
2009
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PARIS
HEPATITIS
CONFERENCE
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Faculty

New endpoints.
Back to the future with Australia Antigen

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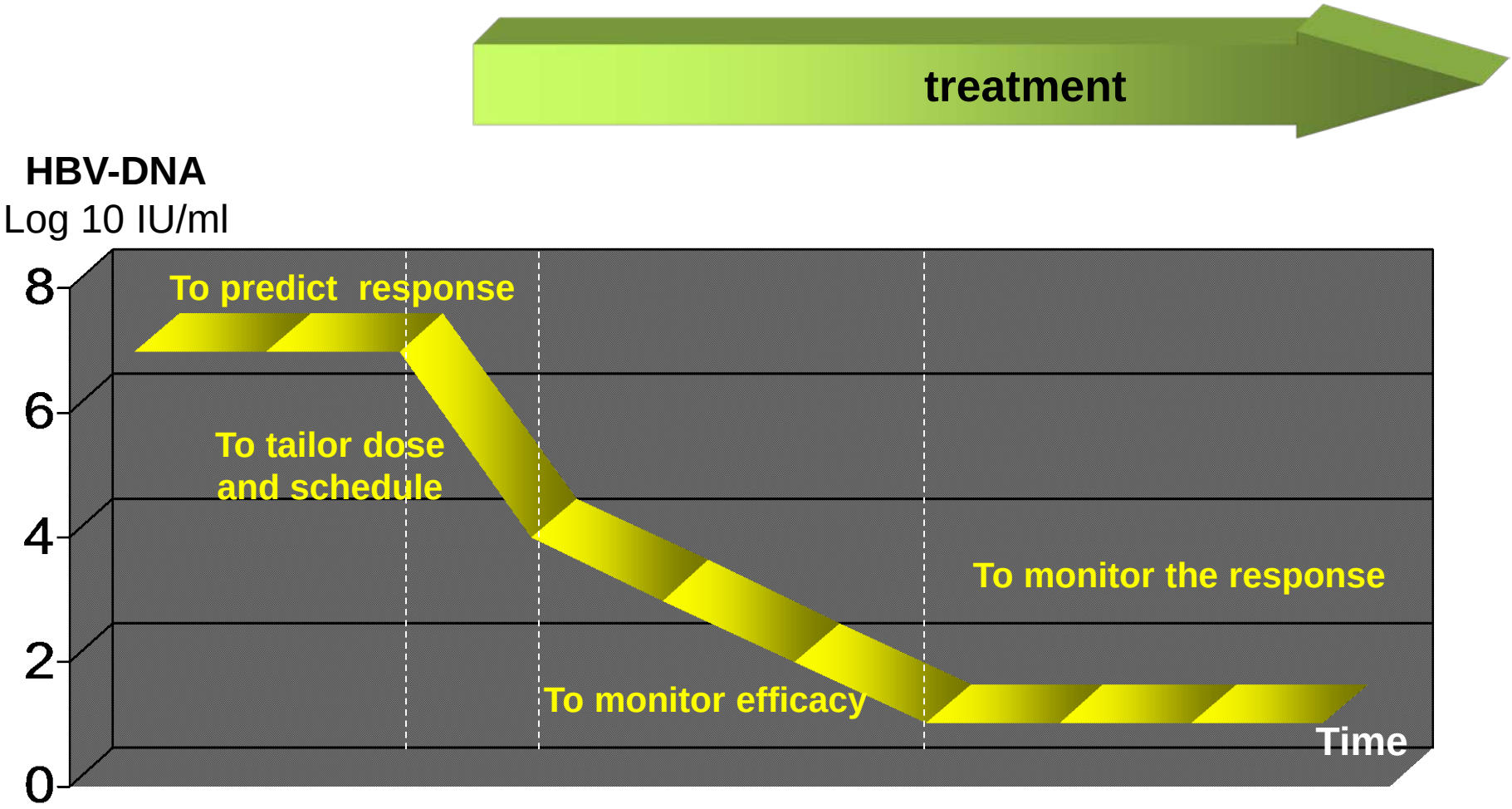
HBV-DNA monitoring during antiviral therapy



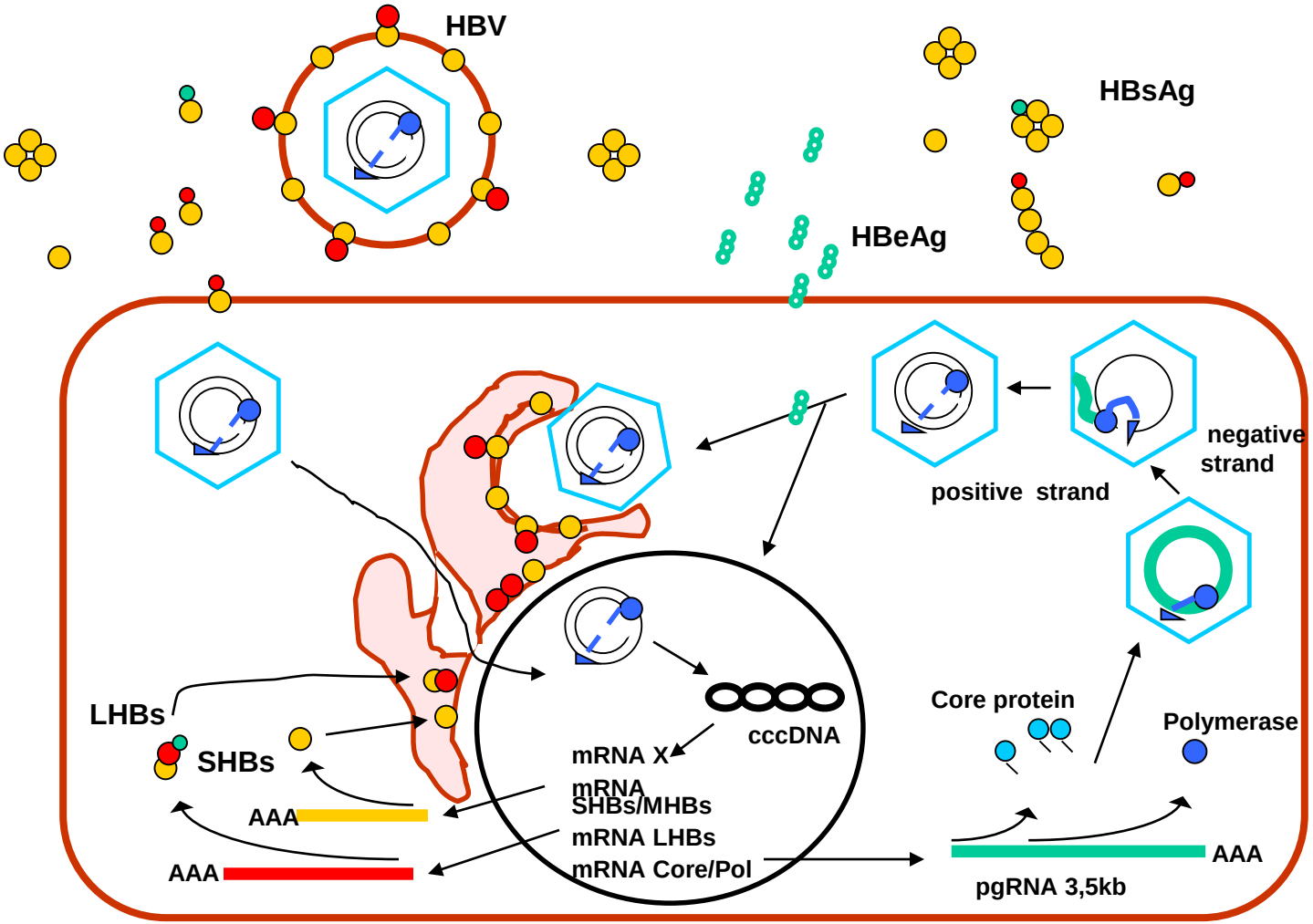
To identify patients to be treated

Undetectability of HBV-DNA even with highly sensitive assays does not mean absence of circulating virus and/or clearance of viral infection

Time point evaluations of viral load are used as markers of antiviral treatment effectiveness and persistence of response

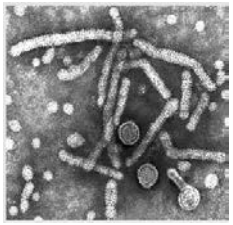
But, at the moment we are missing markers identifying the achievement of an efficacious HBV infection control by the host's immune system

Hepatitis B virus infection and HBsAg production



***Serum HBsAg*:**

Virions	42 nm
Filaments	22 nm
Spherical particles	22nm



Defective particles exceed virions by a factor of 10^3-10^5

HBsAg measuring

Qualitative screening assay for HBsAg universally available

Commercial tests

- Elecsys II – Roche
- Architect – Abbott
- ADVIA Centaur HBsAg Assay – Bayer
- Hepanostika HBsAg – Biomerieux

In-house assays widely used in some countries

Quantitative HBsAg measurement with some assays after dilution

Architect HBsAg (QT)

- **Dynamic Range: 0 – 250.0 IU/ml (WHO)**
- **Specimens with values exceeding 250 IU/ml are flagged and may be diluted with the Manual Dilution Procedure.**
- **Operator enters the dilution factor in the Patient or Control order screen. System uses this dilution factor to automatically calculate the concentration of the sample before dilution and report the result.**
- **Dual epitope capture format, detects all known HBsAg mutants**

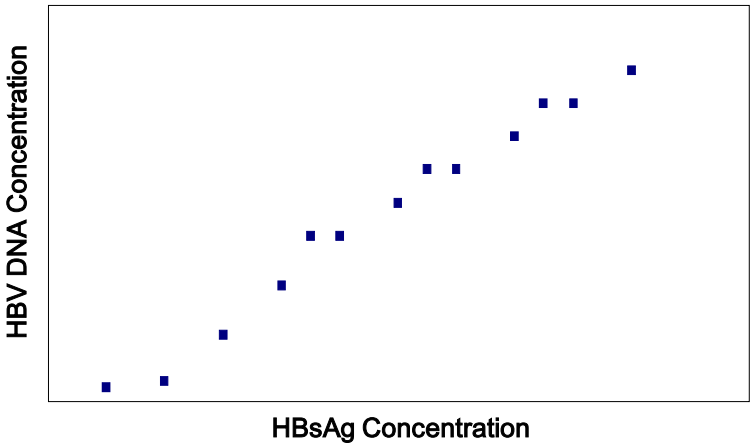
Quantitative HBsAg Assays

Sample factors that may impact HBsAg quantification

- anti-HBs presence, titer, epitope recognized (specific interaction)
- rheumatoid factor, HAMA or non-specif binding factors (low affinity)
- lipid levels or particulate matter (physical sequestering)
- sample integrity/HBsAg stability issues
- HBV mutant or variant

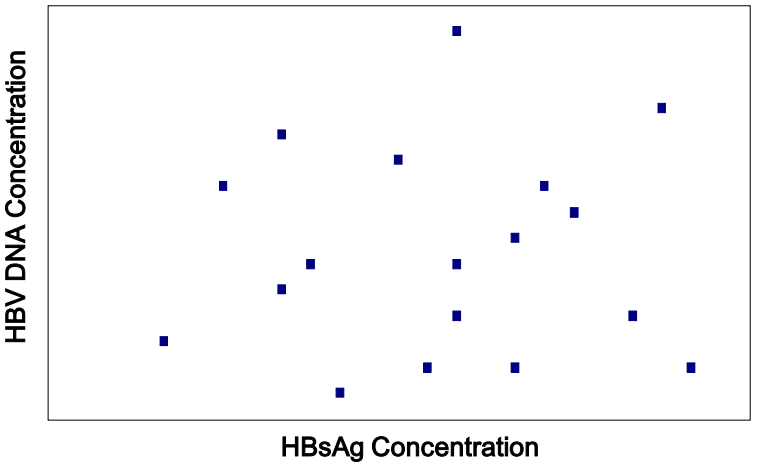
Relationship between HBV DNA and HBsAg concentrations according to Stage of Infection

Early Acute



Good correlation in the rump-up phase

Chronic

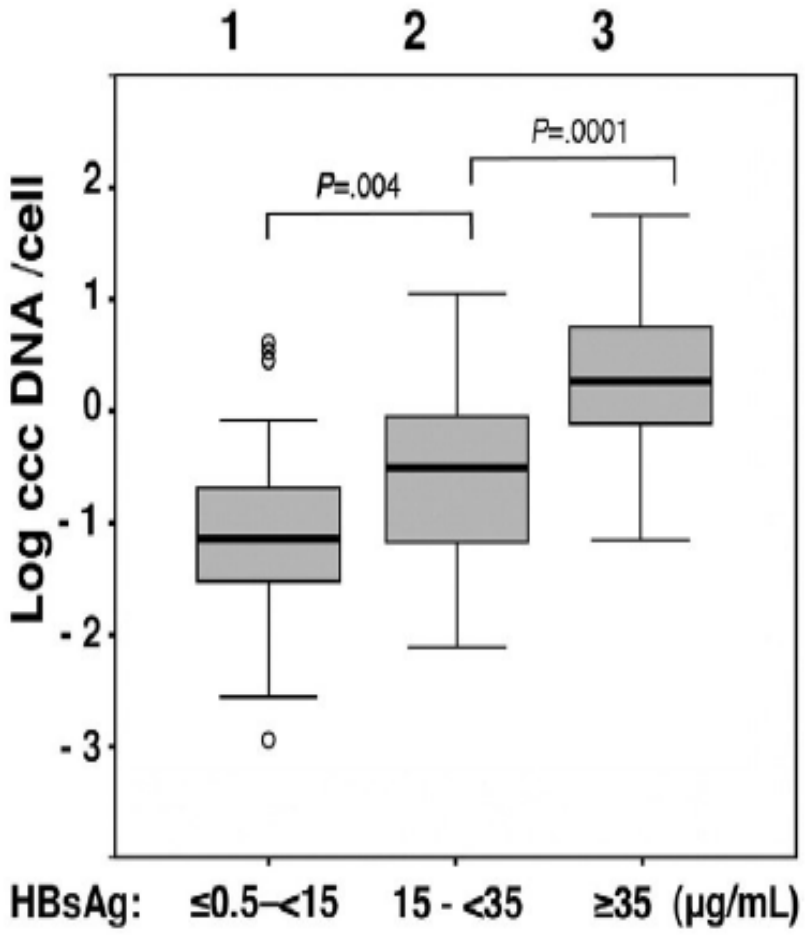


The concentration of serum HBsAg does not correlate with levels of serum HBV-DNA

Kuhns and Busch, Mol Diag Ther 2006;10(2):77-91.

Impaired Intrahepatic Hepatitis B Virus Productivity Contributes to Low Viremia in Most HBeAg-Negative Patients

T.Volz et al. GASTROENTEROLOGY 2007;133:843–852



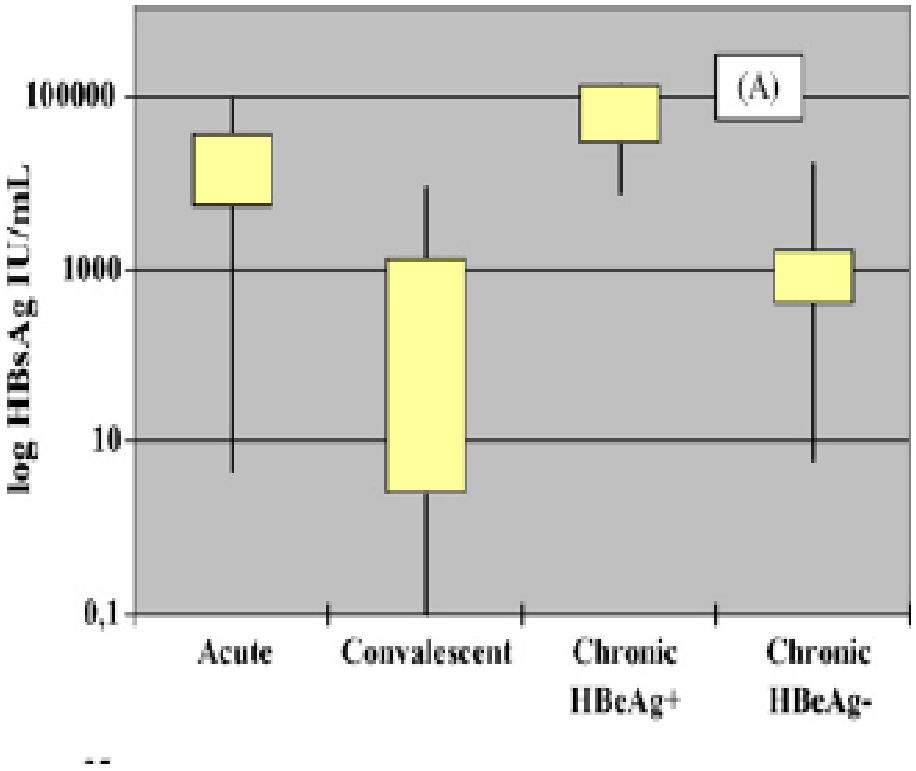
Relationship between HBsAg serum levels (by the Laurell test) and intrahepatic cccDNA amounts (median values 0.07, 0.3 and 1.8 cccDNA copies/cell)

Differences between groups were highly significant by Mann-Whitney test

Low serum HBsAg levels correlate with low intrahepatic cccDNA amounts

Quantitative analysis of HBsAg, IgM anti-HBc and anti-HBc avidity in acute and chronic hepatitis B

Rodella A et al Journal of Clinical Virology 37 (2006) 206–212



Acute phase samples:
Mean value 25,767 IU/ml (5-90,575)
Recovery phase samples:
Mean value 1351 IU/ml (0.05-8495)

HBeAg positive samples:
Mean value 78,756 IU/ml
Exceeding 10,000 IU/ml 90%
HBeAg negative samples:
Mean value 2192 IU/ml
Exceeding 10,000 IU/ml 3.12%

Impaired Intrahepatic Hepatitis B Virus Productivity Contributes to Low Viremia in Most HBeAg-Negative Patients

T.Volz et al. GASTROENTEROLOGY 2007;133:843–852

Table 1. Comparison of Patients With or Without Detectable HBeAg

	HBeAg+	HBeAg−	*P*
Male/Total	31/42	58/77	ns
Age (*years*)	32.5 (19–64)	40 (20–63)	$P = .0001$
Serum			
HBV-DNA (*copies/mL*)	4.5×10^7 ($<10^2$–4×10^9)	7×10^3 ($<10^2$–4×10^8)	$P < .0001$
ALT (ULN)	2.1 (0.4–15.8)	1.5 (0.3–13)	$P = .064$
HBsAg (*μg/mL*)	41 (<0.5–324)	7.6 (<0.5–62)	$P < .0001$
Intrahepatic			
Total HBV-DNA (copies/cell)	95 ($0.6–2\times10^3$)	0.72 (0.036–73)	$P < .0001$
cccDNA (copies/cell)	1.8 (0.008–54)	0.09 (0.001–15)	$P < .0001$
cccDNA/Tot.HBV-DNA	2% (0.09–44%)	10.4% (0.05–100%)	$P < .0001$
HBsAg+ staining (% of cells)	30% (0–95)	10% (0–80)	$P = .001$
HBcAg+ staining (% of cells)	5% (0–90)	0% (0–20)	$P < .0001$
Grading (Desmet)	2 (1–3)	1 (0–4)	ns (0.91)
Staging (Desmet)	1 (0–4)	1 (0–4)	ns (0.35)

Does serum HBsAg quantitative measurement represent a new tool to monitor and guide antiviral therapy in CHB patients?

Quantitative HBsAg analysis in HBeAg-negative CHB enrolled in the PEGASYS study

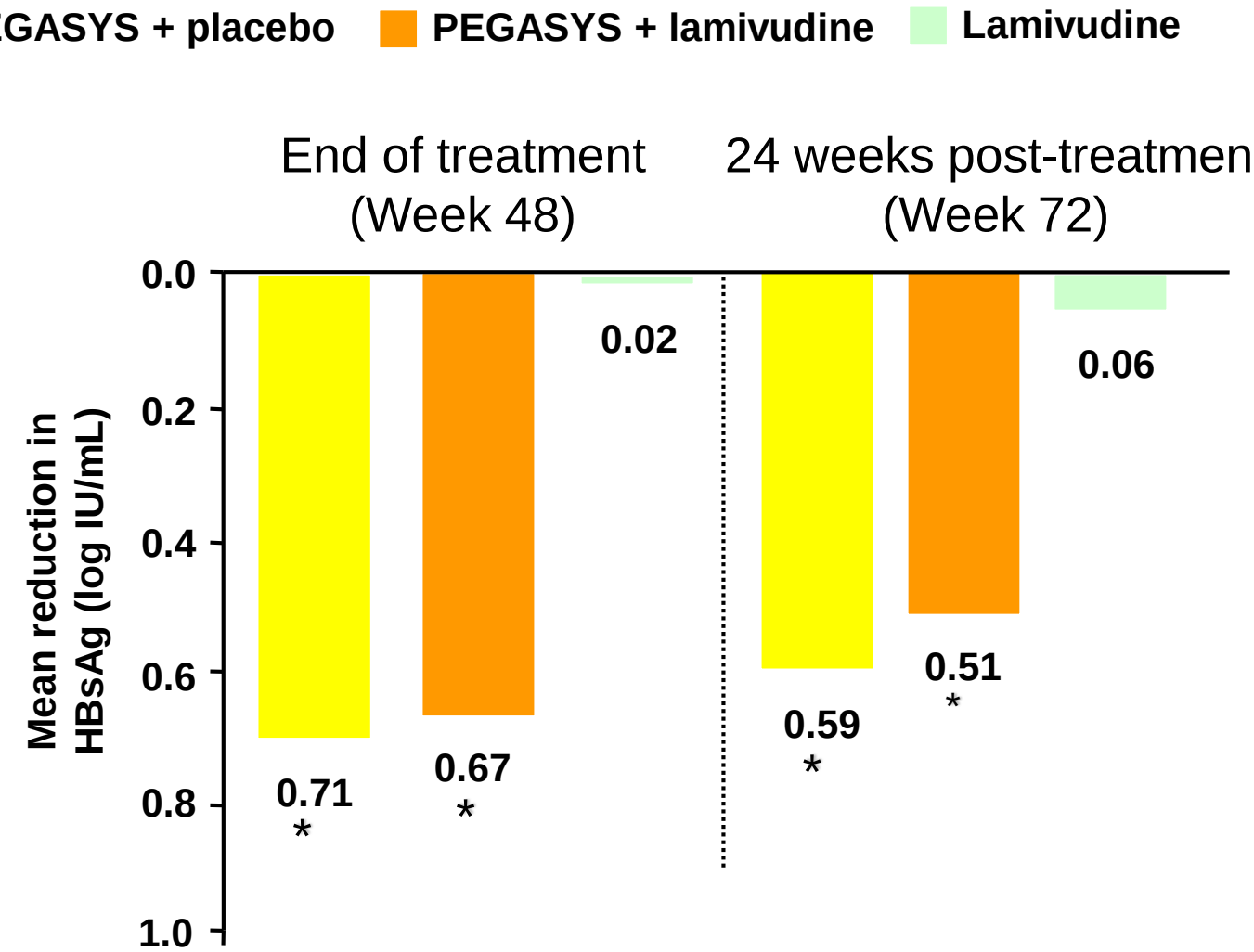
HBsAg levels were determined in a subset of patients with available sera:

- Prior to treatment, on treatment and during follow-up
- Using the Architect HBsAg assay (Abbott Laboratories; range 0.05–250.0 IU/mL) after 1:100 dilution

	PEGASYS 180 µg qw + placebo	PEGASYS 180 µg qw + LAM 100 mg qd	LAM 100 mg qd
Original study population, n	177	179	181
qHBsAg sub-population, n	**127**	**137**	**122**
Long-term roll over study 3 years , n	116	114	85
qHBsAg sub-population in roll over study, n	**97**	**101**	**–**

Brunetto et al., Hepatology in press

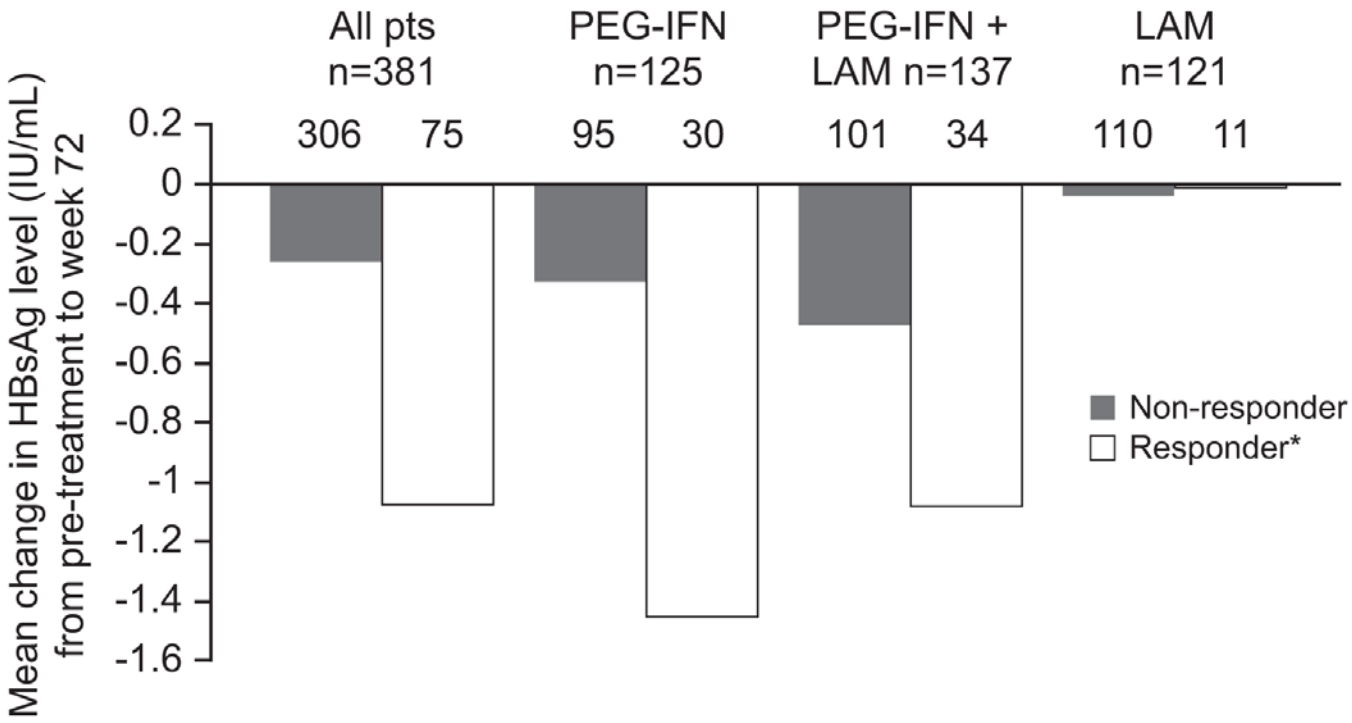
HBsAg level reduction compared with baseline



* P<0.01 vs baseline

Brunetto et al., AASLD 2006 and Hepatology in press

HBsAg levels decline from pre-treatment to EOT according to virological response at 6 months



*__HBV DNA <400 copies/ mL__ 6 months post -treatment

Brunetto M et al, Hepatology in press

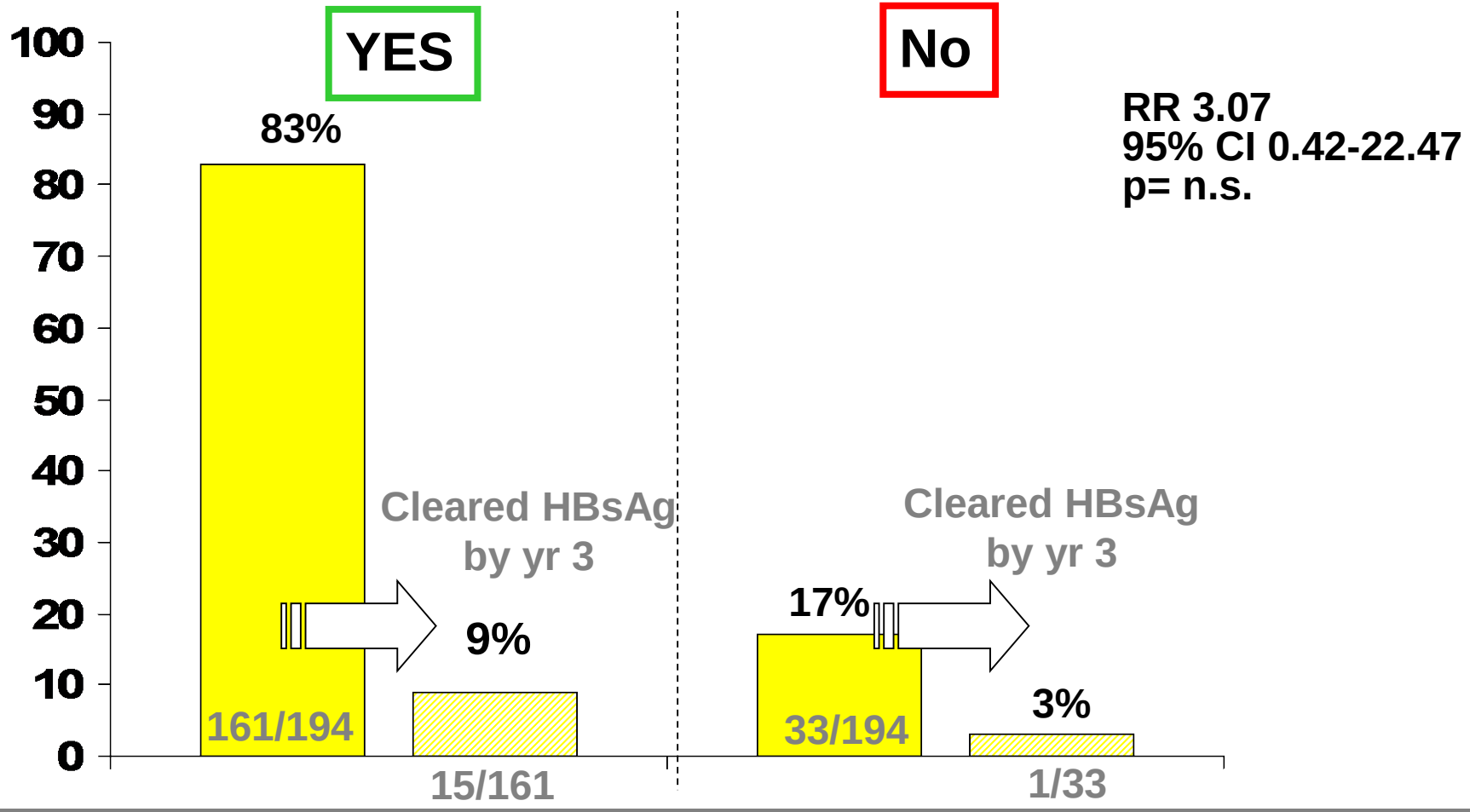
Identification of Predictors of HBsAg clearance 3 years post-treatment by Logistic regression analysis

	OR*	95% CI value	P value
Age	1.09	0.99–1.20	ns
PEG vs PEG + LAM	0.38	0.06–2.53	ns
BL ALT	1.00	0.99–1.01	ns
BL log HBV DNA	1.10	0.66–1.85	ns
Wk 48 log HBV DNA	1.58	0.37 –6.77	ns
Wk 48 log HBsAg	0.12	0.04–0.37	0.0002
Change in log HBsAg from BL to wk 48	0.22	0.10– 0.50	0.0003

For n=65 (n=64) patients with available data and HBsAg loss at 3 years post-treatment

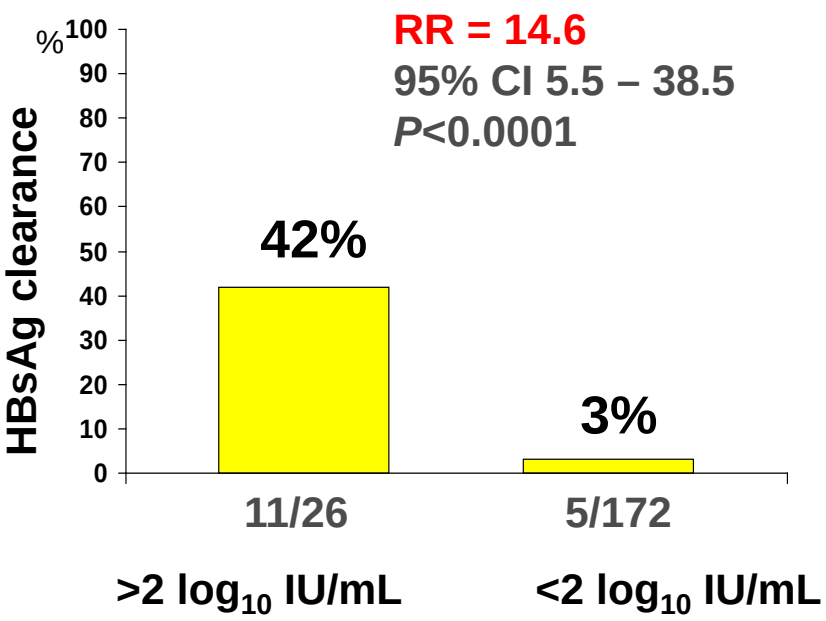
No correlation between HBV DNA <400 cp/mL at EOT year 3 sustained HBsAg clearance

HBV DNA <400 cp/mL at week 48 (EOT)

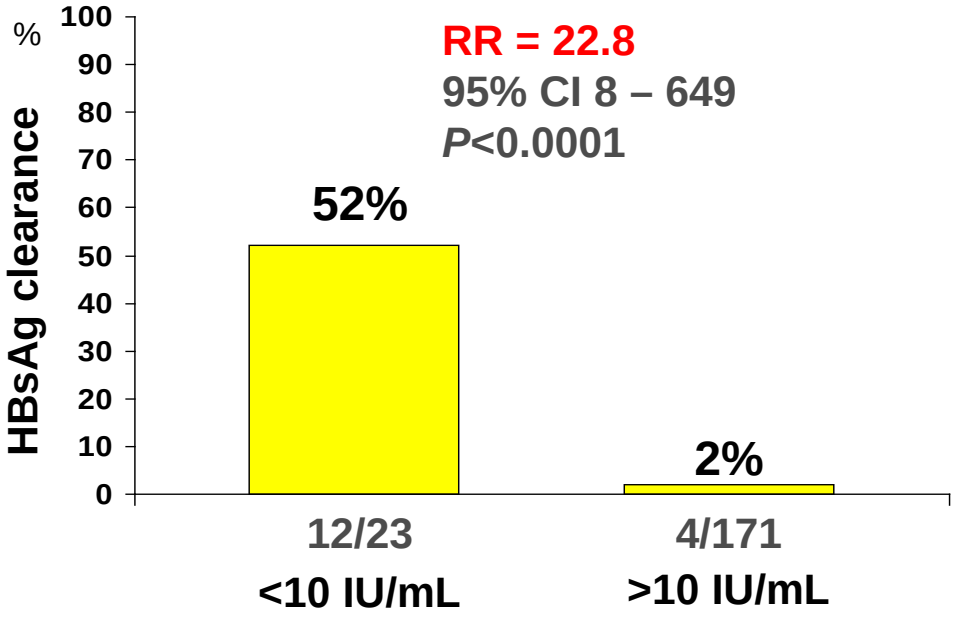


HBV DNA suppression is required, but is not sufficient, for HBsAg clearance

Predictive value of HBsAg reduction at week 48 for sustained HBsAg clearance by year 3



HBsAg reduction from BL to week 48



HBsAg level at week 48

These findings, together with viral dynamics studies,[1] suggest that PEGASYS, even without the high level inhibition of HBV replication shown by NAs, has a higher impact on the clearance of the infected cells, as a consequence of its immune-modulation activity

1. Colombatto et al, ATV 2006

Parameters	ρ	ω	$Log10\Psi_{asy}/\Psi_0$	δ_0	$I_{t/2}$ (days)	I_0/H (%)	k	$Log_{10} I_{eot}/I_0$
PegIFN	0.120	0.73	1.14	0.10	10.4	19	0.32	-3.31

ρ fraction of residual viral production (it describes the antiviral effect of IFN)

ω inverse of the time constant for viral production decline

Parameters	ε	λ	$V_{t/2}$ (hours)	ϕ	$Log10\Psi_{asy}/\Psi_0$	δ_0	$I_{t/2}$ (days)	I_0/H (%)	k	$Log_{10} I_{eot}/I_0$
Lam	0.88	1.91	9.5	0.34	2.22	0.077	12.88	20	0.36	-3.31
Lam + Peg	0.87	2.79	8.24	0.44	2.36	0.091	9.76	21	0.28	-5.02

On treatment kinetics of HBsAg serum levels in HBeAg negative CHB to predict SVR

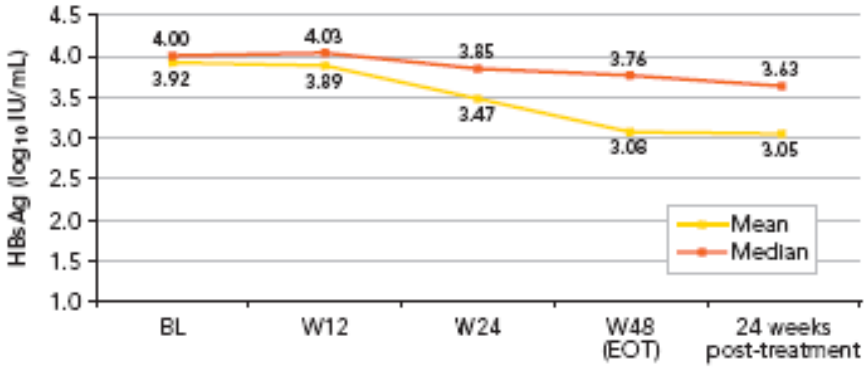
		SVR	PPV
Week 12 ↓ HBsAg ≥ 0.5 Log IU/mL	9 pts	8	89%
Week 24 ↓ HBsAg ≥ 1 Log IU/mL	12 pts	11	92%

		no SVR	NPV
Week 12 ↓ HBsAg < 0.5 Log IU/mL	39 pts	35	90%
Week 24 ↓ HBsAg < 1 Log IU/mL	36 pts	35	97%

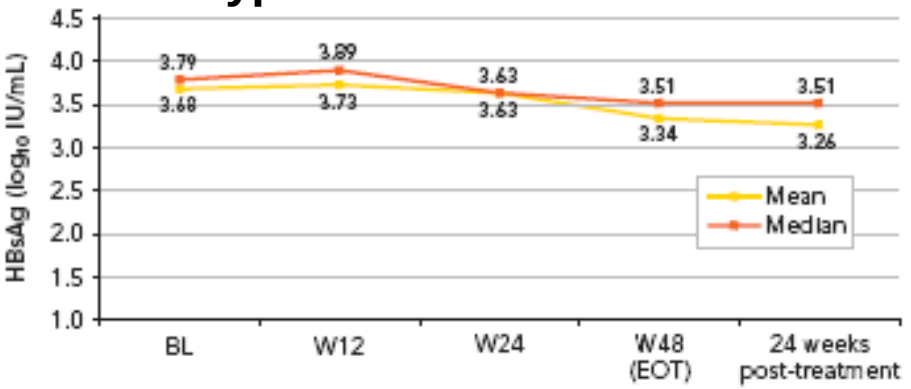
Moucari R. et , AASLD 2008

HBsAg decline over time according to genotype

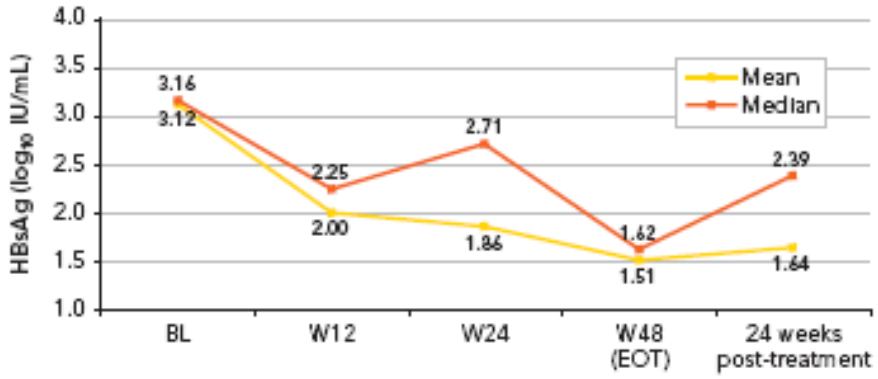
Genotype A n=16



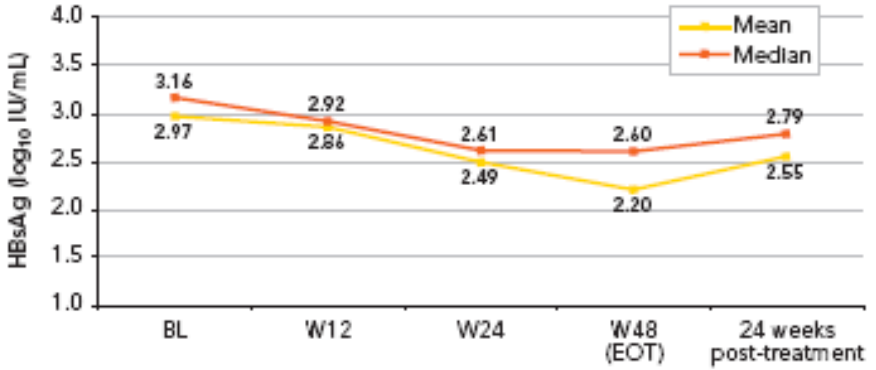
Genotype D n=38



Genotype B n=7

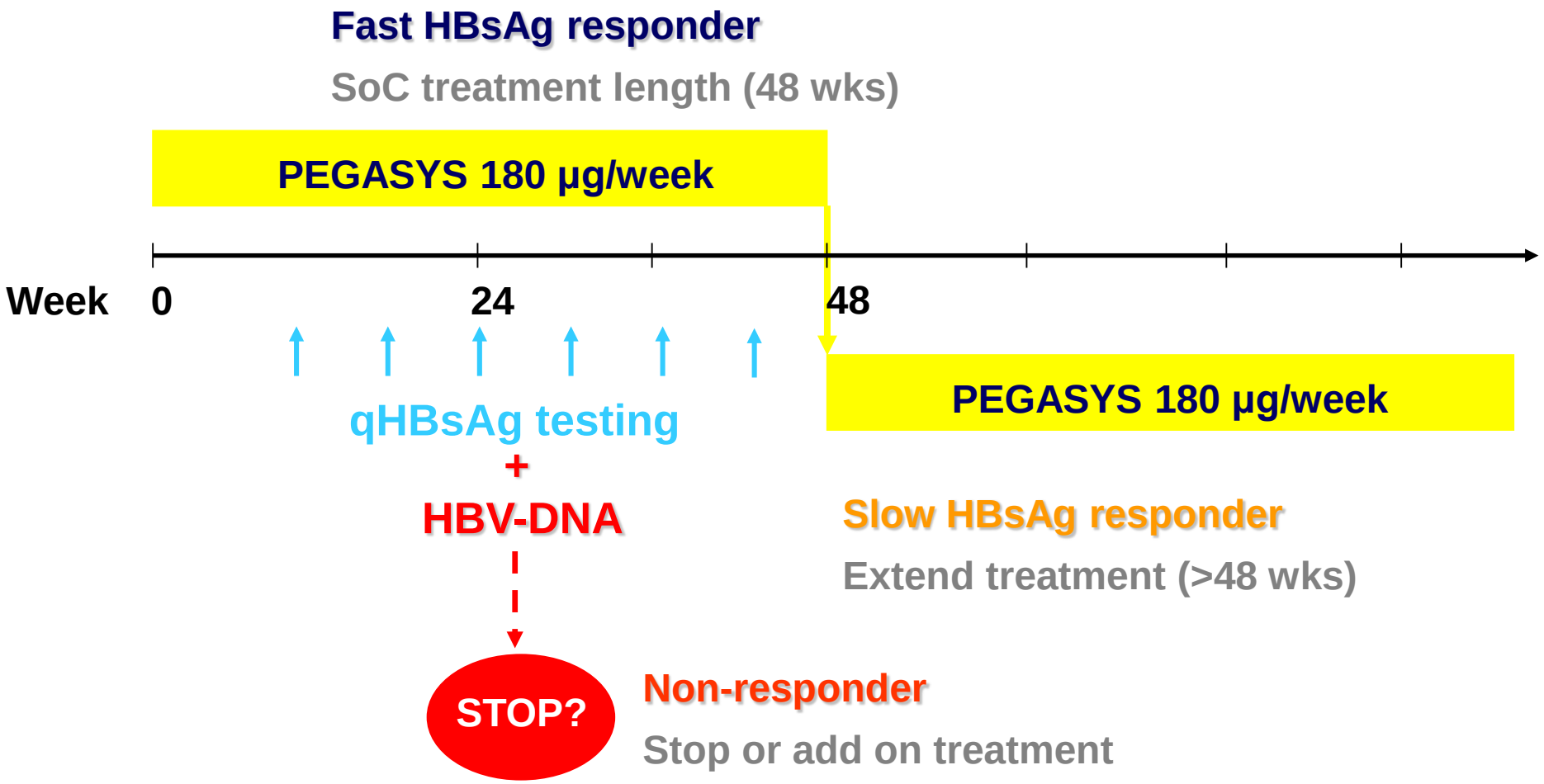


Genotype C n=20



Brunetto MR et al, AASLD 2008

Future perspective: quHBsAg as a way to optimise patient management?



Brunetto et al. *EASL* 2008 Poster

HBsAg loss with NAs

- HBsAg loss following short-term NA therapy is rare
  - ETV study 2% in HBeAg-positive CHB
- Longer treatment with NAs – up to 4 or 5 years – doesn't appear to improve this
  - Suggests immunological control is not achieved
  - Need more than potent HBV DNA suppression to achieve HBsAg loss
- However, there are recent reports of HBsAg loss with Tenofovir and Clevudine

Prediction of treatment-related HBsAg loss in HBeAg-negative chronic hepatitis B: a clue from serum HBsAg levels

Serum HBsAg quantified using ADVIA Centaur® (Bayer)

Rate of HBsAg decrease was significantly higher in IFN-treated patients compared with LAM-treated patients (p=0.022)

- **IFN** responder/relapsers + IFN sustained responders: **median 155 IU/month**
- **LAM** responders: **median 7.7 IU/month**

Median estimated time to HBsAg undetectability

- **IFN** 65.3 (36.3–95.0) months (**5.4 years**)
- **LAM** 127 (87.6–263.5) months (**10.6 years**)

Manesis EK et al, Antiviral Therapy 2007

Serum HBsAg is the closest meaning we have to a ‘cure’ of Chronic Hepatitis B

- HBsAg serum levels appear to be correlated with intrahepatic cccDNA levels, that are a marker of HBV infected cells

- Reduction of infected hepatocytes is the hallmark of stronger control of HBV infection, that is a pre-requisite to achieve a off therapy sustained response

qs HBsAg serum levels monitoring could become the best tool to tailor antiviral therapy

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In chronic hepatitis B HBsAg levels correlate with:

1. **HBV-DNA serum levels**

2. **ALT levels**

3. **Intrahepatic cccDNA levels**

The kinetics of serum HBsAg during antiviral treatment appear to be the most accurate marker of:

1. **Drug effectiveness**

2. **Viral suppression**

3. **Capacity to achieve a sustained control of the infection (HBsAg clearance)**