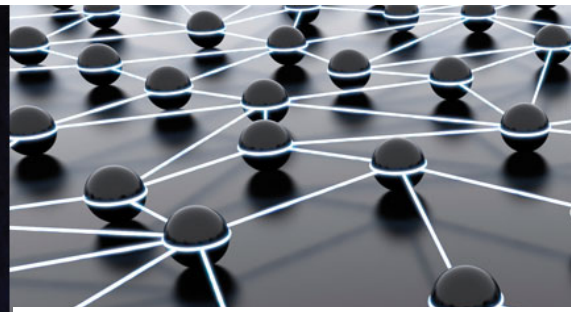
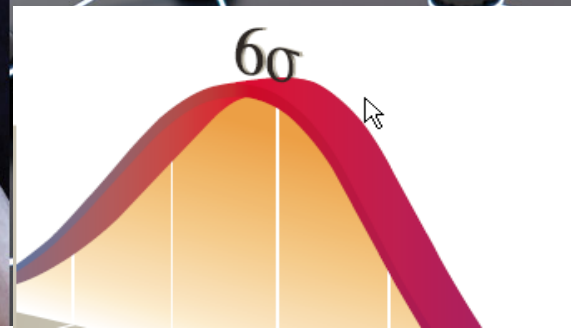
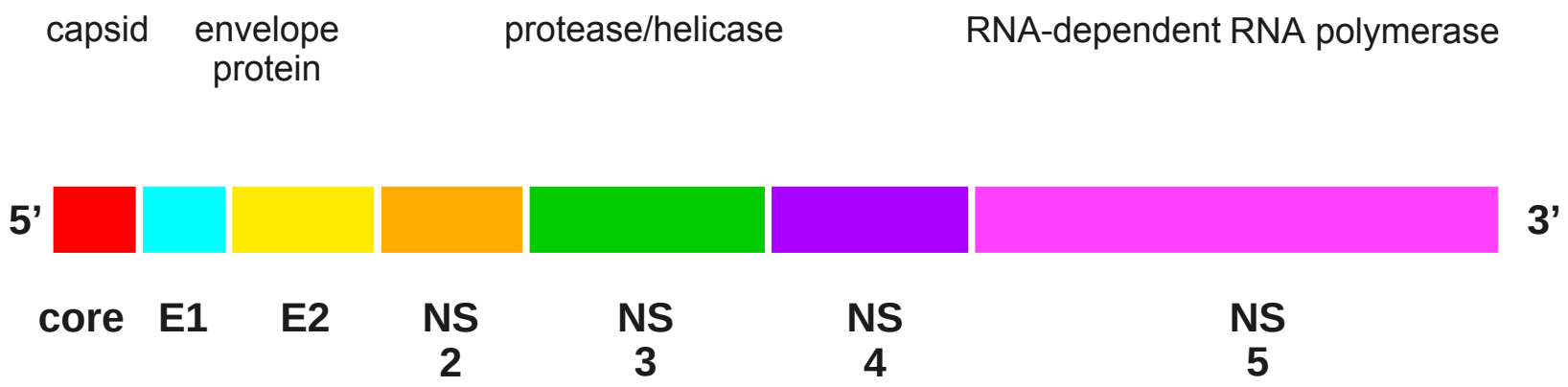
HCV Patient Management: Translating Molecular Diagnostics into Clinical Care





Hepatitis C

- Family Flaviviridae
- Enveloped, RNA virus
  - Highly mutable genome
  - Rapid mutation in a hypervariable region of the genome coding for the envelope proteins and escapes immune surveillance by the host
- Occurs *in vivo* as a group of “quasispecies”



Hepatitis C

- Hepatitis C virus is the most common chronic blood-borne viral infection in North America.

- HCV is a major cause of chronic hepatitis.
  - Causes progressive hepatic fibrosis which leads to cirrhosis and an increased risk of hepatocellular carcinoma

- Chronic Hepatitis C is the leading indication for liver transplantation

The Clinical Burden of Viral Hepatitis

Virus	Geographical spread	Transmission	Symptoms	Long-term prognosis	Chronic infections	Treatment
A[1,6,7]	Endemic in Africa, Asia, Central and South America	Faecal–oral	Fever, malaise, diarrhoea, nausea, Jaundice, cholestasis	Recovery within 6 months; no chronic form	N/A	Treatment is usually not required
B[2,6,7]	Africa, Asia, Central and South America	Percutaneous or permucosal	Dark urine, extreme fatigue, nausea, vomiting and abdominal pain, cholestasis	Cirrhosis, liver failure, hepatocellular carcinoma (HCC)	350 million	Vaccine gives >95% protection *Acute:* No specific treatment *Chronic:* Interferon (IFN), nucleos(t)ide analogues
C[3]	Africa, central and south-eastern Asia, South America	Percutaneous or permucosal	Asymptomatic (50-90%)	Cirrhosis, liver failure, HCC	130–170 million	Pegylated-IFN + ribavirin (RBV); direct-acting antivirals (DDAs)
D[4,6,7]	South America, South Pacific, western Africa, Mediterranean, Middle East and central Asia	Percutaneous or permucosal	Jaundice, anorexia,,nausea and vomiting and fever, cirrhosis	Cirrhosis, liver failure, HCC; HBV co- or super-infection; asymptomatic	10 million	HBV vaccine protects against HDV Otherwise: treatment with IFN
E[5]	Endemic in Asia, Middle East, Africa and Central America	Faecal–oral	Jaundice, anorexia,,nausea and vomiting and fever	No chronic form	N/A	Treatment is rarely required

1. WHO. Hepatitis A. Fact sheet 328. 2008; 2. WHO. Hepatitis B. Fact sheet 204. 2008; 3. WHO. Hepatitis D. WHO/CDS/CSR/NCS/2001;
4. WHO. Hepatitis C. Fact sheet 164. 2011; 5. WHO. Hepatitis E. Fact sheet 280. 2005. 6. CDC. Viral hepatitis (http://www.cdc.gov/hepatitis)
7. Zieve D. (http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001263/)

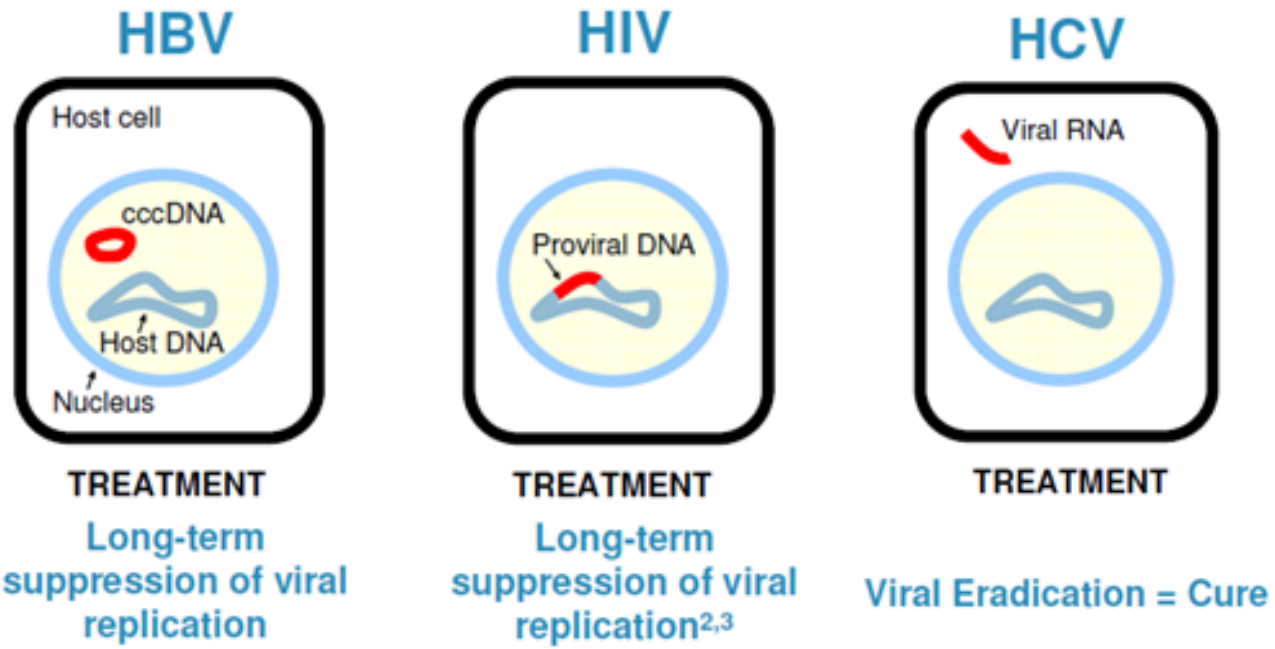
Hepatitis C – Impact and Challenges

- **Major public health problem**
  - A leading cause of chronic liver disease[1]
  - Global epidemic with regional variance
- **Uncertainty around transmission**
  - Intravenous or nasal drug use, mother-to-child transmission, medical or surgical procedures, sexual transmission
  - 30% of infections are unexplained[2]
- **Often a 'silent' disease**
  - Asymptomatic in 50−90% of cases[3]
  - Over 30% have normal enzymes
  - Screening based on exposure to transmission risk factors[4]
- **Progressive disease**
  - High percentage of patients will develop chronic infection (55–85%)[5,6]

1. Williams R. Global challenges in liver disease. Hepatology 2006;44:521−26;
2. Pol S, et al. Contrib Nephrol. 2012;176:1−92;
3. EASL. J Hepatol. 2011;55(2):245−64;
4. Alter MJ, et al. Ann Intern Med 2004;141:715−17;
5. McHutchison JG. Am J Manag Care 2004;10(2 Suppl):S21−9;
6. Seeff LB. Hepatology 2002;36(5 Suppl 1):S35−46.

HCV Is Curable!

- **The Goals of HCV treatment are different to those of HBV and HIV!**



1. Pawlotsky JM. *J Hepatol* 2006;44:S10-S13; 2. Siliciano JD, Siliciano RF. *J Antimicrob Chemother* 2004;54:6-9;
3. Lucas GM. *J Antimicrob Chemother* 2005;55:413-416

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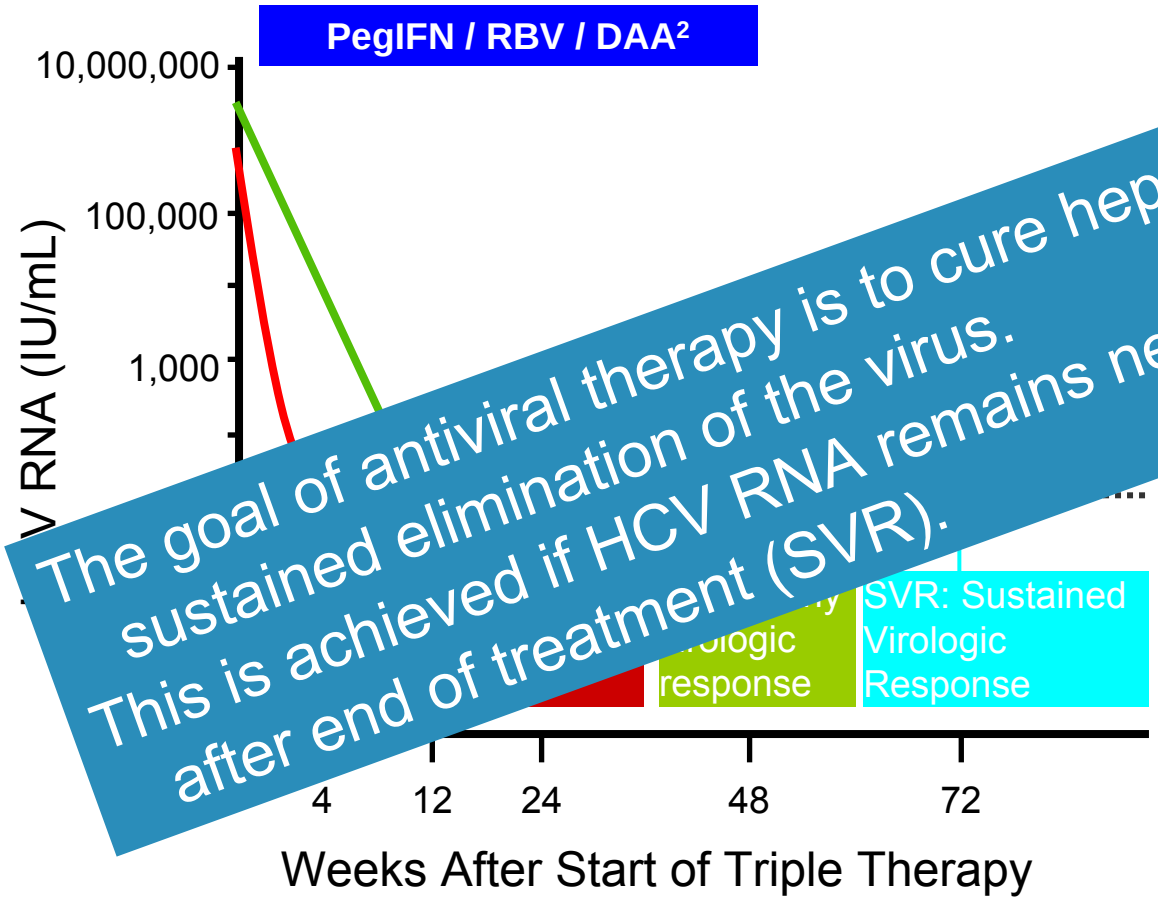
Patterns of Virologic Response Related to Triple Therapy[1]



The goal of antiviral therapy is to cure hepatitis C via a sustained elimination of the virus. This is achieved if HCV RNA remains negative six months after end of treatment (SVR).

Rapid virologic respons R)
HCV RNA negati

r TVR
BOC

se (EVR)
$\log_{10}$ decline at week 12.

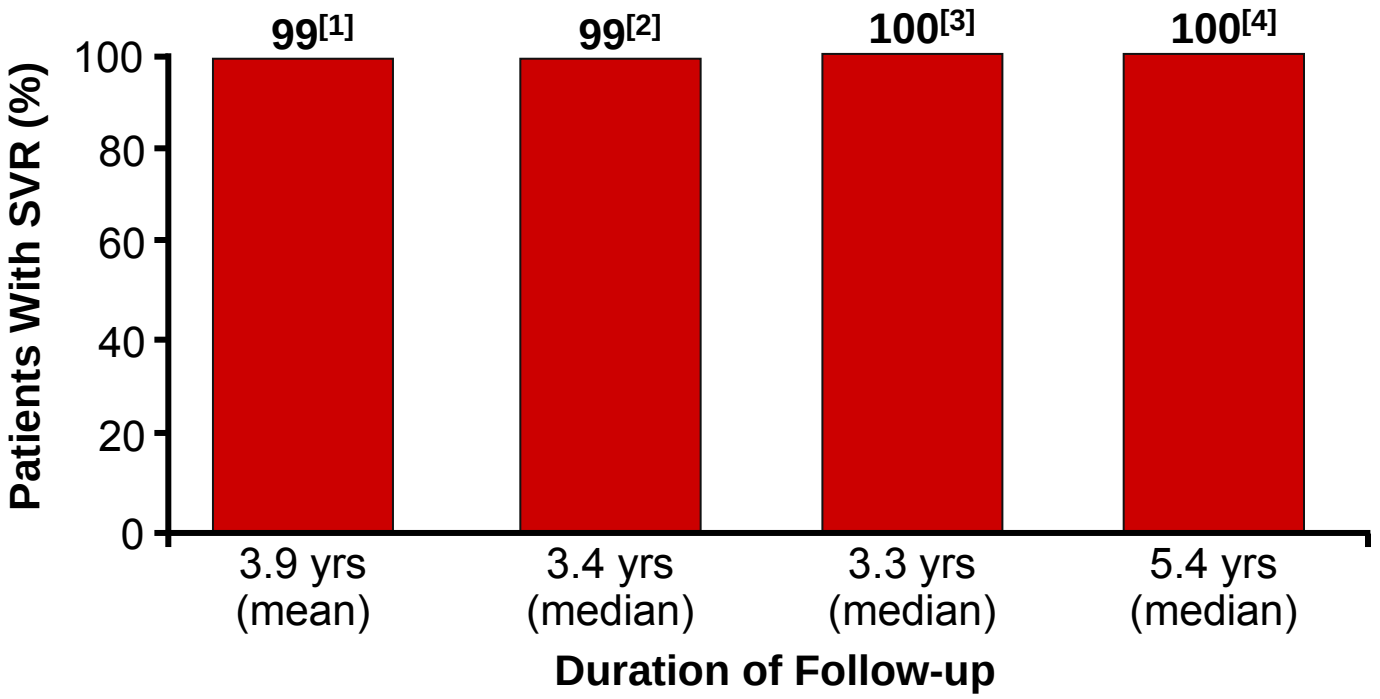
Sustained virologic response (SVR)
HCV RNA negative at month 6 after end of treatment

1) Yee et al., 2012, The American Journal of GASTROENTEROLOGY
2) DAA: direct-acting antiviral; PegIFN: peginterferon; RBV: ribavirin

Why is SVR So Important?

SVR Equivalent to Viral Cure

- Nearly 100% of patients who achieve SVR remain undetectable during long-term follow-up[1-4]



1. Swain MG, et al. Gastroenterology. 2010;139:1593-1601. 2. Giannini EG, et al. Aliment Pharmacol Ther. 2010;31:502-508. 3. Maylin S, et al. Gastroenterology. 2008;135:821-829. 4. George SL, et al. Hepatology. 2009;49:729-738.

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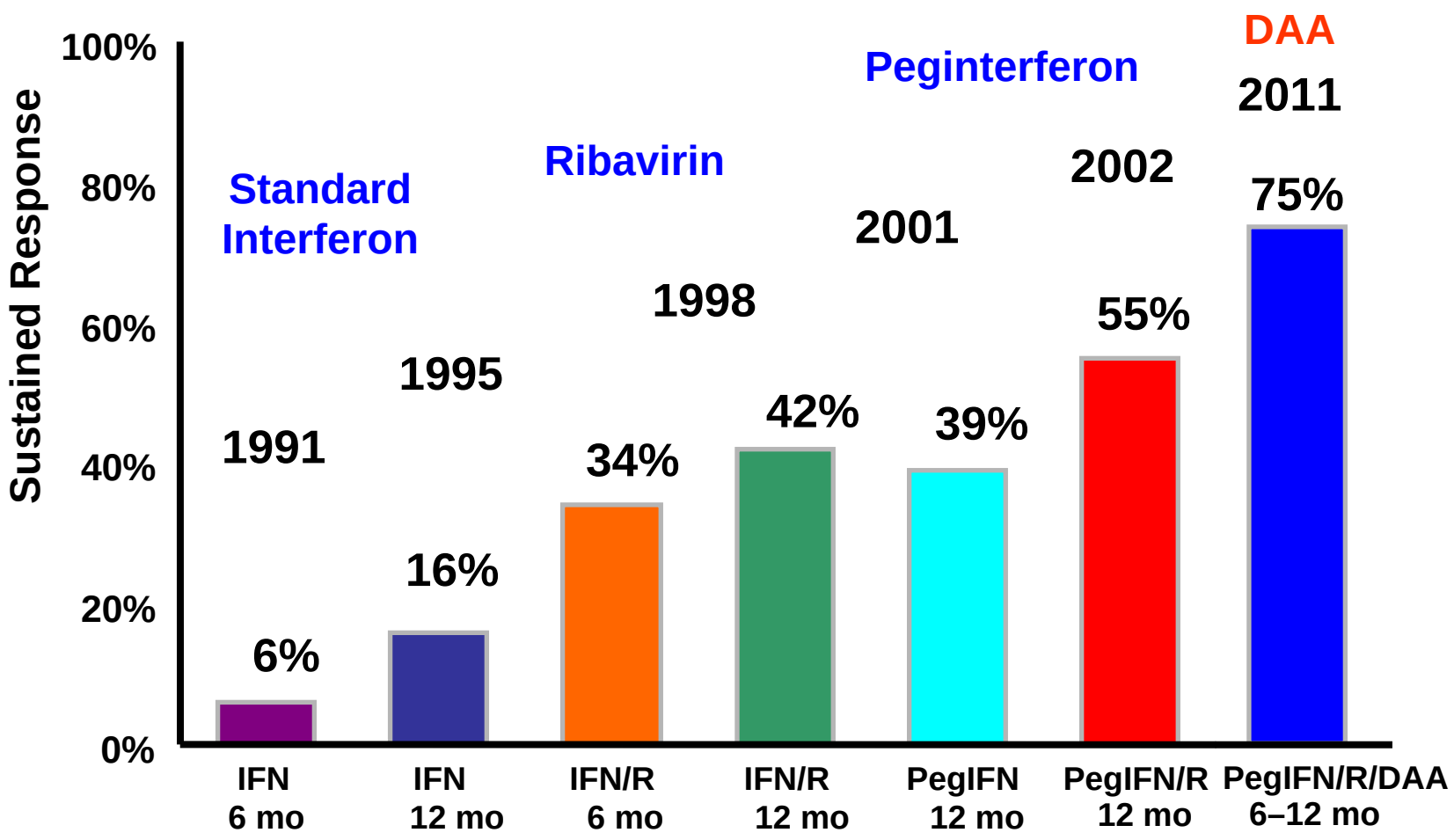
Improved Efficacy of Treatments for HCV



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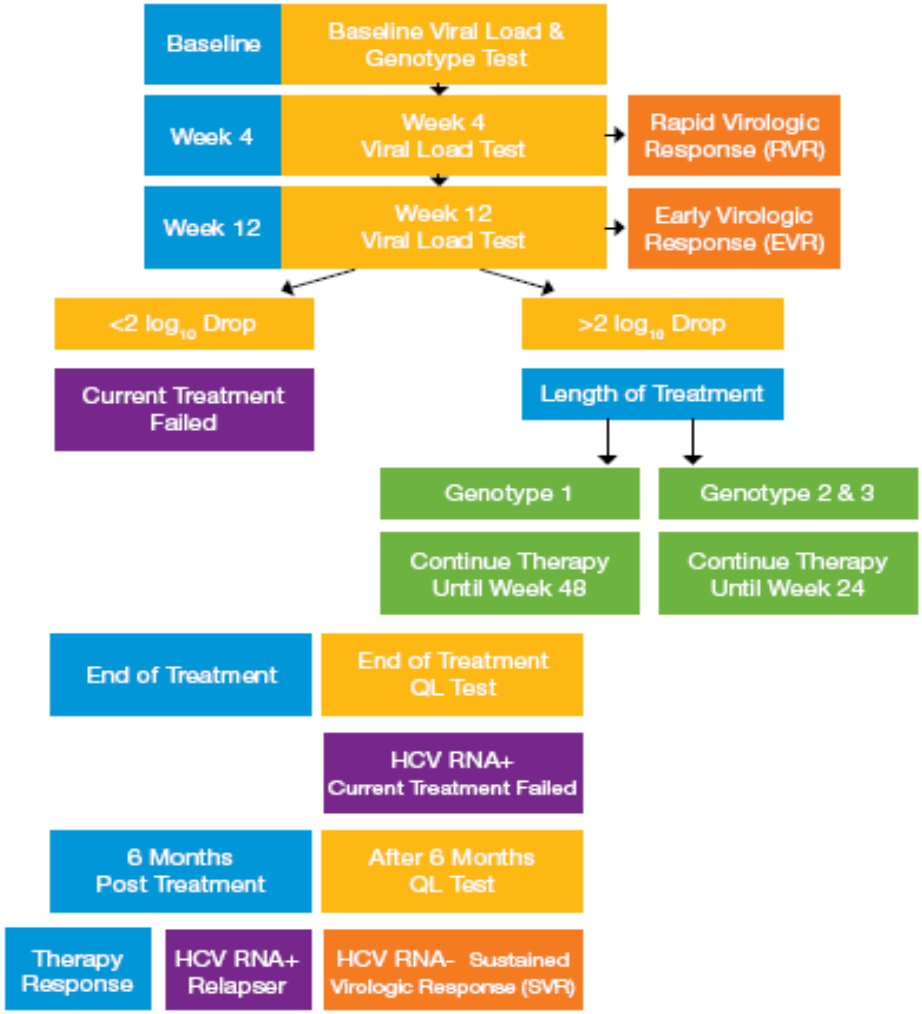
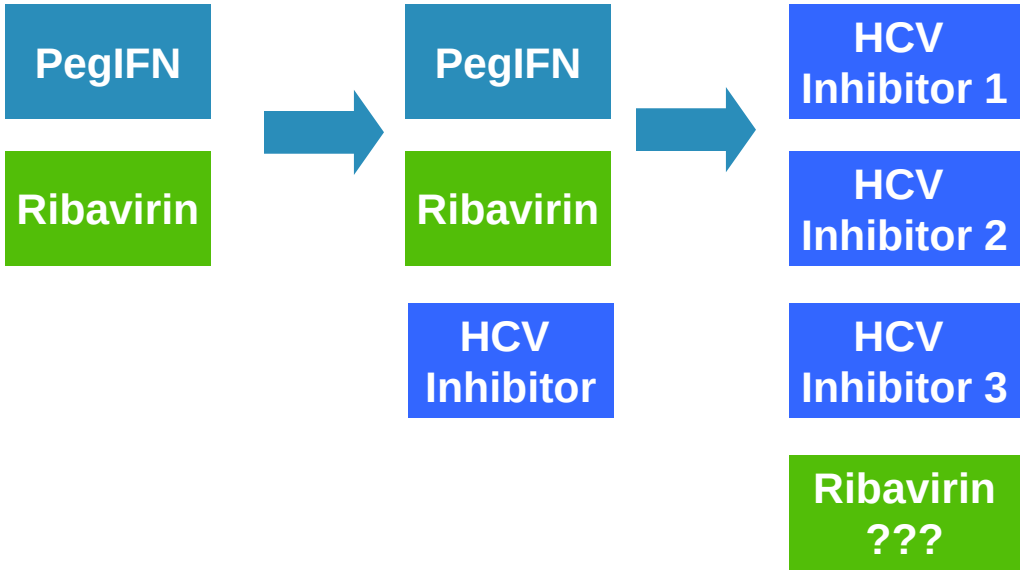
PEG IFN/r HCV Treatment Guidelines



Sources: Ghany MG, et al. Hepatology. 2009;49:1335-1374.
AASLD guidelines

Evolution of HCV Therapy



- Once daily dosing
- Pan-genotype
- No concern about resistance

The aim will be an interferon-free, oral treatment that eradicates the virus in every patient without side effects!

2011 – A New Era in HCV Therapy

Approval of first specific HCV antivirals (DAA): HCV protease inhibitors

boceprevir

telaprevir

- Triple therapy will become the new standard therapy
- Approved for genotype 1 infected patients
- SVR rates ↑ in treatment-naïve and in treatment-experienced patients

<u>But</u>

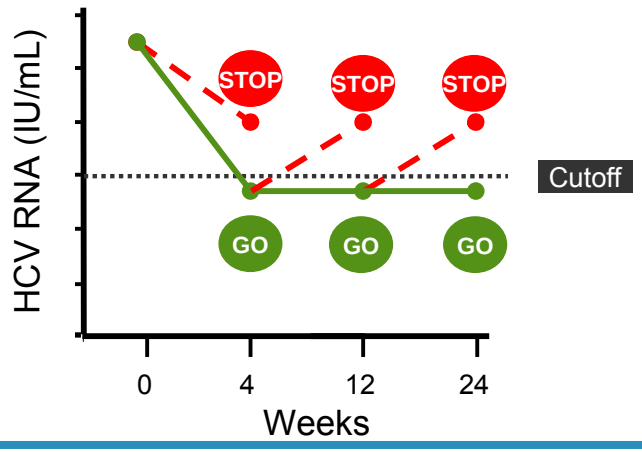
- Side effects
- Resistance development
- Complicated treatment algorithm

HCV RNA is THE Key Indicator

- Analysis of HCV RNA levels in plasma or serum is critical for assessing the efficacy of antiviral therapy for chronic hepatitis C virus (HCV) infection.
- The primary goal of anti-HCV therapy is achievement of a sustained virologic response (SVR), traditionally defined as undetectable serum or plasma HCV RNA 24 weeks following completion of treatment.
- The use of on-treatment HCV RNA measurements to guide treatment duration, termed response-guided therapy (RGT), has become a key component of patient management.
- During HCV treatment, a rapid HCV RNA decline may justify a shorter treatment duration without significantly compromising efficacy. Conversely a slow HCV RNA decline may warrant an extended duration of treatment to maximize the chances of achieving SVR, and little or no HCV RNA decline may warrant early treatment cessation due to futility.

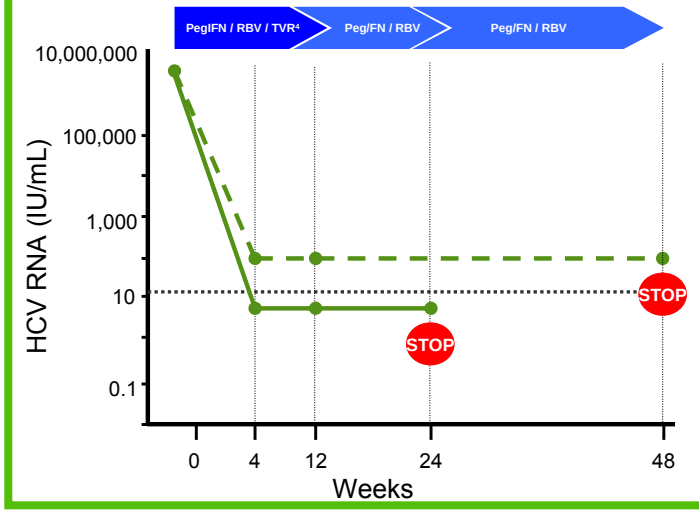
Harrington et al, Hepatology 2012

DAA – Clinical Decision Points
Stopping Rules / Truncated Therapy Rules



Stop triple therapy if HCV RNA is…

- >1000 IU/mL @ wk 4 or wk 12 (TVR[1])
- ≥100 IU/mL @ wk 12 (BOC[2])
- Detectable @ wk 24 (TVR & BOC)

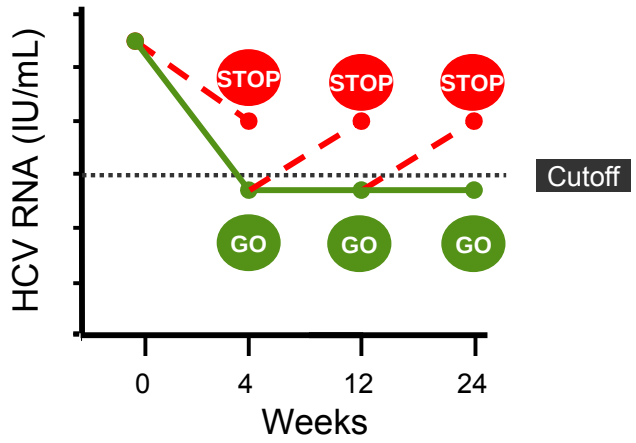


Shorten therapy by 20/24 wks if HCV RNA is…

- Undetectable @ wk 4 and wk 12/24 (TVR / BOC)

1) TVR: Telaprevir
2) BOC: Boceprevir

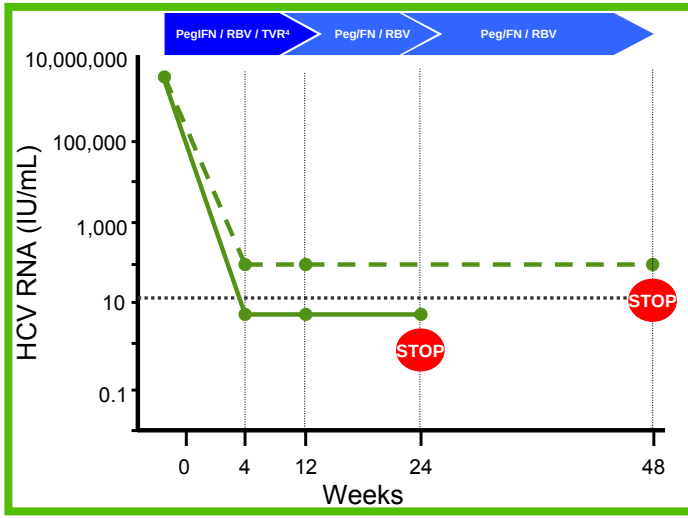
New HCV Drugs
Implications for HCV RNA Measurement



Stop Rules

Measure precisely, accurately, and with high sensitivity

- To reduce uncertainty of stopping or continuing therapy



Therapy Duration Rules

Measure with high sensitivity enabling detection of minimal residual viremia

- To provide confidence that the virus is indeed cleared and that therapy can be truncated if HCV RNA is "undetectable"

HCV RNA assays: Does sensitivity matter?

YES!

In particular for response-guided therapy

16

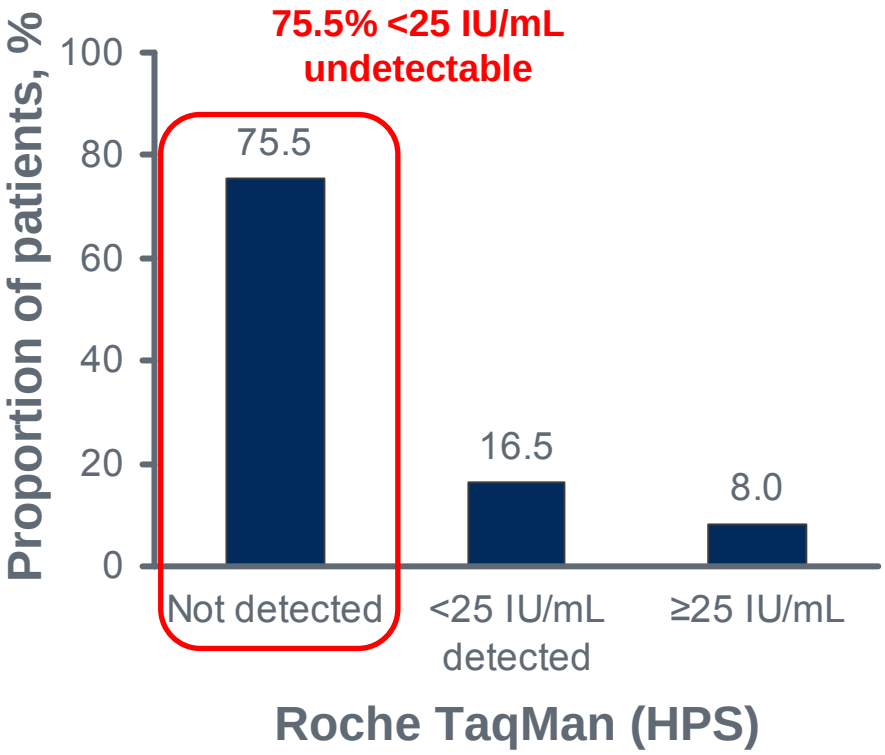
Controversy 1: Sensitivity of HCV RNA Assays

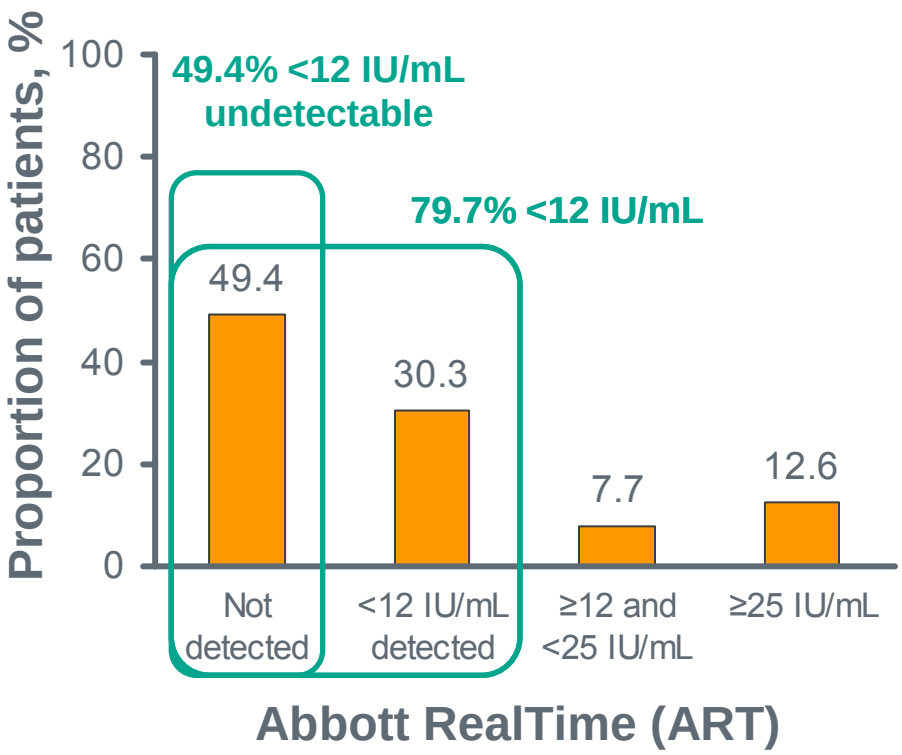
Supplier	Assay	Limit of Detection* (IU/ml)	Lower Limit of Quantification* (IU/ml)	Upper Limit of Quantification* (IU/ml)	
Abbott	Abbott RealTi*me* HCV[1]	12	12		/mL, detected /mL, detected not detected
Roche	Roche High-Pure-System/COBAS® TaqMan assay v2 (HPS)[2]			10^8	"Reported value", detected <25 IU/mL, not quantifiable not detected
Roche	COBAS® Ampliprep/C		43	6.9×10^7	"Reported value" IU/mL, detected <43 IU/mL, not quantifiable not detected
R	uantitative rsion 2.0[4]	15	15	10^8	"Reported value" IU/mL, detected < 15 IU/mL, detected not detected
	RSANT HCV RNA 1.0 (for use on kPCR system)[5]	15	15	Not available	Not available
Qiagen	*artus* HCV QS-RGQ[6]	36.2	67.6	1.8×10^7	Not available

Protease inhibitors approval trials were conducted with Roche HPS/COBAS TaqMan assay v2 - a manual assay not commonly used in routine labs.
Need to translate cutoffs created with HPS into cutoffs for automated assays.

* Performance claims as per respective package inserts or websites. 1. Abbott RealTime HCV (LN 4J86; 51-602124/R4);2. Roche High-Pure-System/COBAS® TaqMan assay v2 (HPS); 3. Roche: COBAS TaqMan HCV, 03543544001-01; 4. COBAS® AmpliPrep/COBAS® TaqMan® HCV Quantitative Test, version 2.0; 5. Siemens: http://www.medical.siemens.com; 6. Qiagen: *artus* HCV QS-RGQ, LN 4518366, 1060924EN

Simulation of RGT duration based on Week 4 result: HPS vs ART*





•At Week 4, 197/261 (75.5%) samples were undetectable by HPS whereas 129/261 (49.4%) were undetectable and 79/261 (30.3%) <12 IU/mL detectable by ART, leading to a different determination of RVR in 68/261 (26.1%) of cases

PILLAR study data presented at EASL 2012 by C. Sarrazin
RVR, HCV RNA undetectable at Week 4

Evaluation of low end sensitivity

Tab.1: (A) Results of five different HCV quantification assays in extremely low viremic range of diluted PEI standards at nominal dilution of 25 IU, 10 IU and 5 IU per ml. (B) Performance for 3rd WHO standard dilutions, respectively.

(A) PEI standard

	25 IU/ml	10 IU/ml	5 IU/ml
RealTime HCV	31, 32, 41	<12, <12, <12	<12, <12, nd
artus HCV QS-RGQ	<36, 91, 98	<36, nd, nd	<36, nd, nd
Versant kPCR HCV	46, 50, 100	nd, nd, nd	nd, nd, nd
CAP/CTM HCV	<43, <43, <43	<43, <43, <43	<43, <43, nd
CAP/CTM HCV v2	<15, <15, 21	<15, nd, nd	nd, nd, nd

(B) WHO standard

	25 IU/ml	10 IU/ml	5 IU/ml
RealTime HCV	15, 28, 33	<12, <12, <12	<12, <12, nd
artus HCV QS-RGQ	185, 150, nd	<36, nd, nd	<36, nd, nd
Versant kPCR HCV	23, 49, 50	23, <15, <15	<15, nd, nd
CAP/CTM HCV	<43, <43, <43	<43, <43, <43	nd, nd, nd
CAP/CTM HCV v2	18, <15, nd	nd, nd, nd	nd, -, -

* nd = not detected - = invalid

Case #1

- 55 year old Caucasian female with GT 1 HCV
  - Naïve to therapy
  - IL28B CT
  - Pretreatment viral load 1,400,000
  - F_2 fibrosis

- HCV therapy initiated with Telaprevir Ribavirin and Peginterferon

Courtesy of Dr. Sid Barritt, UNC Hepatology Dept.

Case #1

Time Point	HCV RNA
Pretreatment	1,400,000
Week 4	Detected/Below Limit Of Quantification (BLOQ)
Week 12	Undetectable
Week 24	Undetectable

Is this patient eligible for RGT?

Courtesy of Dr. Sid Barritt, UNC Hepatology Dept.

Assessing eRVR
(extended rapid virologic response)

Telaprevir

- Check HCV RNA at weeks 4 and 12
  - If HCV RNA is undetectable at ***both*** time points, patient is eligible for RGT
  - 24 weeks total therapy

Boceprevir

- Check HCV RNA at weeks 8 and 24
  - If HCV RNA is undetectable at ***both*** time points, patient is eligible for RGT
  - 28 weeks total therapy for naïve patients
  - 36 weeks total therapy for prior relapsers and partial non-responders

Courtesy of Dr. Sid Barritt, UNC Hepatology Dept.

Detectable/BLOQ ≠ Undetectable?

Clinical Relevance of Detectable but Not Quantifiable Hepatitis C Virus RNA During Boceprevir or Telaprevir Treatment

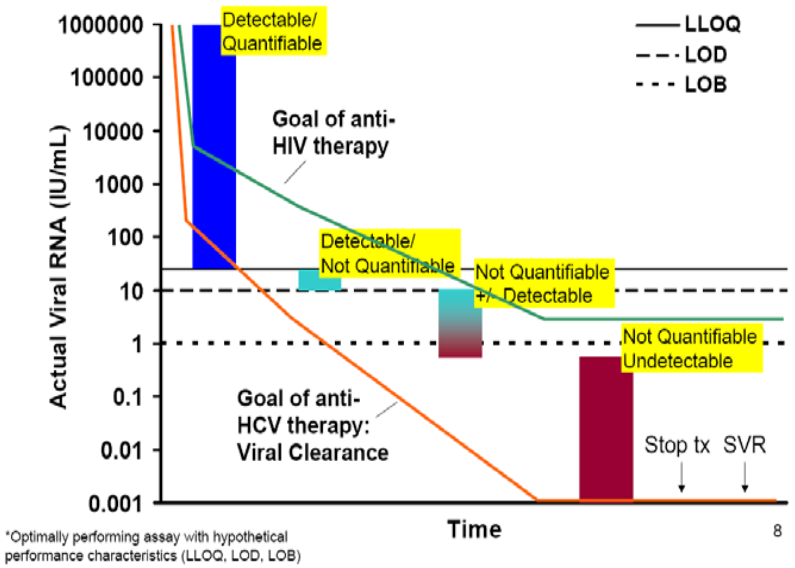
Patrick R. Harrington,[1] Wen Zeng,[2] and Lisa K. Naeger[1]

- Analyzed boceprevir and telaprevir clinical trial data
- Detectable/BLOQ were reported for 10-20% of all on-treatment HCV RNA measurements

Harrington et al, Hepatology 2012

Detectable/BLOQ ≠ Undetectable!

- Subjects with Detectable/BLOQ at RGT specific time points had ~20% lower SVR rate compared to patients with undetectable HCV RNA
- This patient should receive a total of 48 weeks of therapy for optimum chance at SVR



Harrington et al, Hepatology 2012

Case #1 Summary

- **Detectable means Detectable!**
  - Detectable/BLOQ should not be used to make RGT decisions
  - SVR rates ~20% lower when BLOQ/detectable used instead of undetectable

- Current HCV treatment guidelines recommend using assays with LOQ of <25IU/ml and LOD 10-15IU/ml for viral load monitoring

- This recommendation is a critical component of HCV treatment as less sensitive assays in the case of this patient would suggest shortened treatment when this is not beneficial to the patient

Courtesy of Sid Barritt, UNC Hepatology Dept.

Case # 2

Time point	HCV RNA	Lab	LLOQ	LLOD
Pretreatment	1,800,000	A	12 IU/mL	7.3 IU/ mL
Week 8	undetectable	B	25IU/mL	9.3 IU/mL
Week 24	undetectable	A	12 IU/mL	7.3 IU/ mL

- Is this patient eligible for RGT?

Courtesy of Dr. Sid Barritt, UNC Hepatology Dept.

HCV RNA levels and Lab Assays

- Analysis of HCV RNA levels is critical for assessing efficacy of therapy for chronic HCV infection
- RGT guidelines for boceprevir and telaprevir are based on undetectable viral load and specific time points
- Protocols for other trials have incorporated secondary endpoints based on achieving <LLOQ viral load rather than an undetectable viral load

Assay Name	LLOQ
Roche COBAS® AmpliPrep/COBAS® TaqMan® HCV Test	43 IU/mL
Roche COBAS® TaqMan® HCV test, v2.0	25 IU/mL
Abbott RealTime HCV Assay	12 IU/mL

Courtesy of Dr. Sid Barritt, UNC Hepatology Dept.

Case # 2

- Possible that week 8 HCV RNA assay performed by a less sensitive test may yield a result that would be detectable/BLOQ in another more sensitive assay
- Possible risk for relapse, lower SVR rate as in case #1
- Discussion of risks and benefits with patient

Courtesy of Dr. Sid Barritt, UNC Hepatology Dept.

Case #2 Summary

- Know your lab and HCV RNA assay characteristics
  - Some labs may still use older less sensitive assays
  - Residual viremia at RGT time points may lead to increased relapse rates and reduces SVR

Courtesy of Dr. Sid Barritt, UNC Hepatology Dept.

HCV RNA assays: Does Precision matter?

YES!

In particular for response-guided therapy

30

Controversy 2: Precision
Assay Comparison Study

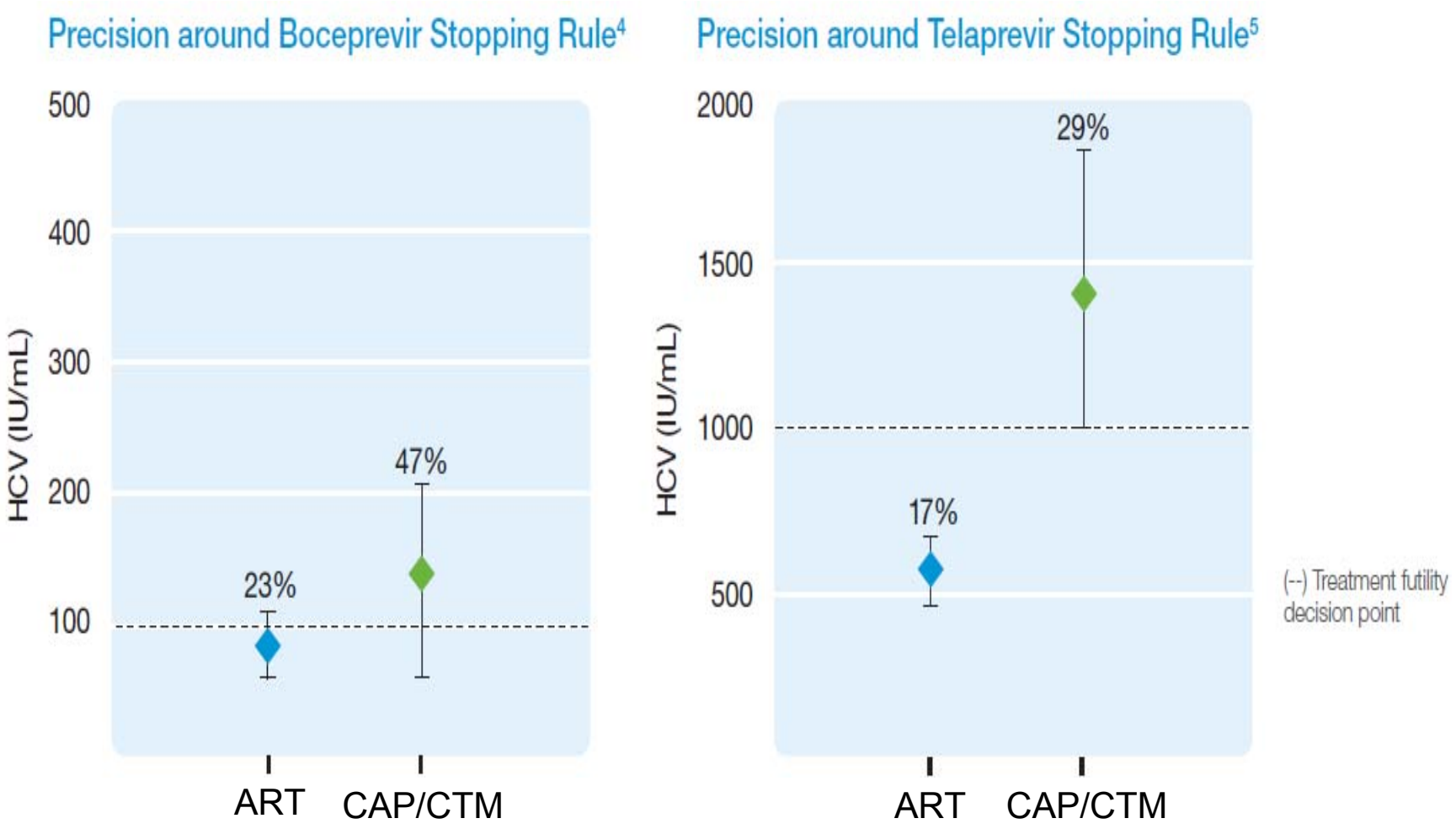
Study Design

Investigate **low end precision, accuracy** and **sensitivity** across different HCV genotypes at clinical decision points of new DAAs comparing

- **RealTi*me* HCV**
- **Roche CAP/CTM HCV 1.0**
- **Roche CAP/CTM HCV 2.0**
- **QIAGEN *artus* HCV QS-RGQ**
- **Siemens HCV kPCR**

Naeth et al., ESCV 2012

Precision at clinical decision point



Precision at clinical decision point

HCV panels targeting 700 IU/ml and 80 IU/mL were tested on four separate occasions at six different commercial clinical laboratories.

Assay	HCV Target Concentration (IU/mL)	Mean Viral Load (IU/mL)	Range (IU/mL)	Standard Deviation (IU/mL)	% Coefficient of Variation (IU/mL)
Abbott RealTi*me*	80	58	43 - 77	10	17
	700	585	414 - 777	113	19
Roche CAP/CTM HCV v1	80	131	61 - 278	68	52
	700	1617	964 - 3050	678	42

1. Adapted from Lucic et al., abstract at ESCV 2012

Panel precision 100 IU/ml

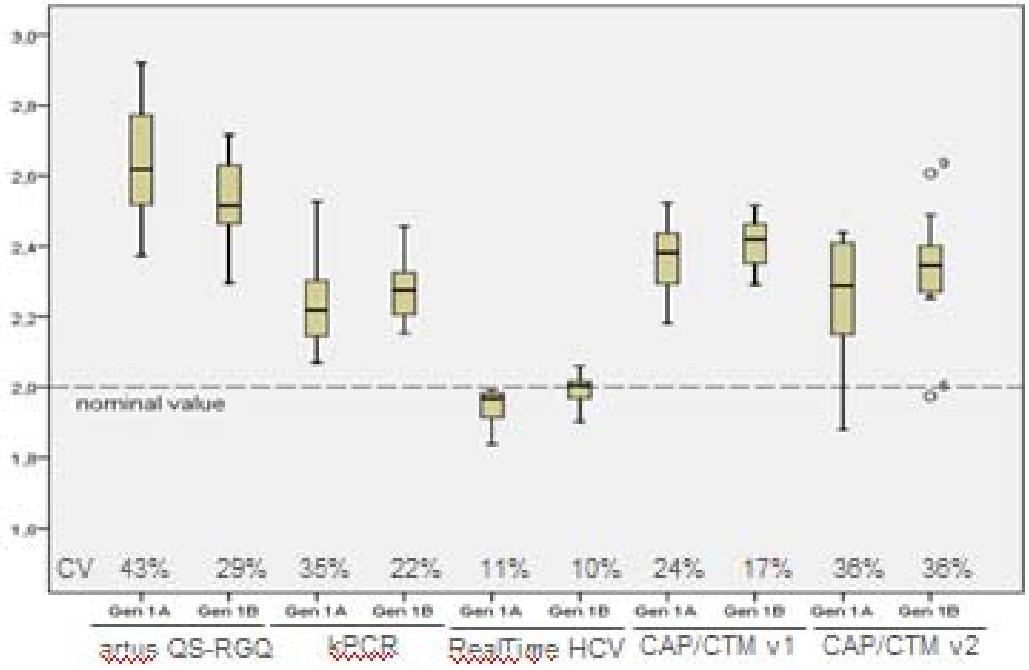


Fig. 3: Box Plot illustration of inter-assay variation analysis: 10 replicates of each 2 clinical samples (Genotype 1A + 1B) were analysed with all five quantitative HCV assays. Coefficients of variation are illustrated below the bars.

Case #3

- 48 year old Caucasian male with HCV cirrhosis
  - MELD 12
  - No decompensation events
  - Prophylactically listed for liver transplantation
  - Relapser to prior therapy, IL28B CT

- HCV therapy initiated with Telaprevir Ribavirin and Peginterferon

Courtesy of Dr. Sid Barritt, UNC Hepatology Dept.

Case #3

Time point	HCV RNA	HGB	Platelets	Comment
Pretreatment	2,800,000	13	75,000	RBV 1000, P 180
Week 1		10	40,000	RBV 600, P 135
Week 2		9	35,000	RBV 400, P 90
Week 4	1034	8	35,000	RBV held, P 90

This patient has had >3 log drop in HCV RNA, should we continue?

Courtesy of Dr. Sid Barritt, UNC Hepatology Dept.

Case #3

- All medications stopped
- While this patient has had a significant drop in the HCV RNA, viral load remains above the threshold for continuing treatment
- Likely represents protease resistant virus, plus innate poor response to Interferon and Ribavirin

Courtesy of Dr. Sid Barritt, UNC Hepatology Dept.

Case #3

- Importance of adherence to stopping rules
  - Patients who do not respond to 1st generation protease inhibitors carry a dominant strain of protease resistant virus at the time of breakthrough
  - Wild type strain reemerges months after therapy discontinued
  - Return of wild type strain may be an indication that patients may be re-challenged in the future with multidrug cocktails that include protease inhibitors.

Courtesy of Dr. Sid Barritt, UNC Hepatology Dept.

Pawlotsky JM, *Hepatology* 2011

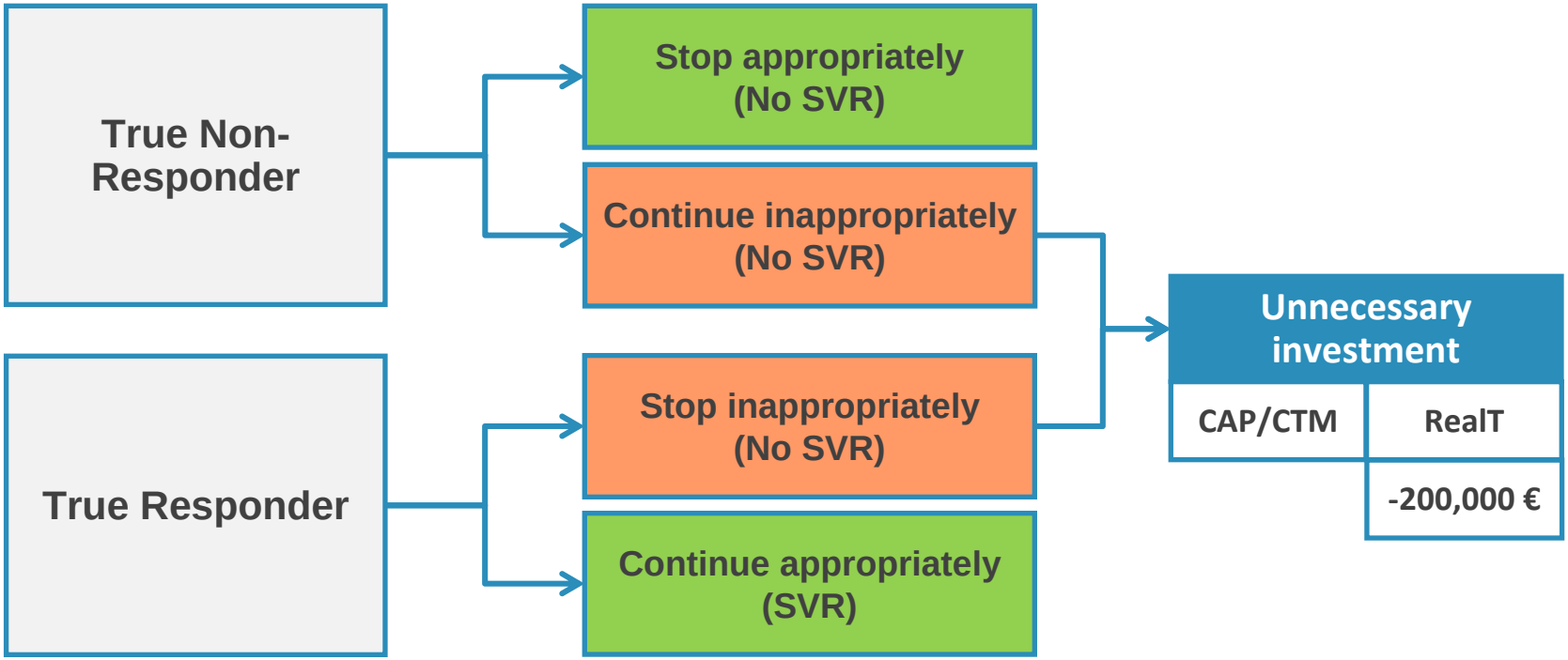
Case #3 Summary

- Stopping rules for futility are important
  - Reduce exposure and expense for patients when chance for SVR is minimal
  - May preserve chance for SVR with future therapies
- Important feature of viral load assay is precision across the full linear range especially around TEL and BOC stopping points

Courtesy of Dr. Sid Barritt, UNC Hepatology Dept.

Clinical & Economic Implications Of A Non-Ideal Test

•Any inappropriate decision would waste resources



* Based on a population size of 1000 patients on treatment. Implications of treatment futility such as risk for progression to cirrhosis, 2nd line therapy etc. are not considered. Direct treatment costs until wk 48 based on average European tariffs with estimated weekly costs of 1,000 €.

Graph: Abbott

Case # 4

- 60 year old African American female
  - Completed 48 weeks of boceprevir based triple therapy

Time Point	HCV RNA
Pretreatment	400,000 IU/mL
Week	150 IU/mL
Week 24	Undetectable
Week 48	Undetectable

At what time point can we assess SVR?

Courtesy of Dr. Sid Barritt, UNC Hepatology Dept.

Case # 4

- Traditional measure of SVR has bee six months (24 weeks) after the completion of therapy.
- New push for faster recognition of cure
  - 12 week assessment of SVR as good as 24 weeks

Table 2. Serum HCV-RNA Outcome During the 24 Weeks Posttreatment Follow-up

	Patients*	SVR†	PPV‡ (95% CI)
Week +4§	252	242	96.0% (93.1-98.1)
Week +12	409	408	99.7% (99.1-100)
Week +24	408	408	100%

Courtesy of Dr. Sid Barritt, UNC Hepatology Dept.

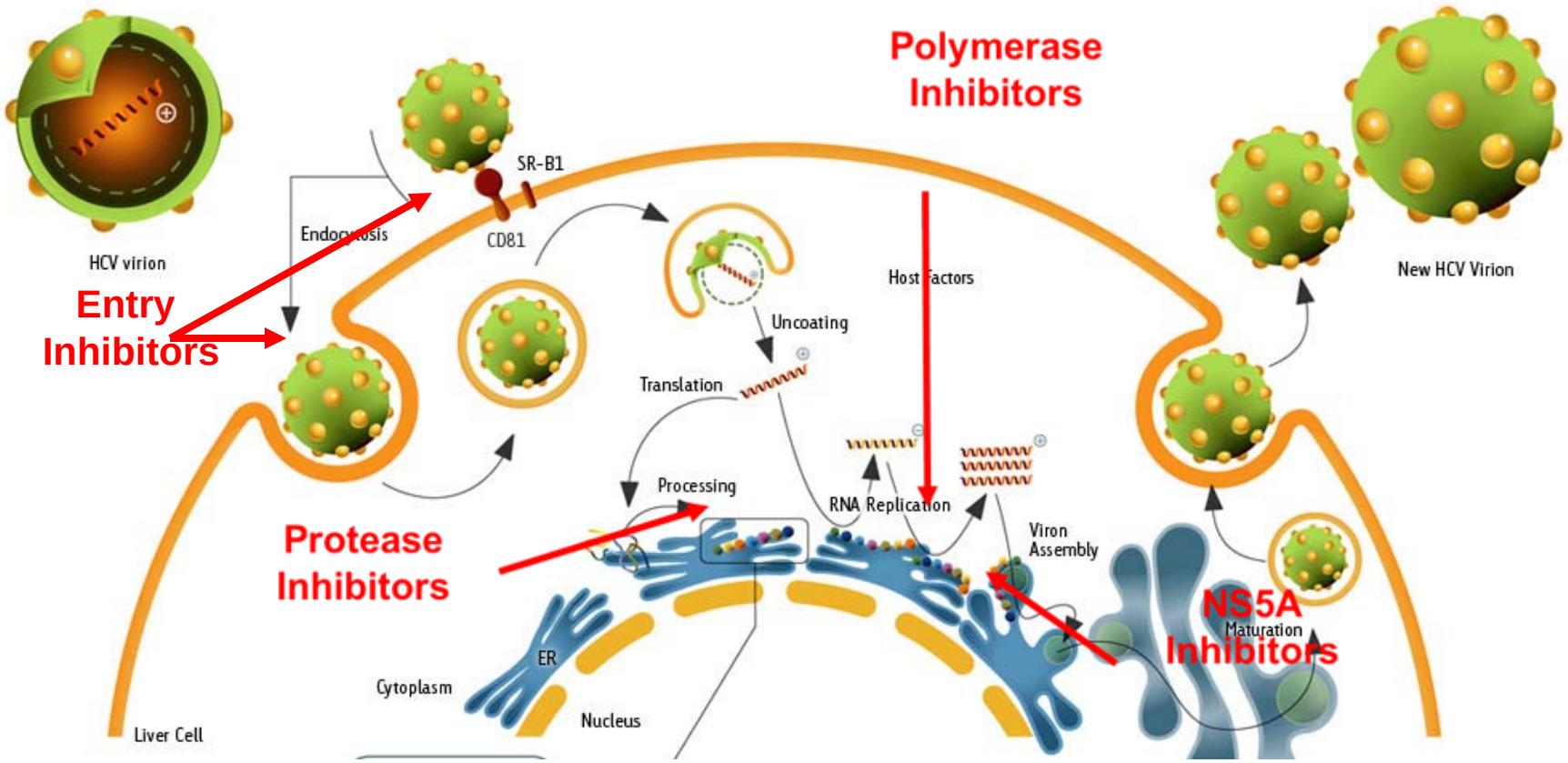
Zeuzem S, et al. J Hepatol 2003.
Martinot-Peignoux M, et al. Hepatology 2010.

Case #4 Summary

- Lesson from Determining SVR at week 12 equivalent to week 24.
  - Patients (and clinical trials) can find out results earlier

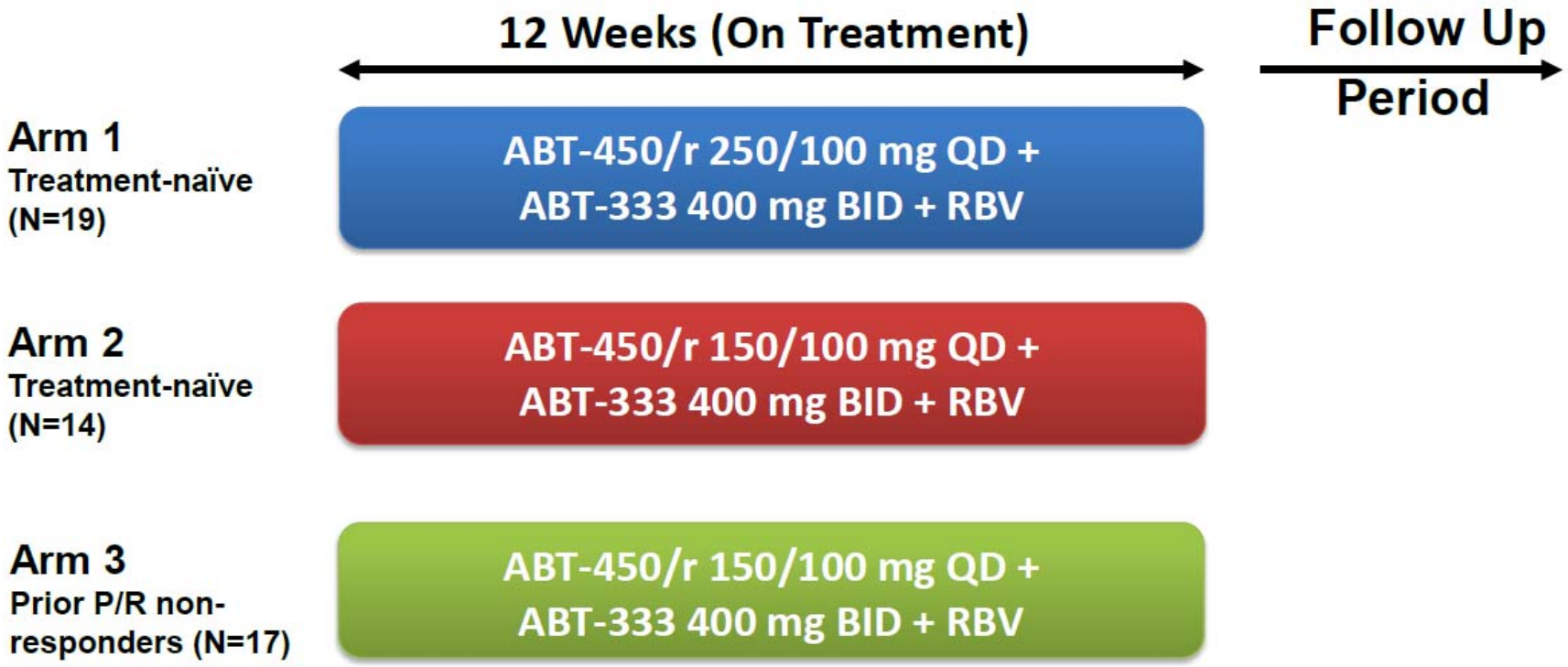
Courtesy of Dr. Sid Barritt, UNC Hepatology Dept.

HCV Lifecycle – Lots of Potential "Druggable" Options



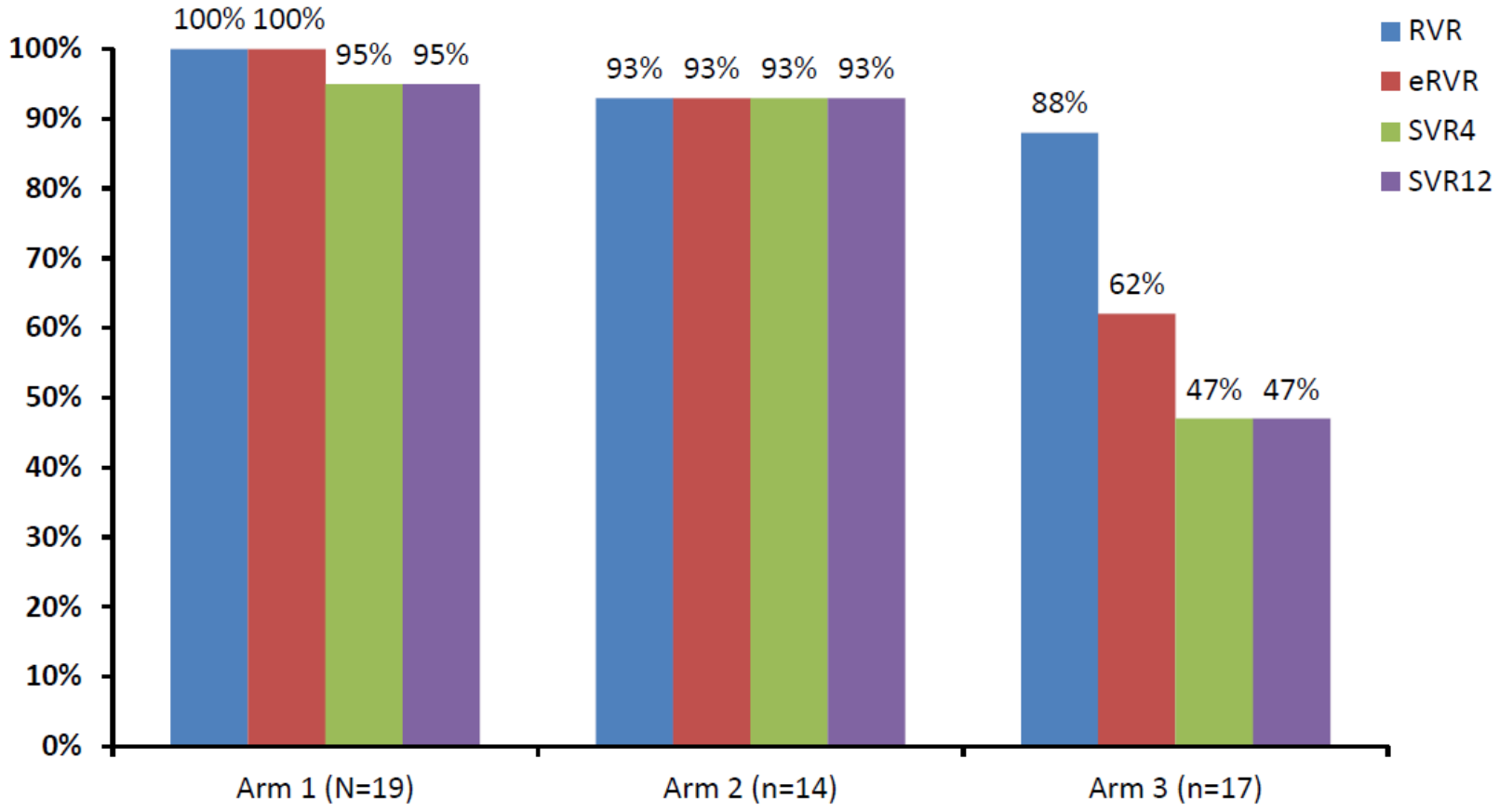
Manns MP, et al. Nat Rev Drug Discov 2007;6:991–1000.

Co-Pilot (M12-746) Study: ABT-450/r+ABT-333



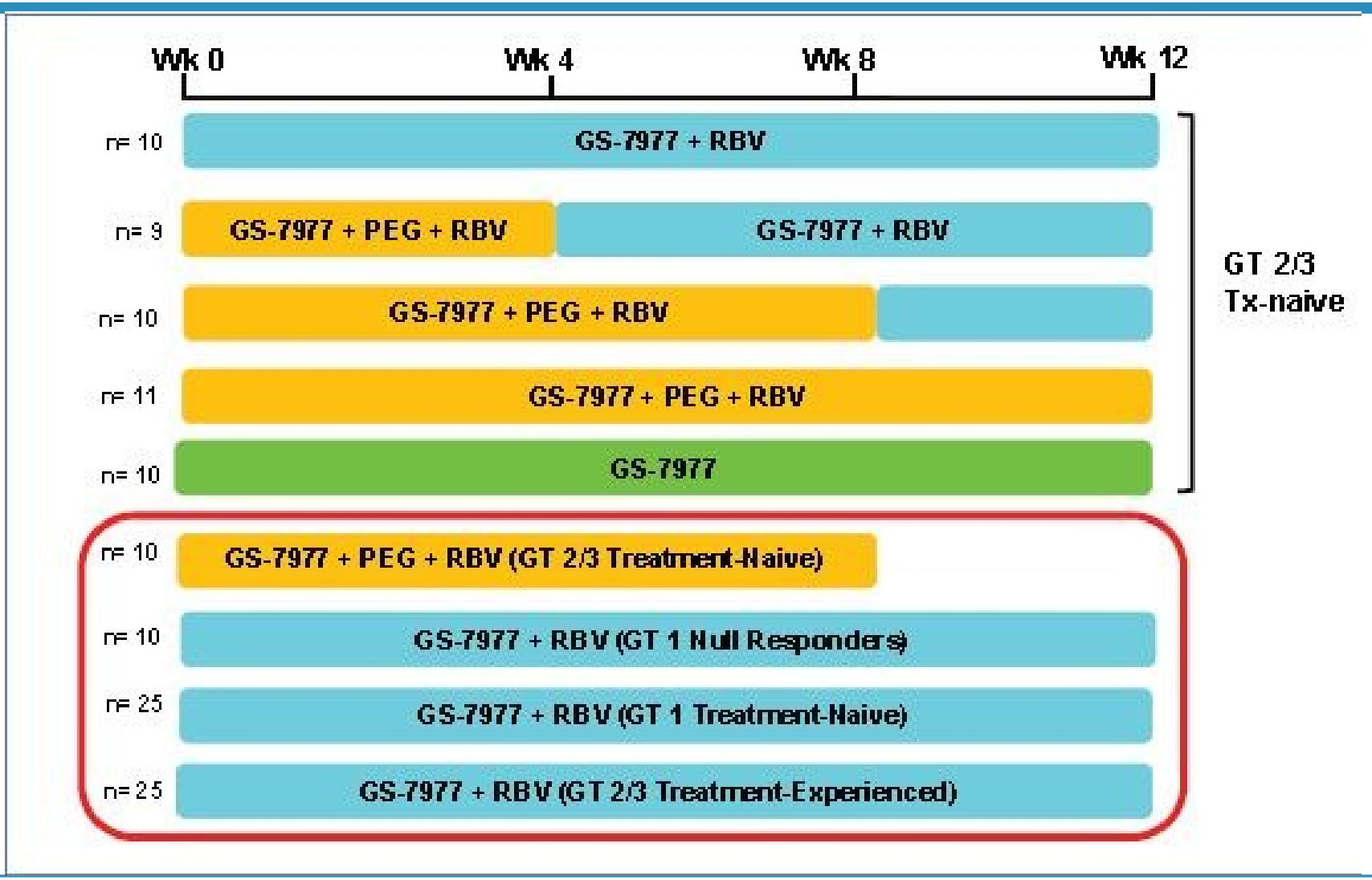
Poordad et al, 47th EASL; Barcelona Spain, April 18-22 2012; Abst 1399

Co-Pilot Study: Virologic Results



Poordad et al, 47th EASL; Barcelona Spain, April 18-22 2012; Abst 1399

GS-7977 ELECTRON - Trial Design



Gane et al., 47th EASL; Barcelona Spain, 2012 Poster # 1113

GS-7977 ELECTRON Study: Virologic Response

Patients with HCV RNA <LOD Over Time, n/N (%)

	GT 2/3 Treatment-naïve 8 wks (N=10)	GT 1 Null Responders 12 wks (N=10)	GT 1 Treatment-naïve 12 wks (N=25)	GT 2/3 Treatment-experienced 12 wks (N=25)
Week 1	6/10 (60)	1/10 (10)	7/25 (29)	8/25 (32)
Week 2	10/10 (100)	7/10 (70)	17/24 (71)	21/25 (84)
Week 4	10/10 (100)	10/10 (100)	25/25 (100)	25/25 (100)
EOT	10/10 (100)	9/9 (100)	25/25 (100)	21/21 (100)
SVR 4	10/10 (100)	1/9 (11)	22/25 (88)	12/15 (80)
SVR 8	10/10 (100)	1/9 (11)	-	
SVR 12	10/10 (100)	-	-	-

Gane et al., 47th EASL; Barcelona Spain, 2012 Poster # 1113

Summary

Laboratories

- The new HCV drugs represent hope for millions of patients who carry the virus.
- Decisions to stop or continue treatment are crucial in determining the outcome and are guided exclusively by the RNA viral load measurements.
- Early stopping helps avoid toxicity in patients unlikely to achieve SVR ; by contrast, continuing treatment for late responders, offers more patients a chance at viral clearance and cure.
- Genotype determination helps to assess a patient's eligibility for new DAA therapy. Subtype determination of 1a from 1b may be used to predict likelihood of development of resistance.