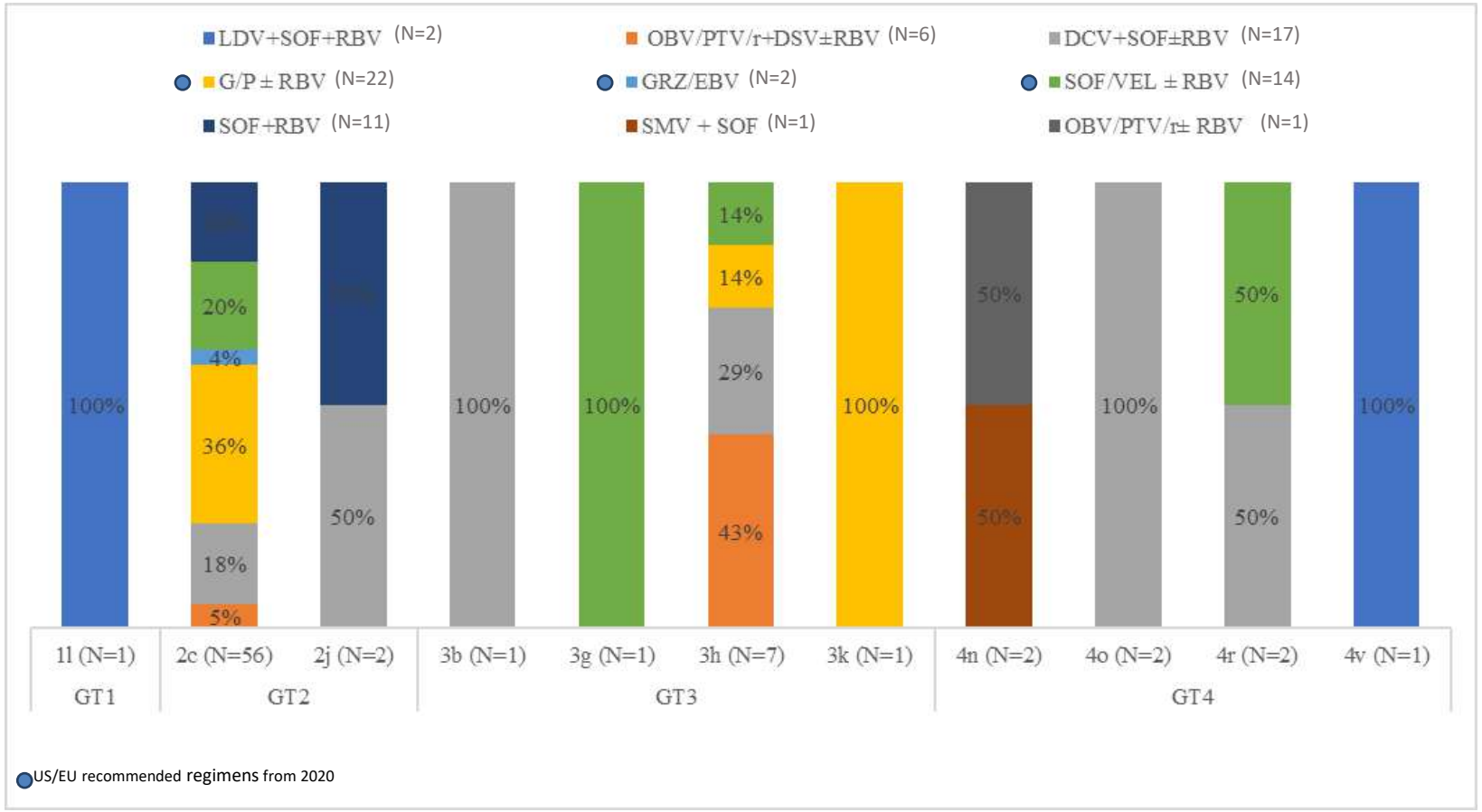
Distribution of the 342 “unusual” HCV subtypes, collected within the Italian Resistance DB VIRONET-C, according to patients country of origin

Continent	Country	GT1 (N=9)				GT2 (N=301)			GT3 (N=13)				GT4 (N=18)								GT5 (n=1)
		c	g	i	l	c	i	j	b	g	h	k	c	i	l	m	n	o	r	v	a
		N=1	N=5	N=1	N=2	N=298	N=1	N=2	N=2	N=1	N=9	N=1	N=1	N=1	N=1	N=1	N=3	N=5	N=5	N=1	N=1
Africa (N=22)	Burundi																			1 (4.5)	
	Cameroon						1 (4.5)														
	Egypt		(18.2)												1 (4.5)	1 (4.5)	2 (9.1)	5 (22.7)	1 (4.5)		
	Eritrea																		2 (9.1)		
	Morocco			1 (4.5)																	
	Nigeria		1 (4.5)		1 (4.5)																
	Congo												1 (4.5)								
America (N=2)	USA					1 (50.0)															
	Argentina					1 (50.0)															
Asia (N=4)	Afghanistan								1 (25.0)												
	Bangladesh									1 (25.0)											
	India					1 (25.0)															
	Pakistan											1 (25.0)									
Europe (N=276)	Italy	1 (0.4)				198 (95.3)					9 (3.3)								2 (0.7)		1 (0.4)
Unknown (N=38)					1 (2.6)	53 (84.2)		2 (5.2)	1 (2.6					2.6			2.6				

Di Maio et al HIVDRU October 2020

Overall, 76 patients infected with “unusual” HCV subtype failed a DAA regimen

38 patients failed a DAA regimen currently recommended by EASL guidelines 2020



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RASs in DAA failures infected with “unusual” HCV subtype

Overall, all DAA failed patients displayed at least one RAS in at least one protein (NS5A, NS3, and NS5B), except for a solo patient infected with GT4n and treated with Ombitasvir/Paritaprevir plus ribavirin. The majority of the patients (88.2%) presented at least one RAS in NS5A, followed (82.0%) by presenting at least one RAS in NS5B, and finally 22.2% with at least one RAS in NS3.

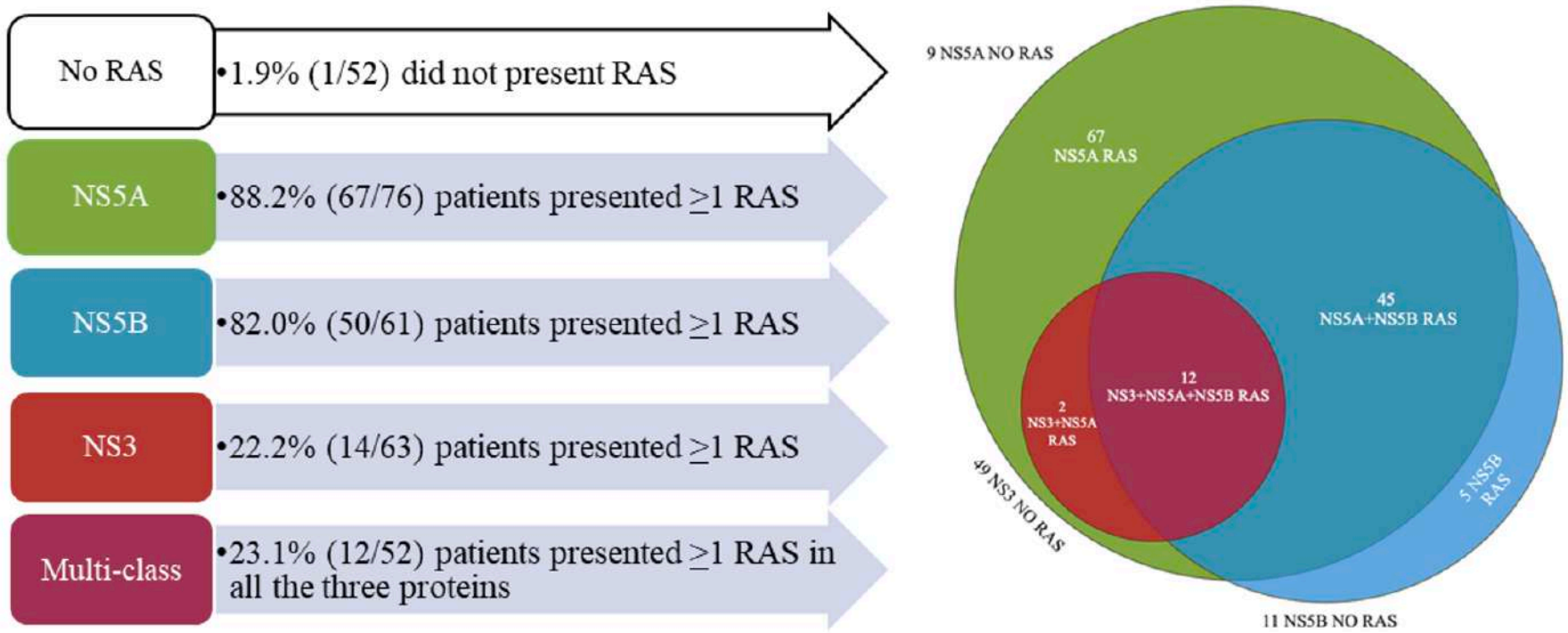

Figure 3. The arrow (left) and Venn (right) diagrams show the prevalence and frequencies of at least 1 RAS in NS5A, NS3, and
NS5B among the 76 patients infected with unusual HCV subtypes who failed several DAA regimens.

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Conclusions

SVR rates are very high with IFN-free regimens (in both mono and co-infected HIV populations).

However, failures can still occur. Previous failures were particularly in cirrhotic patients, individuals infected with rare/difficult to cure subtypes, and when treatment was suboptimal. Today with the new DAAs, highly potent with high genetic barrier with multi-genotypic activity, few failures still may occur in cirrhotic patients but not only.

HCV genotyping and HCV resistance testing at individual level can support **personalized-treatment** by increasing probabilities of response in the context of an experienced multidisciplinary team (complexity: virus, host, clinical aspects, comorbidity, historical therapy, DAAs available: adjustment for duration, RBV-use? choise of regimen) leading to near 100% SVR in all patients.

Aggregation of HCV resistance tests from many HCV individuals generate information at community level: **Surveillance resistance,** molecular analysis for public health intervention.

The big challenge today is to find all individuals with HCV infection, provide appropriate treatment to all and avoid the spread of resistance transmission

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