How to use the virological tools for the optimal management of chronic hepatitis C (including resistance)

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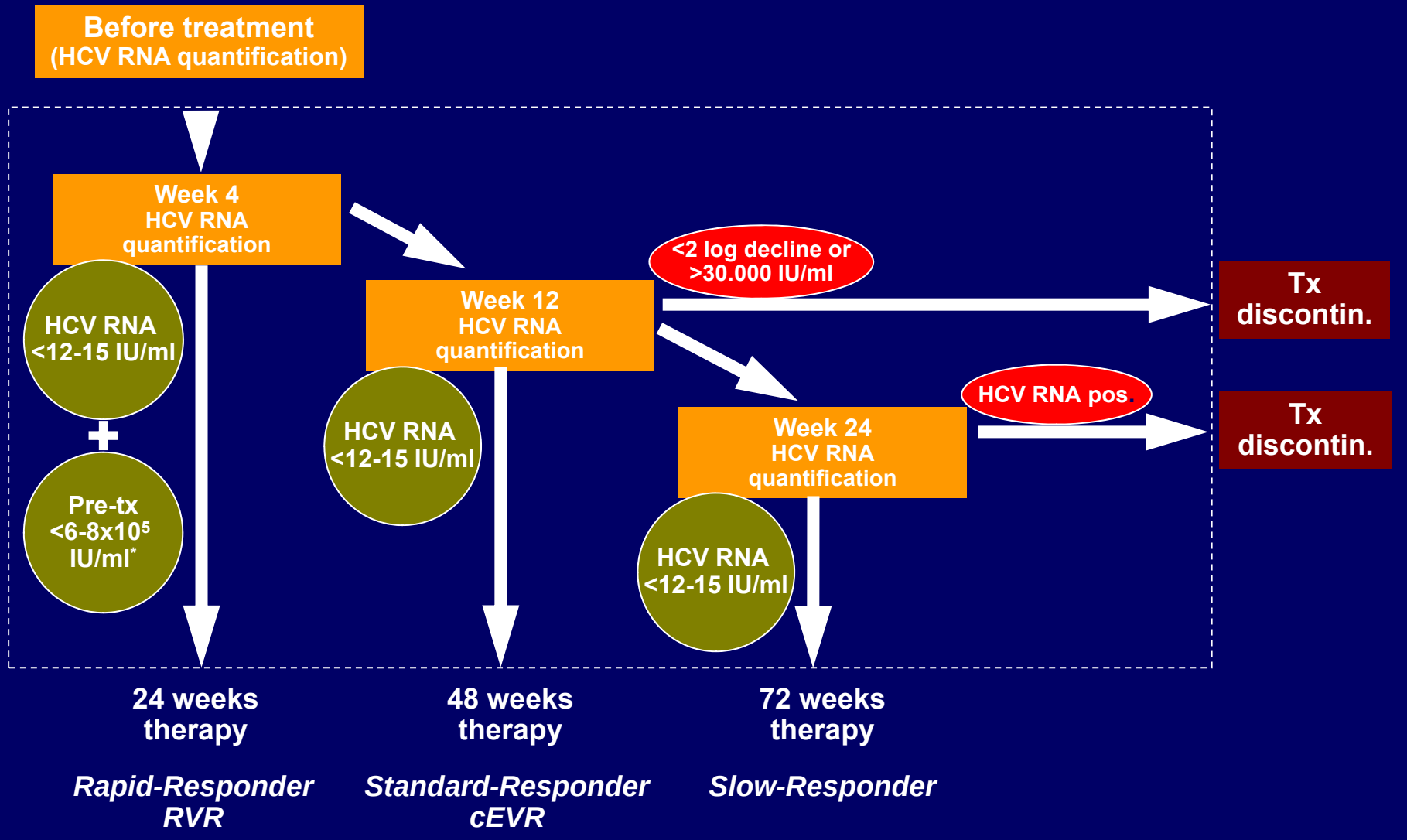
Stefan Zeuzem

J.W. Goethe University Hospital

Frankfurt, Germany

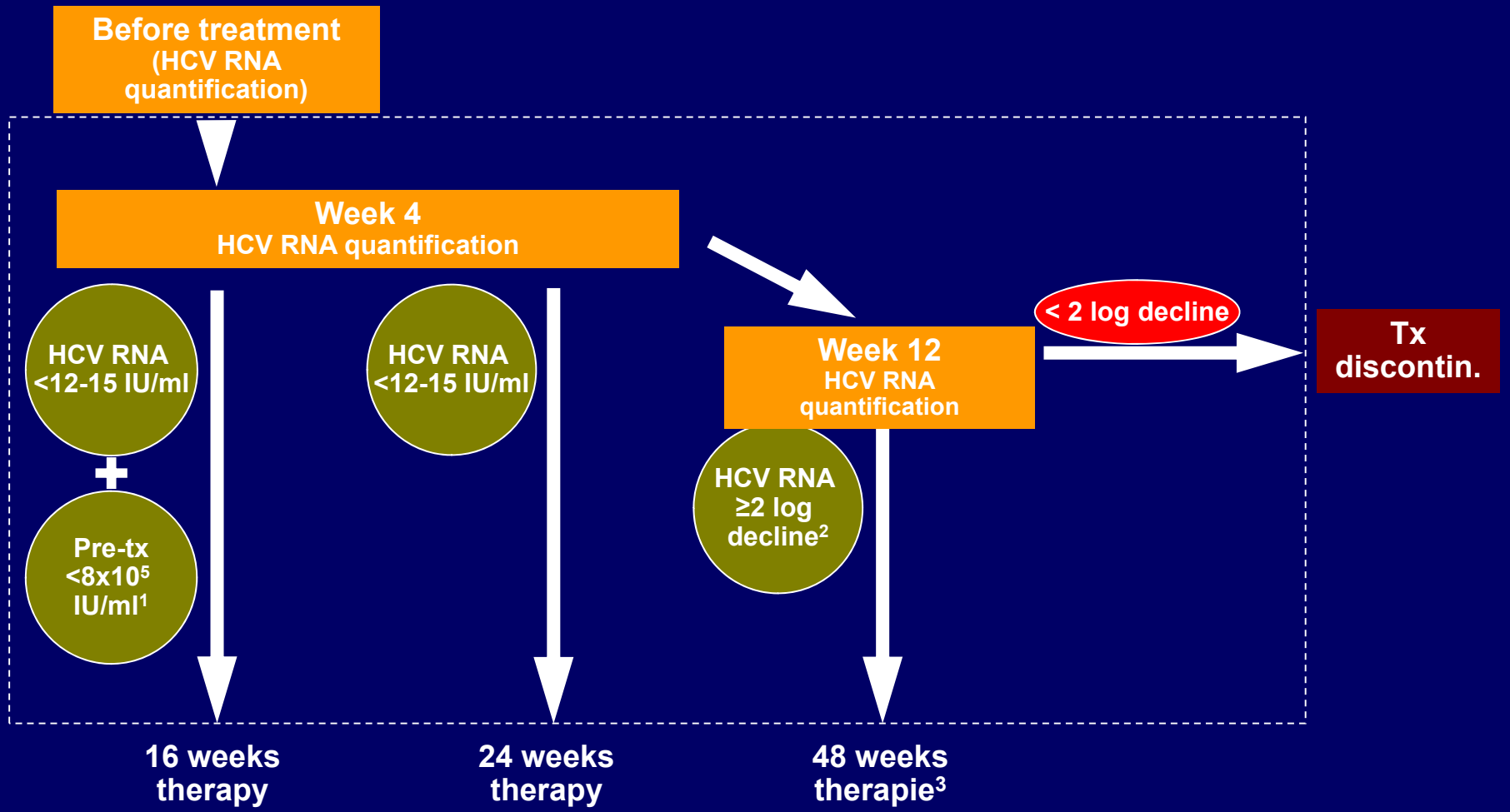
MEDIZINISCHE KLINIK 1

Standard treatment for HCV genotype 1/4



* No treatment shortening in patients with advanced fibrosis, cirrhosis, metabolic syndrome, insulin resistance, HIV/HCV coinfection, etc. No data for patients with persistently normal ALT levels.

Standard treatment for HCV genotype 2/3



1. No treatment shortening in patients with advanced fibrosis, cirrhosis, metabolic syndrome, insulin resistance, HIV/HCV coinfection, etc. No data for patients with persistently normal ALT levels.
2. Detectable HCV-RNA at week 24: discontinuation of treatment.
3. Treatment duration of 36, 48, 72 weeks in „slow-responders“ is currently investigated in prospective trials.

Predictive value of achieving HCV RNA <50 IU/mL at wk 12 (pooled 72 wks vs 48 wks)

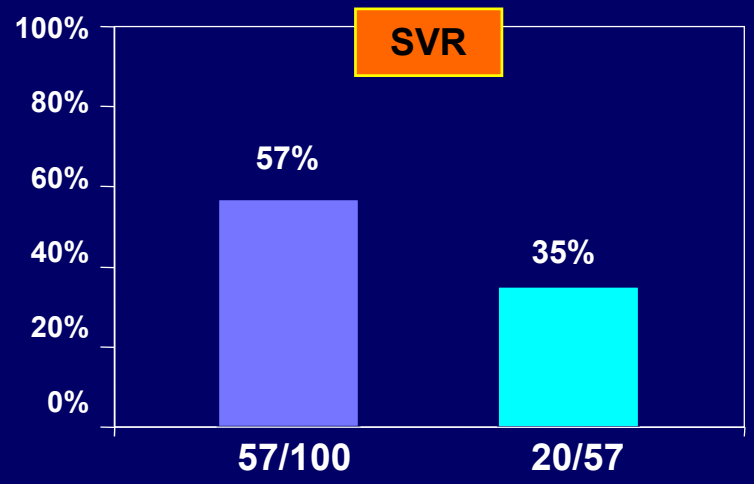
All patients treated (n=942)

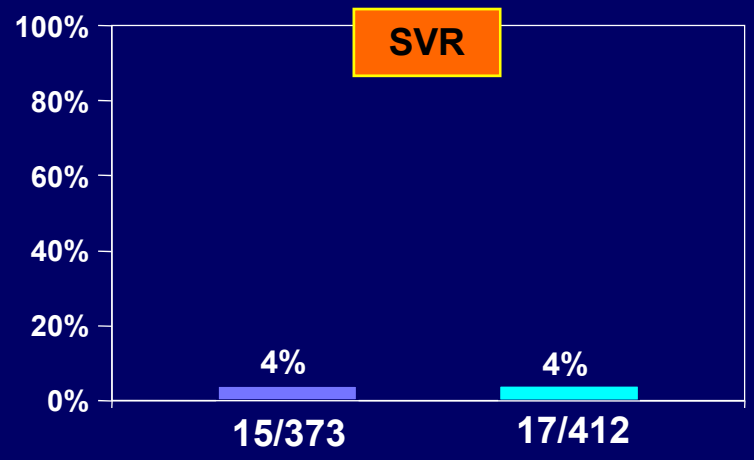
<50 IU/mL at week 12: YES

17%
(157 / 942)

<50 IU/mL at week 12: NO

83%
(785 / 942)





72 weeks (360/180 µg and 180 µg)

48 weeks (360/180 µg and 180 µg)

Jensen et al.,
Ann Intern Med 2009;150:528-40

VIROLOGIC TOOLS IN THE ERA OF PEG-IFN AND RBV

- HCV Genotyping
- HCV RNA detection and quantification

Requirements for HCV RNA assays

- High specificity (risk of false positive results)
- High sensitivity (treatment duration, virologic response)
- Precise quantification (low vs. high VL, 2 log rule)
- Inclusivity for all HCV genotypes
- Comparability between assays (standard. IU)
- One assay for qualitative and quantitative measurement

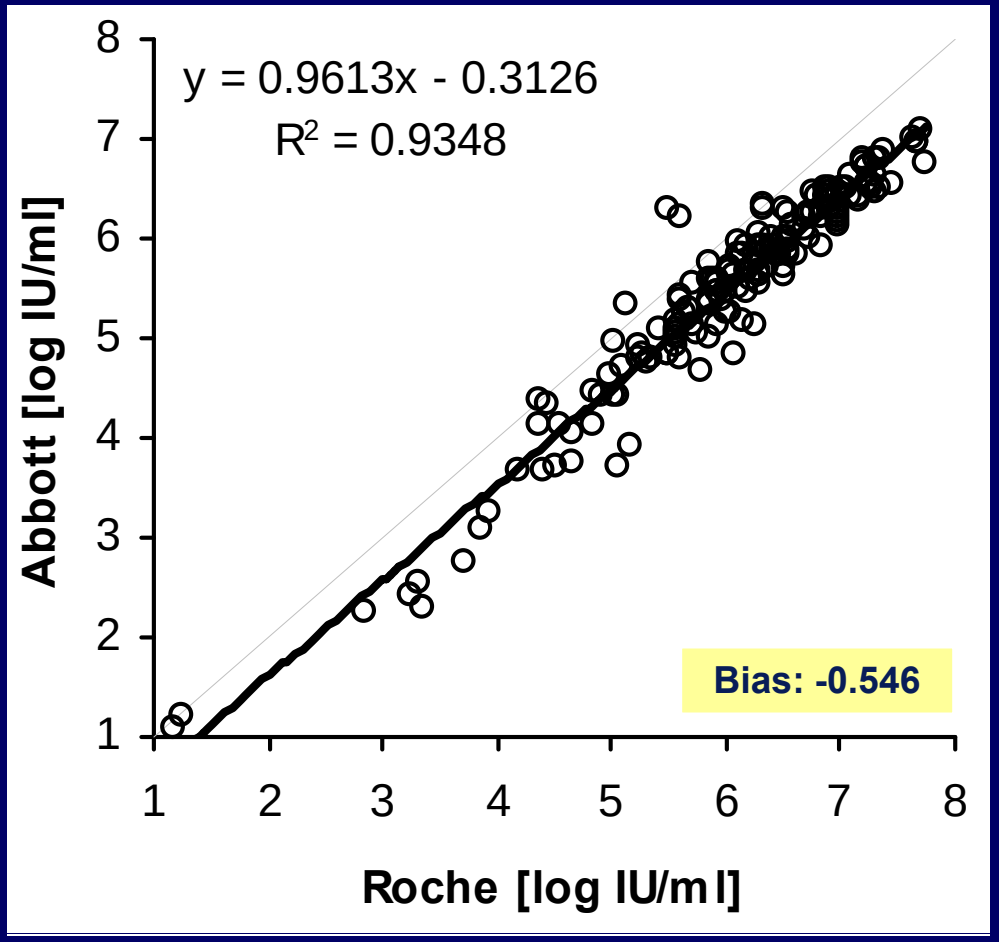
HCV RNA Assays

	negative / undetectable	detectable/ unquantifiable	detectable/ quantifiable
Versant Qual. TMA	neg. (<5-10 IU/ml)		pos. (>5-10 IU/ml)
Versant Quant. bDNA	neg. (<615 IU/ml)		>615 IU/ml
Cobas Amplicor	neg. (<50 IU/ml)		pos. (>50 IU/ml)
Cobas Amplicor Mon.	neg. (<500 IU/ml)		>500 IU/ml
Cobas TaqMan	neg. (<10 IU/ml)	pos. (<15 IU/ml)	>15 IU/ml
RealTime HCV	neg. (<10 IU/ml)	pos. (<12 IU/ml)	>12 IU/ml

Result reporting is not identical in different labs and countries

Differences between commercial assays

RealTime HCV versus Cobas TaqMan



Michelin et al, *J Clin Virol* 2007;38:96-100

Differences in HCV RNA quantification between assay of 0.5 log (factor 3-4) !

Quantification in IU results from Standardization to the WHO Standard

⇨ WHO Standard vs. Cobas TaqMan and RealTime HCV

WHO Standard	RealTime HCV			Cobas TaqMan (CAP/CTM)		
Nominal input IU/mL (log_{10})	Mean (range) IU/mL	Mean (range) log_{10}	Mean difference to WHO Standard	Mean (range) IU/mL	Mean (range) log_{10}	Mean difference to WHO Standard
1,500 (3.2)	920 (674-1102)	3.0 (2.8-3.0)	**- 0.2**	3,064 (2,857-3,096)	3.5 (3.5-3.5)	**+ 0.3**
25,000 (4.4)	13558 (8997-16102)	4.1 (4.0-4.2)	**- 0.3**	43,489 (22085-69852)	4.6 (4.3-4.8)	**+ 0.2**

Mean, mean concentrations or mean log_{10}-transformed concentrations

Vermehren et al., *J Clin Microbiol* 2008;46:3880-91

Quantification differences between commercially available assays

GT	n	AccuGene m-HCV	bDNA		CAP/CTM	
		Mean concn. (IU/mL $\log_{10}$)	Mean concn. (IU/mL $\log_{10}$)	Difference to RealTime HCV	Mean concn. (IU/mL $\log_{10}$)	Difference to RealTime HCV
1	30	5.50	5.48	-0.02	6.22	**0.72**
2	12	5.96	5.74	-0.22	5.99	0.03
3	16	5.58	5.31	-0.27	5.36	-0.22
4	4	5.41	5.22	-0.19	4.14	**-1.27**
5	3	5.36	5.33	-0.03	5.45	0.09

GT, Genotype; Mean concn., mean HCV RNA concentrations

Vermehren et al., *J Clin Microbiol* 2008

Single high viremic HCV genotype 4 samples may be even HCV RNA negative by the Cobas TaqMan assay

Pawlotsky et al., *Hepatology* 2009

Does the sensitivity of the HCV RNA assay matter ?

- CAM (< 50 IU/mL) vs. CAP-CTM (< 15 IU/mL)
- RVR rates highly concordant
- No difference in SVR rates after shorter therapy in pts. with RVR (<50 IU/mL), RVR (<15 IU/mL), and RVR (undetectable by CAP-CTM)
  - 82% vs. 83% vs. 83% (HCV-1, tx duration 24 wks)
  - 95% vs. 95% vs. 94% (HCV-2,3, tx duration 16 wks)

Sarrazin et al., *J Hepatol* 2010;52:832-8

Analysis of the limit of detection (LOD)

Limit of detection for real-time HCV

IU/ml	n	GT1 pos	GT2 pos	GT3 pos	GT4 pos	GT5 pos	GT6 pos
50	12	12	12	12	12	12	12
25	12	12	12	12	12	12	12
12,5	12	12	12	12	12	12	12
6,25	12	12	12	12	10	12	12
3,125	12	8	9	8	10	10	10
LOD (IU/ml)		**5.4**	**5.2**	**5.4**	**8.9**	**4.7**	**4.7**

Limit of detection for CAP/CTM HCV

IU/ml	n	GT1 pos	GT2 pos	GT3 pos	GT4 pos	GT5 pos	GT6 pos
50	12	12	11	12	11	12	12
25	12	11	12	12	12	12	12
12,5	12	12	10	11	8	11	12
6,25	12	12	11	10	5	12	11
3,125	12	11	8	9	4	5	10
LOD (IU/ml)		**3.4**	**44.4**	**14.1**	**40.5**	**11.1**	**7.0**

Vermehren et al., *J Clin Microbiol* 2008;46:3880-91

Positive and negative prediction of SVR at weeks 2 and 4 of SOC

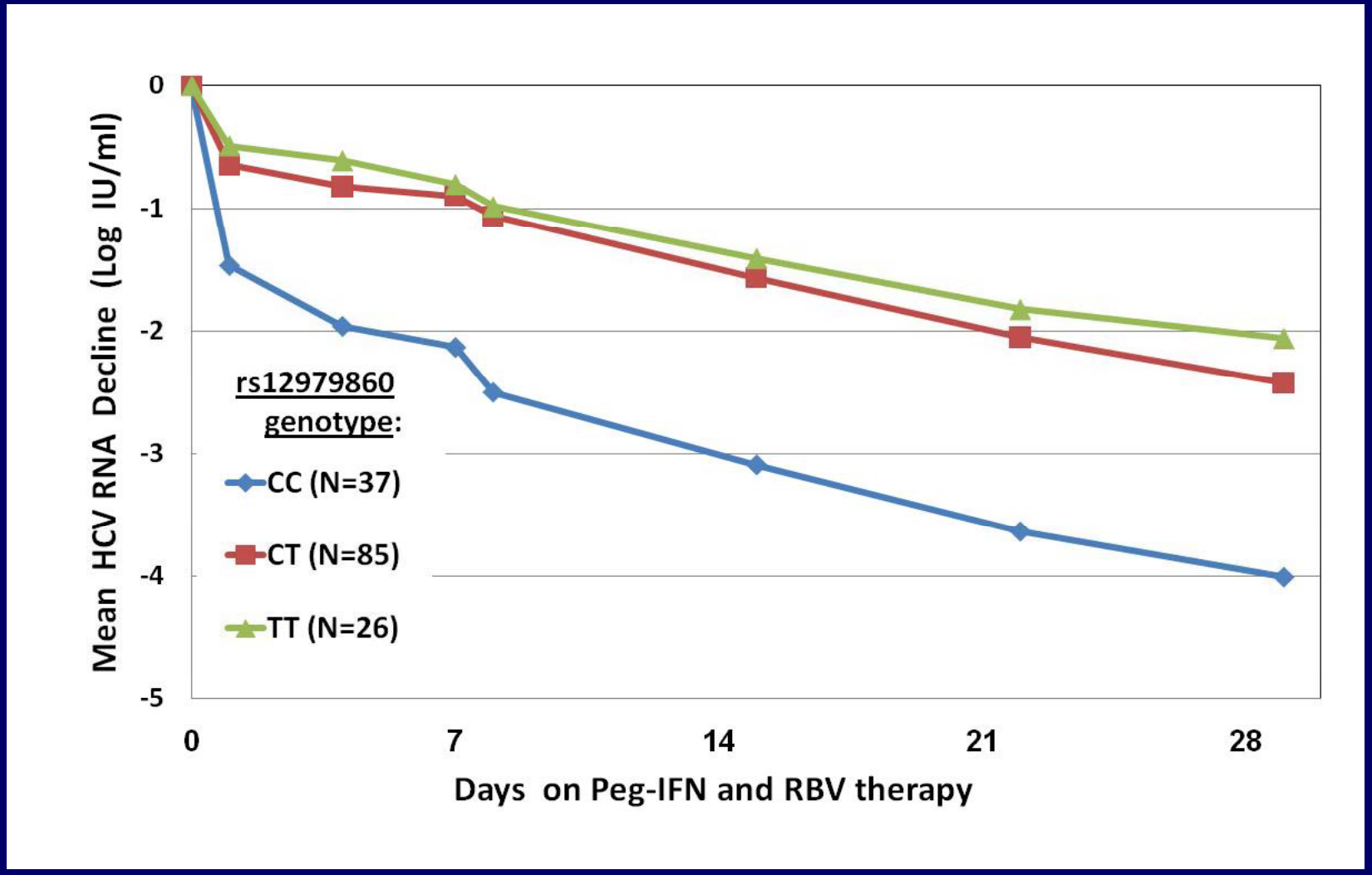
- Treatment-naive patients with GT1 chronic hepatitis C
- Treatment with albinterferon alfa-2b or peginterferon alfa-2a (ACHIEVE 1 trial)
- HCV RNA neg at wk 2: PPV 100%
- HCV RNA > 2log at wk 2: PPV 88-97%
- VL > 6log at wk 2: NPV 82-100%
- VL > 5.5log and HCV RNA decline < 2log at wk 4: NPV 100% (4-13% of pts., specificity 12-29%)

Neumann et al., *J Hepatol* 2009;51:21-28

VIROLOGIC TOOLS IN COMBINATION WITH IL28B IN THE ERA OF PEG-IFN AND RBV

HCV Decline by IL28B SNP Genotype

Patients infected with HCV-1



AU Neumann, S Bibert, B Haagmans, A Soulier, F Negro, M Lagging, C Ferrari, S Zeuzem, J-M Pawlotsky, SW Schalm, P-Y Bochud for the DITTO-HCV group. EASL 2010, Late-breaker poster

Viral Kinetics by IL28B SNP

HCV genotype 1 Caucasian patients

rs12979860 Genotype	N	Baseline HCV-RNA (log IU/ml)	1st phase decline (log IU/ml)	2nd phase slope (log IU/ml/wk)	%RVR
CC	37	6.4	2.03	0.72	32%
CT	85	6.1	0.91	0.56	16%
TT	26	6.0	0.70	0.44	12%
Difference CC vs CT+TT		p<0.01	p<0.0001	p<0.001	p<0.02

AU Neumann, S Bibert, B Haagmans, A Soulier, F Negro, M Lagging, C Ferrari, S Zeuzem, J-M Pawlotsky, SW Schalm, P-Y Bochud for the DITTO-HCV group. EASL 2010, Late-breaker poster

Differences Between IL28B Genotypes in RVR and in 2nd Phase Slope are Driven by the Effect of IL28B SNP on 1st Phase Decline

Patients with 1st phase decline < 1.0 log

rs12979860 genotype	N	2nd phase slope	%RVR
CC	2	0.54-	1/2
CT	50	0.48-	10%
TT	18	0.36-	0%
Difference CC vs CT+TT		NS	NS

Patients with 1st phase decline > 1.0 log

rs12979860 genotype	N	2nd phase slope	%RVR
CC	35	0.73-	31%
CT	35	0.66-	26%
TT	8	0.60-	38%
Difference CC vs CT+TT		NS	NS

Predictive value of IL28B and VK

- CC IL-28B type (rs12979860) is associated with a greater likelihood of RVR (28% vs 5% and 5%; P<0.0001), cEVR (87% vs 38% and 28%; P<0.0001), and SVR (69% vs 33% and 27%; P < .0001) compared with CT and TT.
- CC IL-28B type is the strongest pretreatment predictor of SVR (OR 5.2; 95% CI, 4.1-6.7).
- RVR was a strong predictor of SVR regardless of IL-28B type.
- In non-RVR patients, the CC IL-28B type is associated with a higher rate of SVR (Caucasians, 66% vs 31% and 24%; P < .0001).

Thompson et al., *Gastroenterology* 2010;139:120-9

VIROLOGIC TOOLS IN THE ERA OF PEG-IFN AND RBV PLUS DIRECT ANTIVIRAL AGENTS

Basic Characteristics of Direct Antiviral Agents (DAA)

	Efficacy	Genotype dependency	Barrier to resistance
NS3A (protease inhibitors)	+++	+	+ - ++
NS5A	+++	+ - ++	+ - ++
NS5B (nucleosides)	+ - ++	+++	+++
NS5B (non-nucleosides)	+ - ++	+	+

HCV Genotyping

- Several DAAs (in particular NNI) have different antiviral activity in HCV subtypes (i.e. HCV-1a vs. 1b)
- DAAs may have lower activity in rare geno/subtypes (which are mistyped by assays)
- HCV variants may have different sub/genotypes in different genes (chimera)

On-treatment response definitions

- RVR: rapid virologic response = undetectable HCV RNA at week 4
- pEVR: partial early virologic response = more than 2log decline at week 12
- cEVR: complete early virologic response = HCV RNA undetectable at week 12
- eRVR: extended rapid virologic response = undetectable HCV RNA at week 4 and 12 or week 4-20

Emergence of resistance according to different HCV subtypes

- Different number of nucleotide changes may be required to create a single amino acid change which is associated with a lower susceptibility to a HCV protease inhibitor

- HCV-1a **AGG → AAG**
  (R155) (R155K)

- HCV-1b **CGG → AGG** (R155) (R155) **AGG → AAG** (R155) (R155K)

Emergence of double variants according to different HCV subtypes

- The double variant V36M + R155K is associated with a markedly lower susceptibility to telaprevir and other HCV PIs
- HCV-1a: 2 steps required
  (clinically observed)
- HCV-1b: 4 steps required
  (not yet clinically observed)

Changes In Drug Susceptibility: Detection Of Resistance

- **Sequence Analysis**
  Detects specific mutations that are known to decrease susceptibility to antiviral agents. Requires prior knowledge of these mutations and their individual or combined impact on drug susceptibility.
- **Phenotypic Analysis**
  Determines drug concentrations needed to inhibit viral replication. Inhibitory concentration (IC): drug concentration required to inhibit viral replication by 50% or 90% (IC50 or IC90). Less susceptible (resistant) viruses will require *more* drug to be inhibited, thus an *increase* in IC50 or IC90.

Conclusions

- Optimal antiviral treatment depends on HCV genotyping, HCV RNA quantification, and sensitive detection of HCV RNA
- Currently available assays are good, but not yet optimal
- Further improvements may become important in the era of treatment with DAAs
  - Target-specific genotyping
  - HCV subtyping
  - Standardized and clinically validated HCV RNA cut-off levels for eRVR definitions
  - Resistance testing (genotypic, phenotypic)