Understanding HBV Testing: HBsAg, HBV RNA, cccDNA, HBeAg and HBcrAg in Context of Antiviral Drug Development

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Disclosure

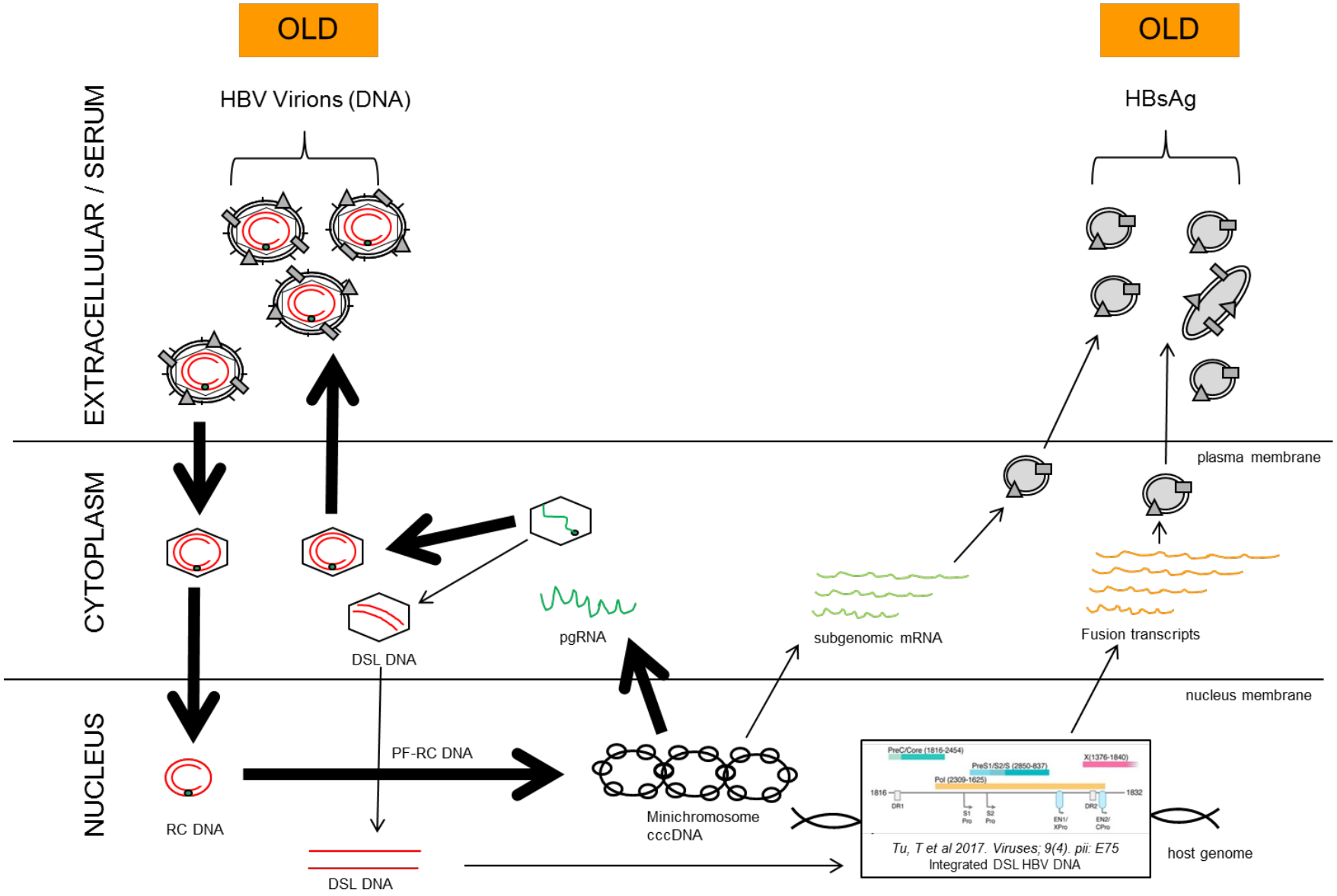
	Gilead Sciences Inc	Arrowhead Research Corp	Spring Bank Pharmaceuticals, Inc.	Roche Molecular	AusBio Ltd	Janssen (J&J)
Consulting Fees (eg. Advisory Boards)	yes	yes		yes	yes	yes
Contract Research (grant)	yes	yes	yes			

Viral Biomarker Scenarios: DAAs and Cytokines

Serum Marker	Possible Interpretation*
HBV DNA	• priming RT [ETV vs TDF] • RT [first-strand] • DNA polymerase [second-strand] • priming RT • Pol-5´-ε binding/encapsidation
HBV RNA: •pgRNA [full length]	• Pol-5´ε binding • priming RT • encapsidation inhibition • nucleocapsid assembly inhibition • cccDNA dependent
Other [Truncated]	• ? splice HBV RNAs • ? chimeric HBV RNAs
HBeAg	• precore mRNA [cccDNA dependent]
HBcrAg [HBcAg; HBeAg; p22cr]	• pregenomic RNA [cccDNA dependent] • precore mRNA [cccDNA dependent] • cccDNA “activity”
HBsAg	• phase of CHB [set-points] • episomal HBV (ccc)DNA [HBeAg-POS] • integrated HBV DNA [HBeAg-NEG]

*** Substantial Overlap**

Biomarkers and MOA of DAAs



Old Concept

- **HBeAg-Pos and HBeAg-Neg Replication Same**
- **Very low level of HBV Integration**

Novel Findings ARC-520 Studies: Predominant Liver HBV DNA Differs in HBeAg Neg and HBeAg Pos Chimps

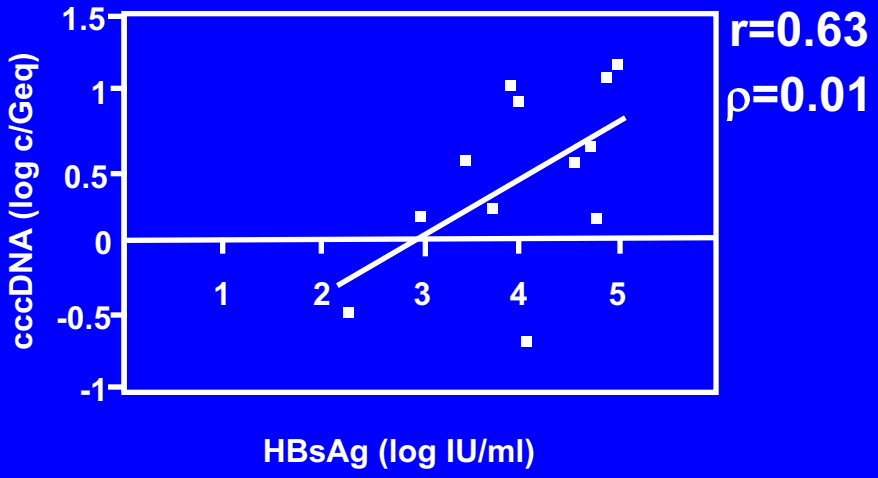
Liver biopsy at initiation of ARC-520 treatment revealed:

- Most HBV DNA in liver of HBeAg pos is cccDNA
- 500-fold less cccDNA in HBeAg neg
  - Only 5% of total HBV DNA in liver in HBeAg neg was cccDNA and total HBV DNA levels were not affected by NUCs
- HBV DNA profile in HBeAg neg chimps is consistent with a high proportion of integrated HBV DNA

Wooddell, C et al. 2017 Sci Transl Med;9(409). pii: eaan0241

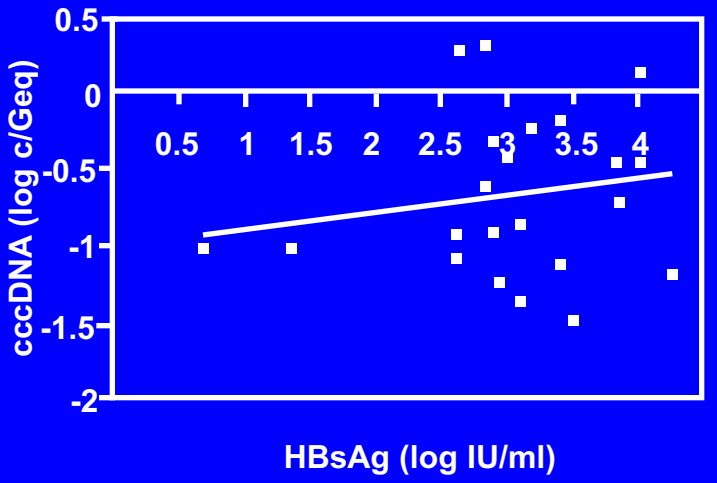
Changes in Serum HBsAg are Correlated with Changes in cccDNA Titer: HBeAg-pos vs neg

HBeAg-positive cohort



r=0.63
ρ=0.01

HBeAg-negative cohort

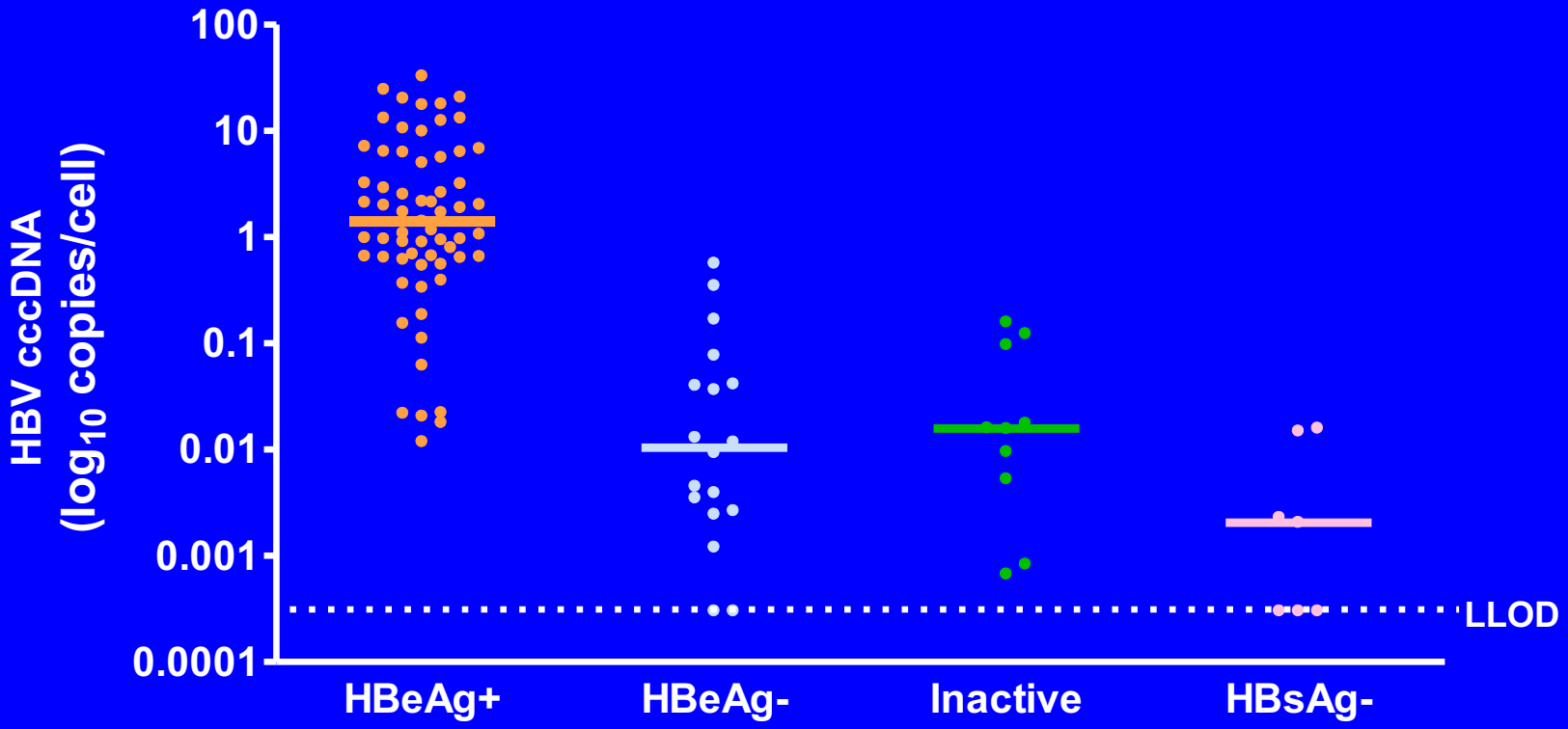


r=0.15
ρ=0.47

HBeAg-NEG different transcriptome than HBeAg-POS

Thompson, A. et al 2010. Hepatology;51(6):1933-1944

Hepatic HBV cccDNA Levels in Different Patient Populations

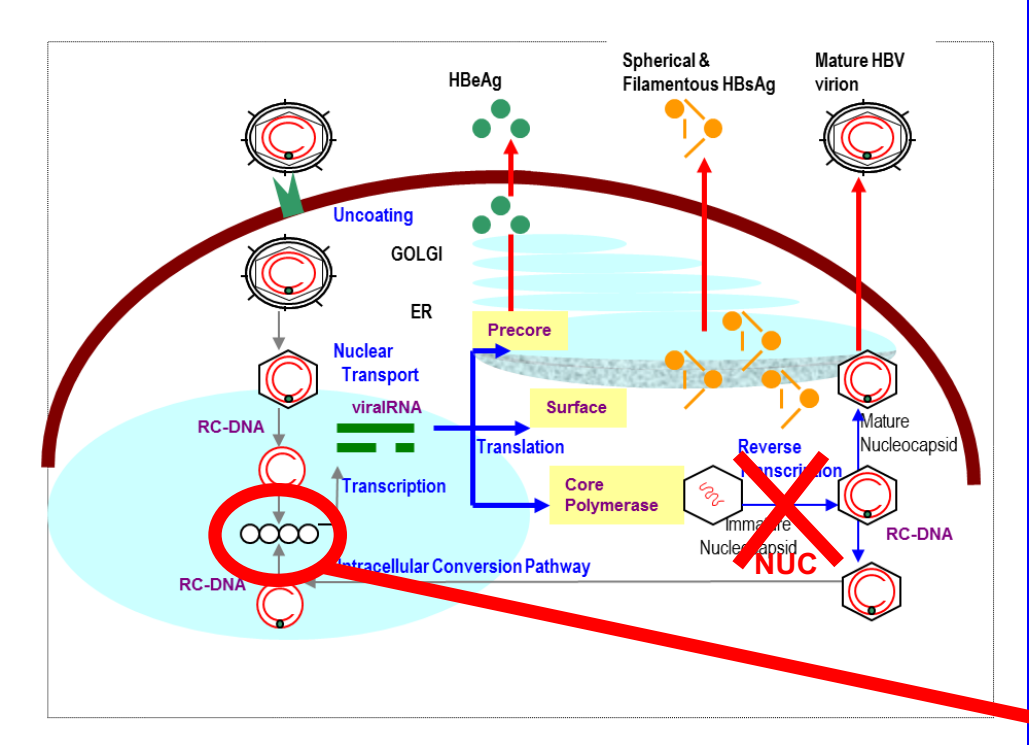


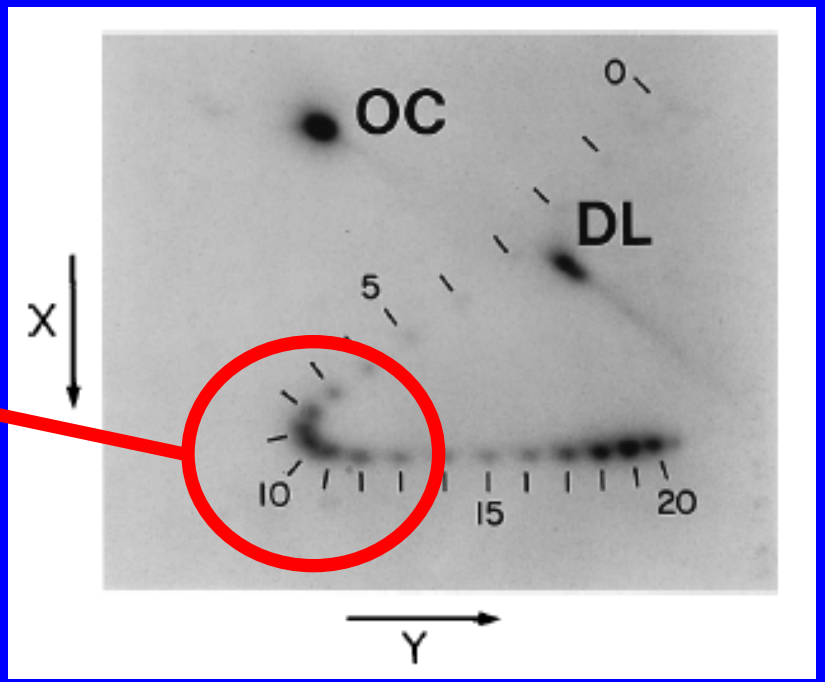
- cccDNA persists through all phases of the natural history of chronic hepatitis B
- PCR Measures **Level** of cccDNA NOT Activity
- **Copy number 0.1-10 cccDNA/hepatocyte**

(Werle-Lapostolle, B. et al 2004. Gastroenterology;126:1750

Partial Reduction of cccDNA by NUCs

Role of Intracellular Conversion Pathway





- **cccDNA = 21 Topoisomers**
  [NOT a single entity]
- **Difference in Transcriptional Activities**

Newbold, J. et al 1995. J.Virol;69:3350

cccDNA Transcriptional Activity

- ***Virion Productivity*** :

"the number of intrahepatic (IH) replicating HBV DNA molecules per cccDNA molecule" *(Volz T, et al 2007. Gastroenterol;133:843-52)*

$$\frac{\text{Total IH HBV DNA - cccDNA}}{\text{cccDNA}}$$

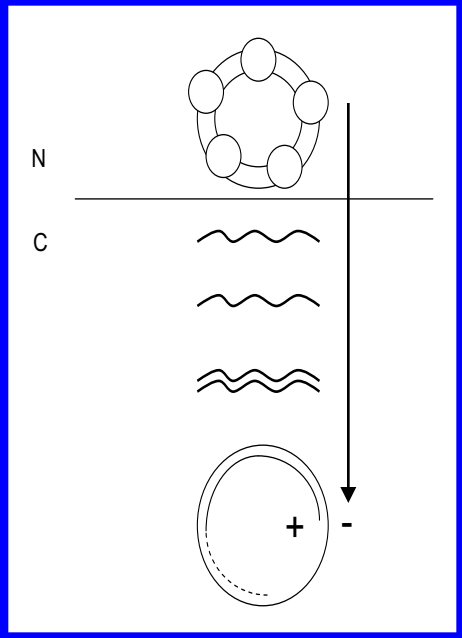
- ***Replicative Activity:***

Intrahepatic pgRNA : cccDNA assay
(Laras, A et al 2006. Hepatol;4:694-702)

- ***Epigenetic State:***

cccDNA acetylation assay *(Pollicino, T et al 2006. Gastroenterol;130:823))*

  - CHIP assay - HBV replication parallels the acetylation status of HBV cccDNA-bound H3 and H4 histones



cccDNA

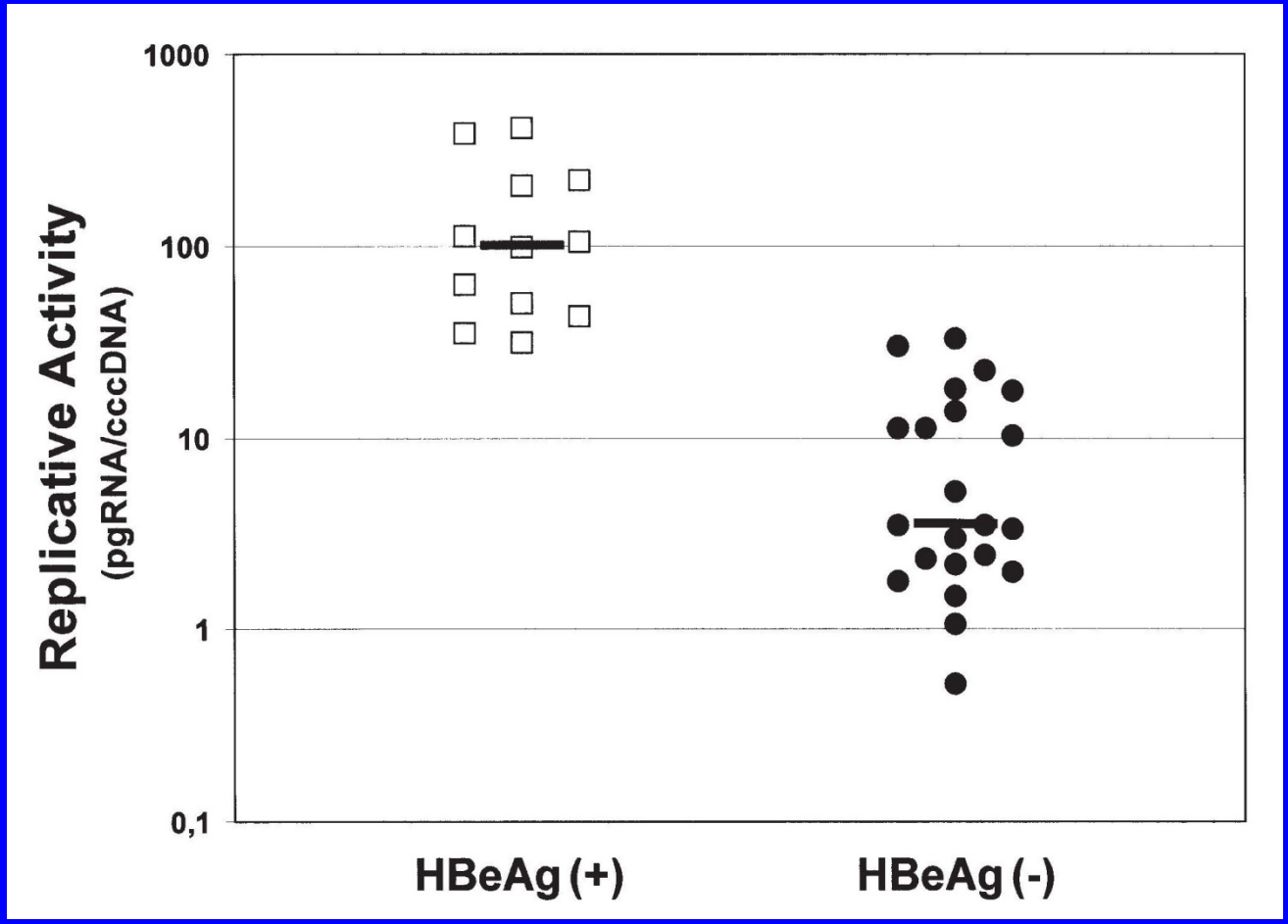
pgRNA

ssDNA (-)

dsDNA

rcDNA

HBV cccDNA Replicative Activity (pgRNA transcripts produced per cccDNA molecule) in HBeAg(+) (open squares) and HBeAg(-) (closed circles) patients

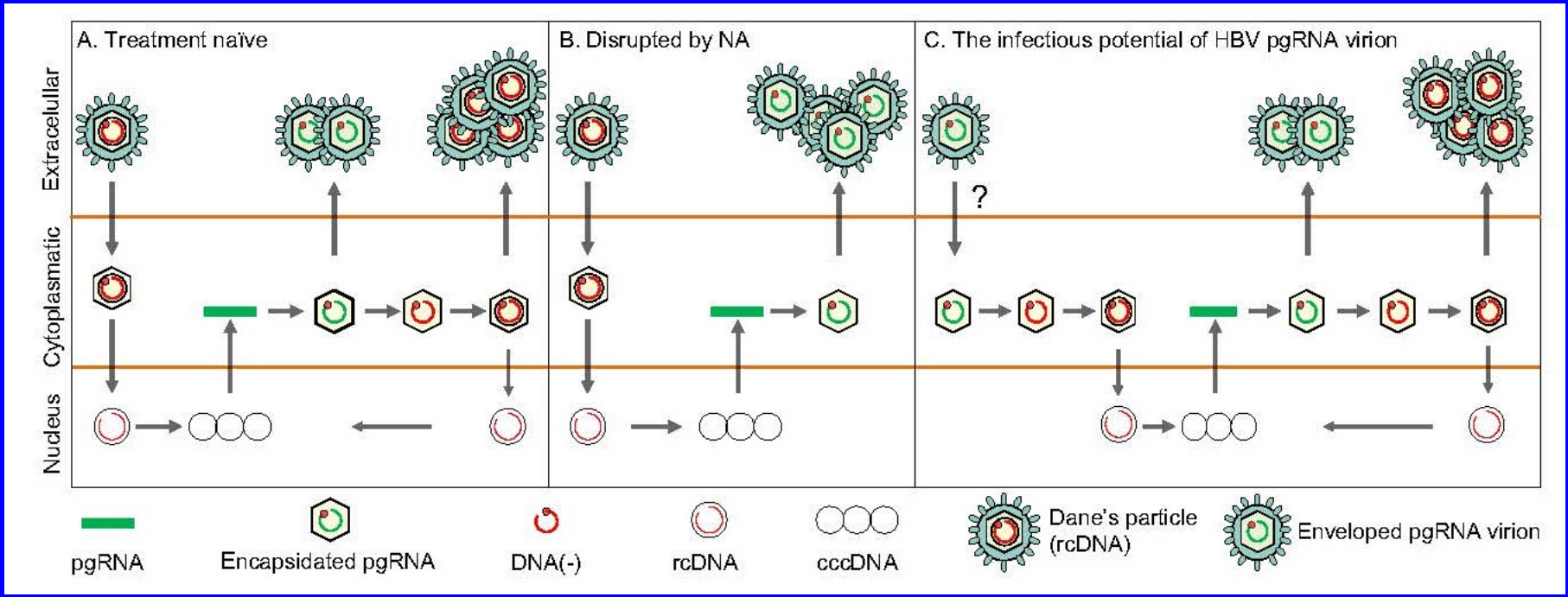


Laras, A et al 2006. Hepatol;44(3):694-702

Serum HBV RNA

- **Mimic what's happening in the liver with cccDNA levels**
- **RNA in serum may reflect the presence and active transcription of cccDNA in the liver** *(Wang J et al. J Hepatol 2016)*
- Typically lower than HBV DNA levels (but abundant)
- Serum RNA levels vary significantly from other viral markers during AV therapy
  - eg. in HBeAg pos pts there is a stronger decline in HBV DNA levels cf with RNA levels
  - highlighting potential as an independent marker in the evaluation of pts with CHB *(Jansen L et al. JID 2015)*
- Persistence of serum HBV RNA associated with risk of viral rebound following discontinuation of NUC therapy (reflect level of intrahepatic cccDNA?) *(Wang et al 2016. J Hepatol;65:700-710)*

Model of the Production of Enveloped pgRNA Virions and Their Infectious Potential: Entry and Re-entry

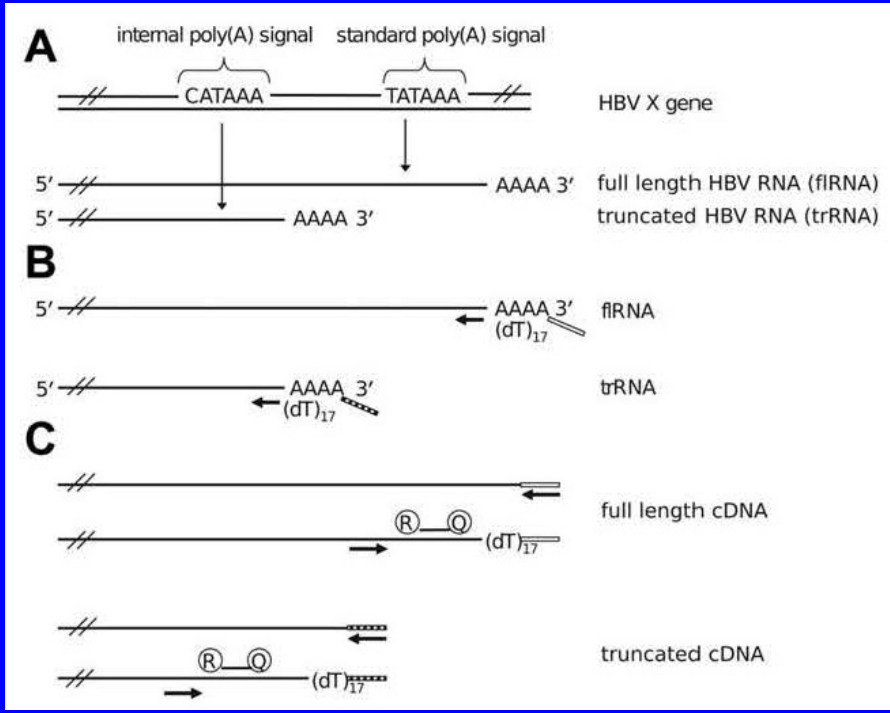


Wang, J et al 2016. J Hepatol;65:700-710

Serum Hepatitis B Virus RNA Levels as an Early Predictor of Hepatitis B Envelope Antigen Seroconversion During Treatment With Polymerase Inhibitors

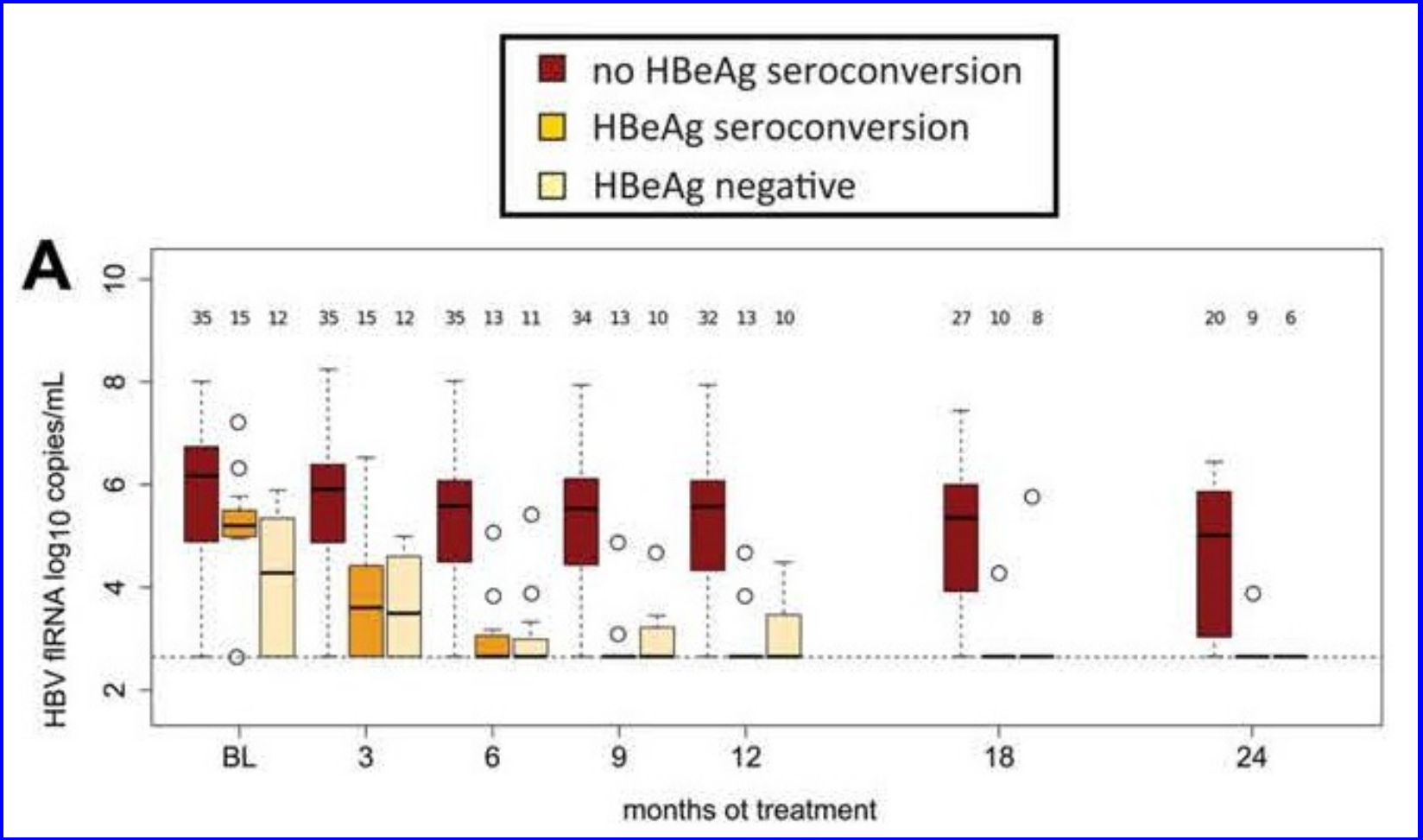
Florian van Bömmel,[1] Anne Bartens,[1,2] Alena Mysickova,[3] Jörg Hofmann,[2] Detlev H. Krüger,[2] Thomas Berg,[1] and Anke Edelmann[2]

HEPATOLOGY 2015;61:66-76



RACE-based RT-PCR technique used for quantitative analysis

HBV Full Length RNA



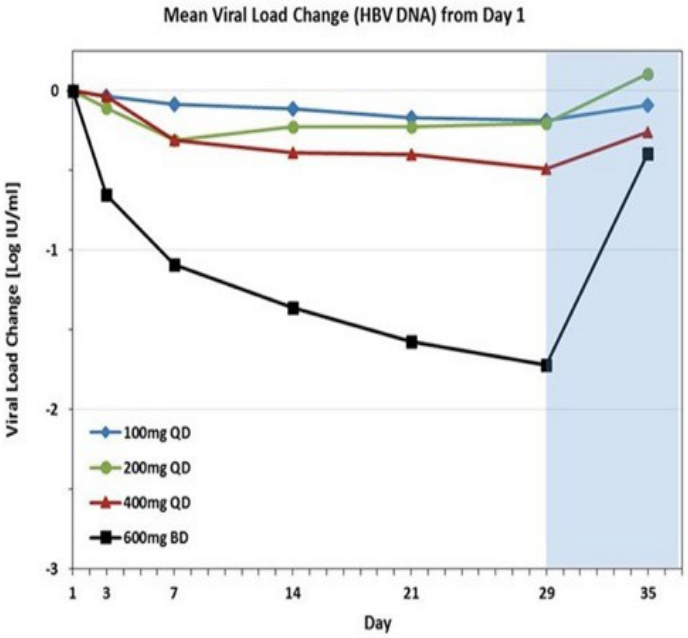
van Bömmel, F et al 2015. Hepatol;61(1):66-76

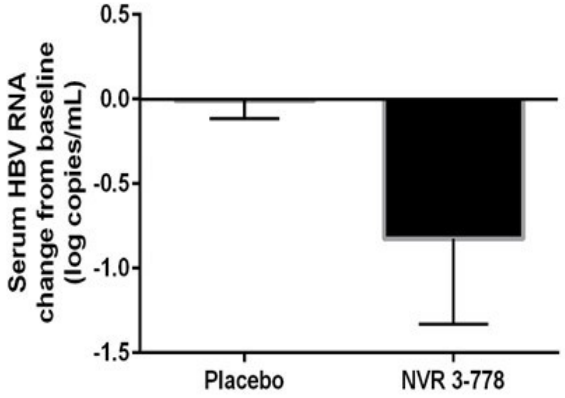
Phase 1b Clinical Trial: CpAM NVR 3-778 Reduces Serum HBV DNA and RNA

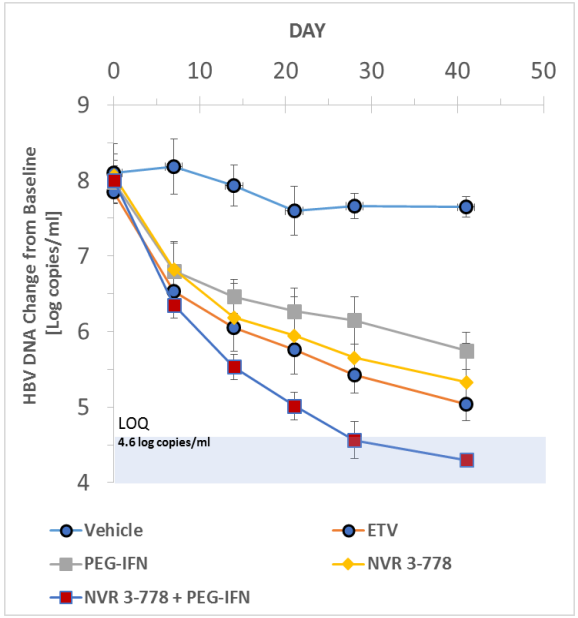
Pre-clinical evaluation in hepatocyte culture and chimeric mouse models Serum HBV DNA: mean

1.7 log reduction (600 mg BID)

Serum HBV RNA: mean 0.86 log reduction (600 mg BID)



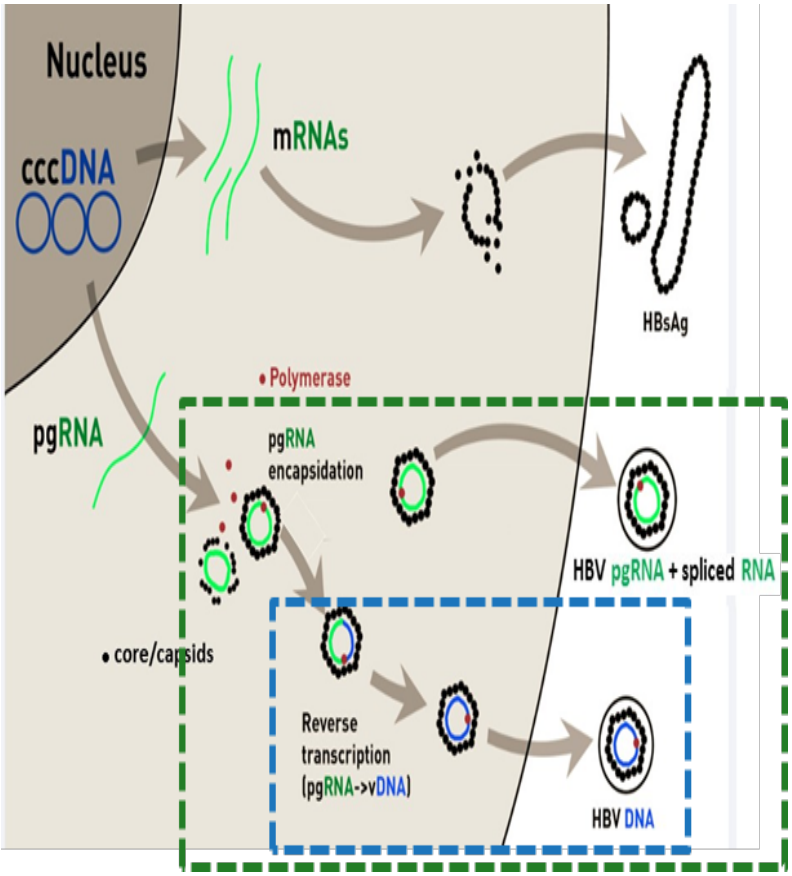


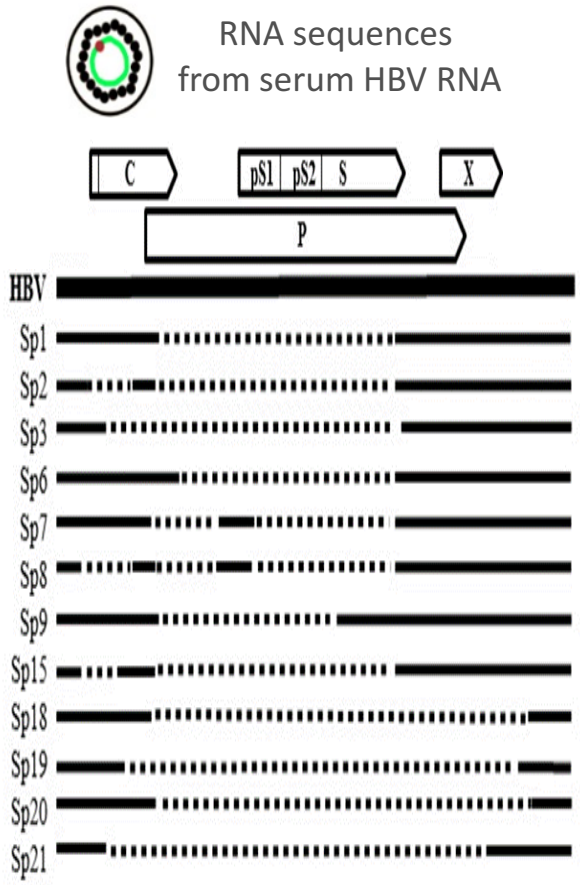


Yuen M-F, et al. AASLD 2015, San Francisco. #LB-10

Lam A, et al. AASLD 2015, San Francisco. #33

HBV RNA from Hepatitis B Patient Sera Contains Significant Amounts of Encapsidated Spliced HBV RNA Variants (2)





- HBV RNA is secreted within virus-like particles consist of envelope and capsid
- Extracellular HBV RNA particles contain pgRNA and spliced RNA variants
- HBV CAM blocks production of pgRNA and spliced RNA containing particles
- 3 new spliced variants identified

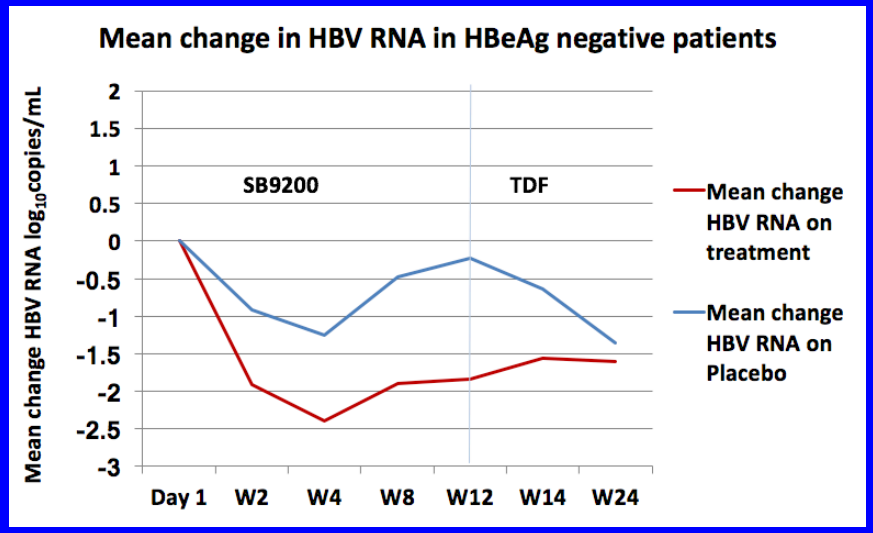
Inhibited by capsid assembly modulators (CAM)

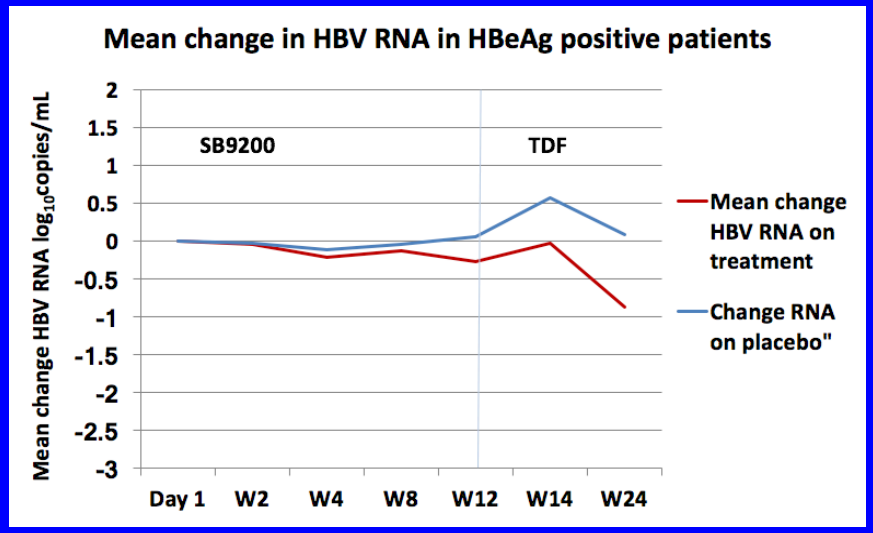
Inhibited by nucleoside analogs

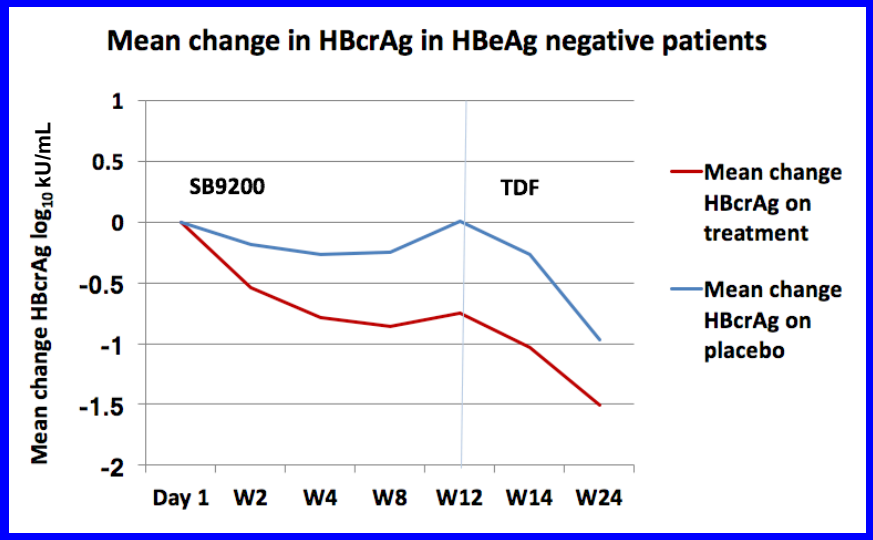
- Patient sera contains encapsidated **spliced** HBV RNA variants (known and novel) and may be potential treatment response biomarkers depending on the DIA

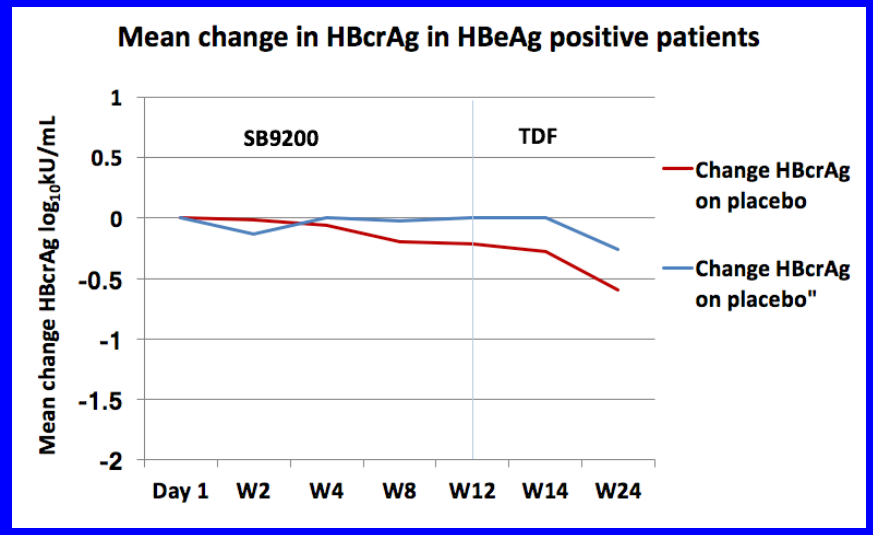
Espiritu C , et al. AASLD 2016, Boston. #17

Antiviral Activity of SB9200 (Inarigivir) HBV RNA and HBcrAg Profile of ACHIEVE Trial





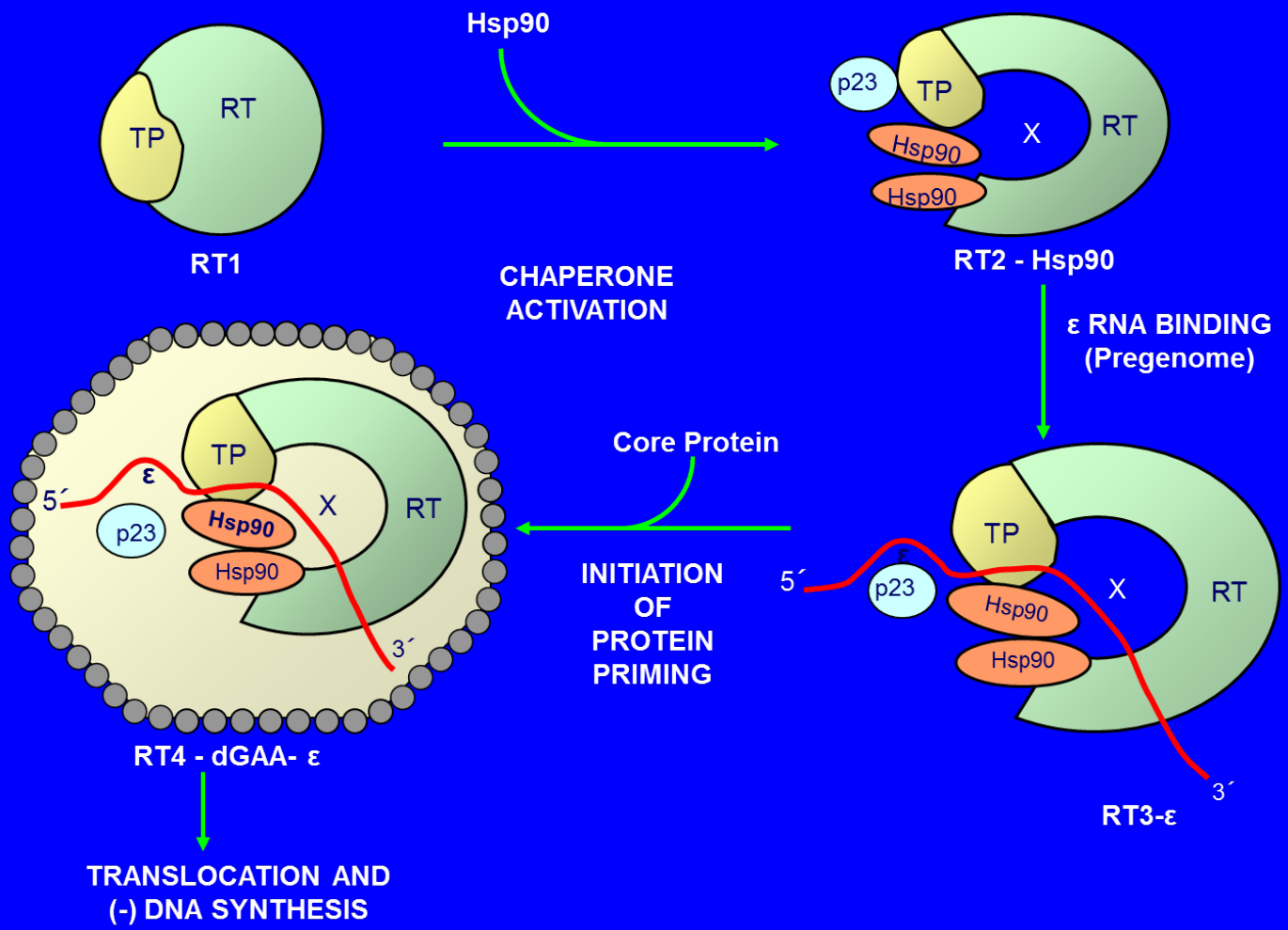




In HBeAg-NEG group: 3 log rapid decline HBV RNA whilst 1 log gradual decline HBV DNA

See AASLD Abstract #39 and Late Breaker Poster

HBV Encapsidation: POL-5´-ε pgRNA Binding



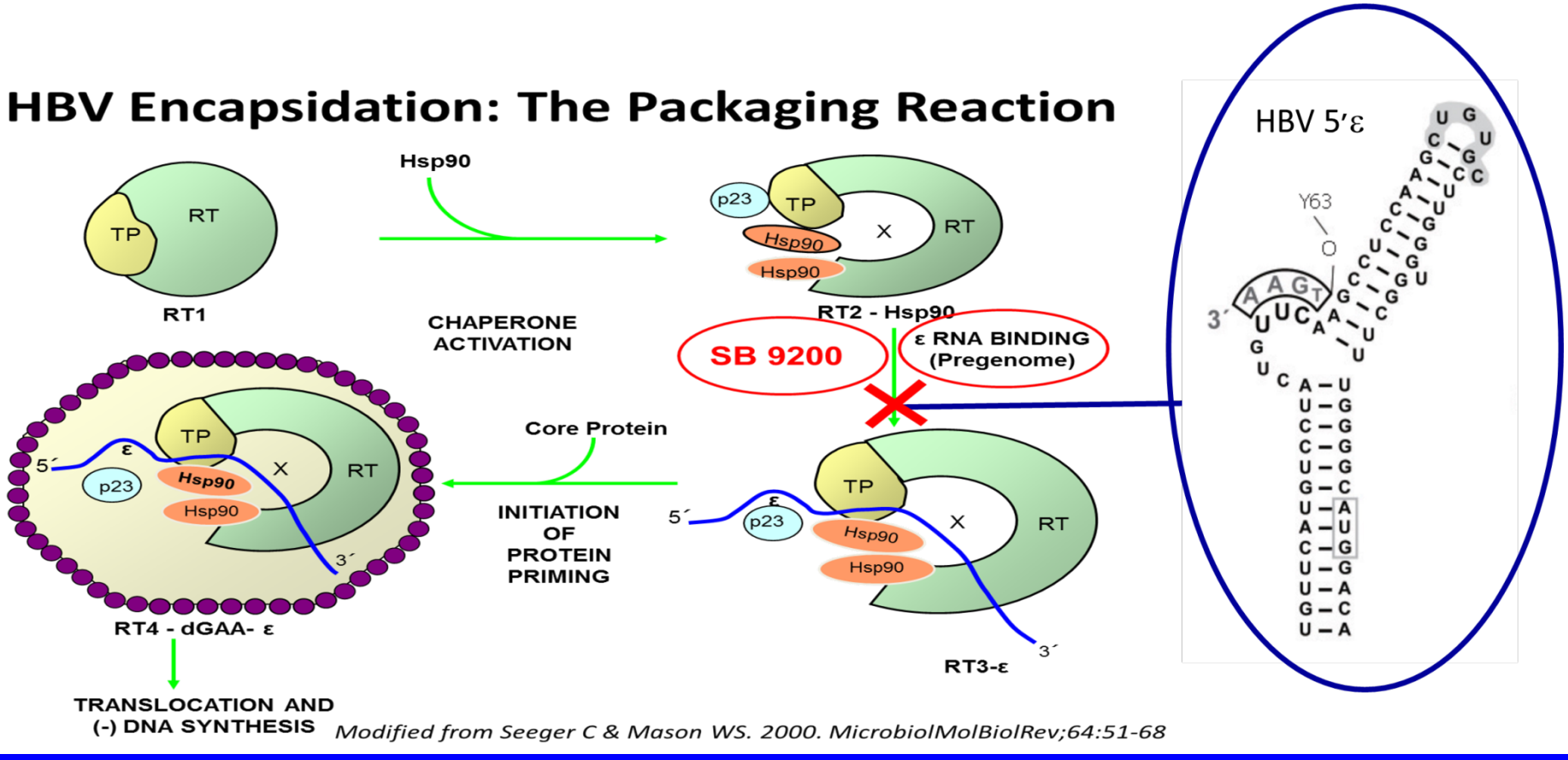
STOICHOMETRIC IMBALANCE: One pgRNA; One Polymerase; 240 Core submits

ABSOLUTE REQUIREMENT: 5´-ε pgRNA

SMALL CAPSID SYNDROME

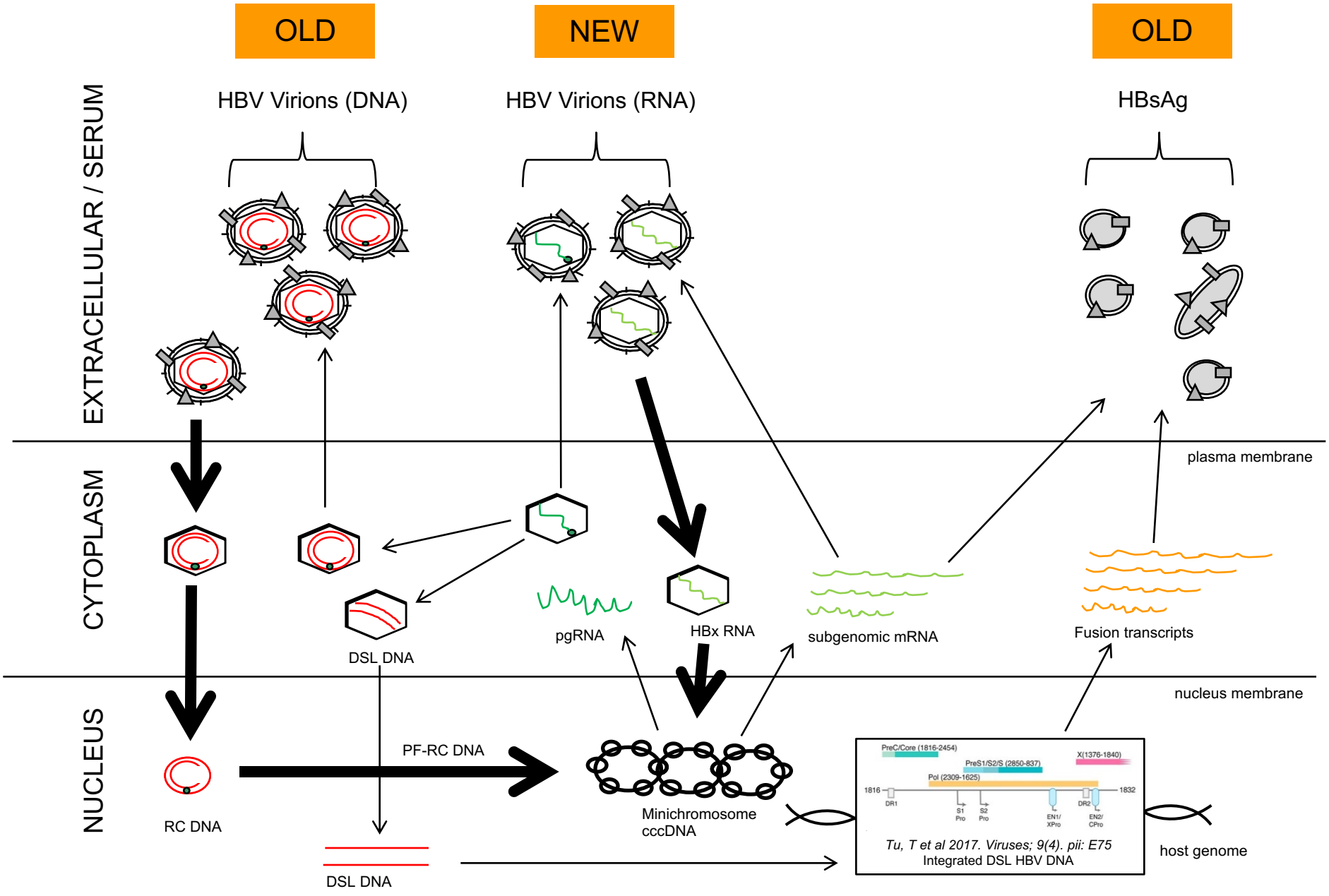
Seeger C & Mason WS. 2000. Microbiol Mol Biol Rev 64, 51-68

Proposed Model for Direct Antiviral Effect of SB 9200



Modified from Seeger C & Mason WS. 2000. MicrobiolMolBiolRev;64:51-68

Biomarkers and MOA of DAAs



Quantitative HBeAg Testing

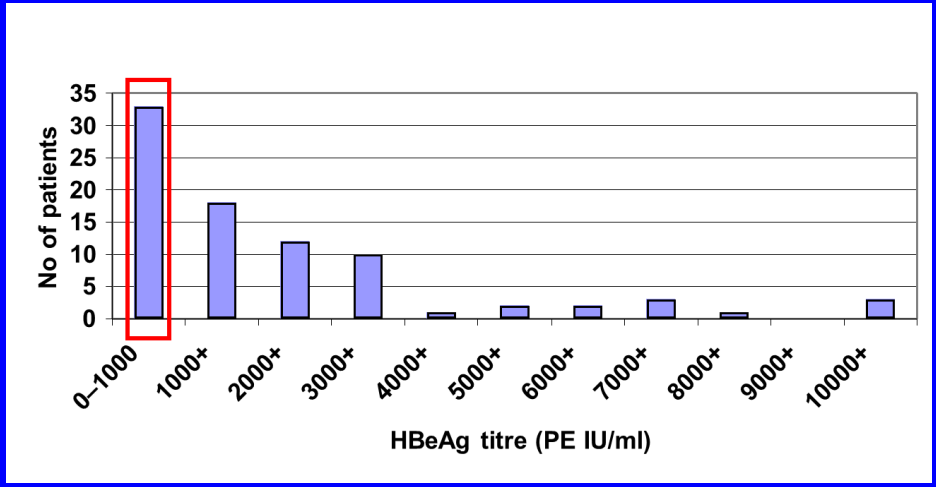
- Role for quantitative HBeAg titre in predicting treatment outcome has been proposed: *(Fried, Hepatology, 2008)*

  Pegylated-interferon therapy:
  - HBeAg seroconversion
    - Baseline HBeAg titre < 31 PE IU/ml – PPV for seroconversion = 51%
    - 24 week HBeAg titre > 100 PE IU/ml – NPV for seroconversion = 96%

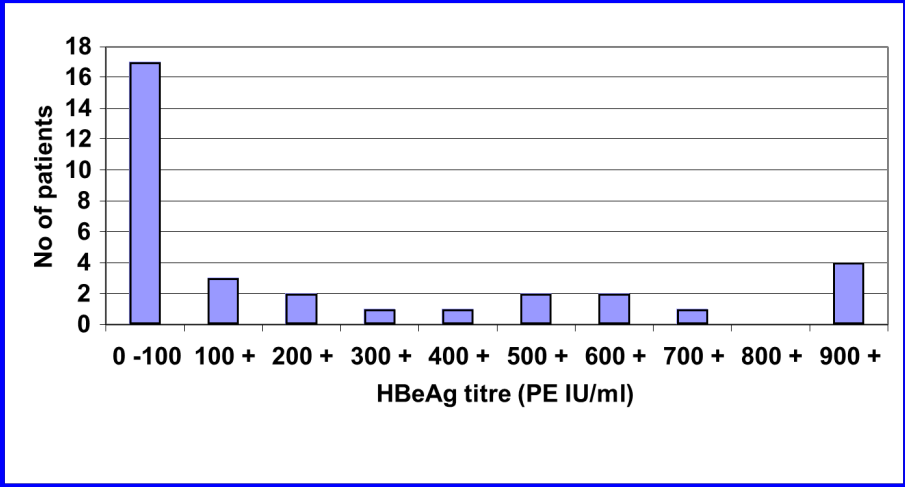
    Vs 24 week HBV DNA > 9 log copies/ml – NPV = 86%

Population Distribution of HBeAg Titre

In 40% the HBeAg titre <1000 PE IU/ml (n=85)

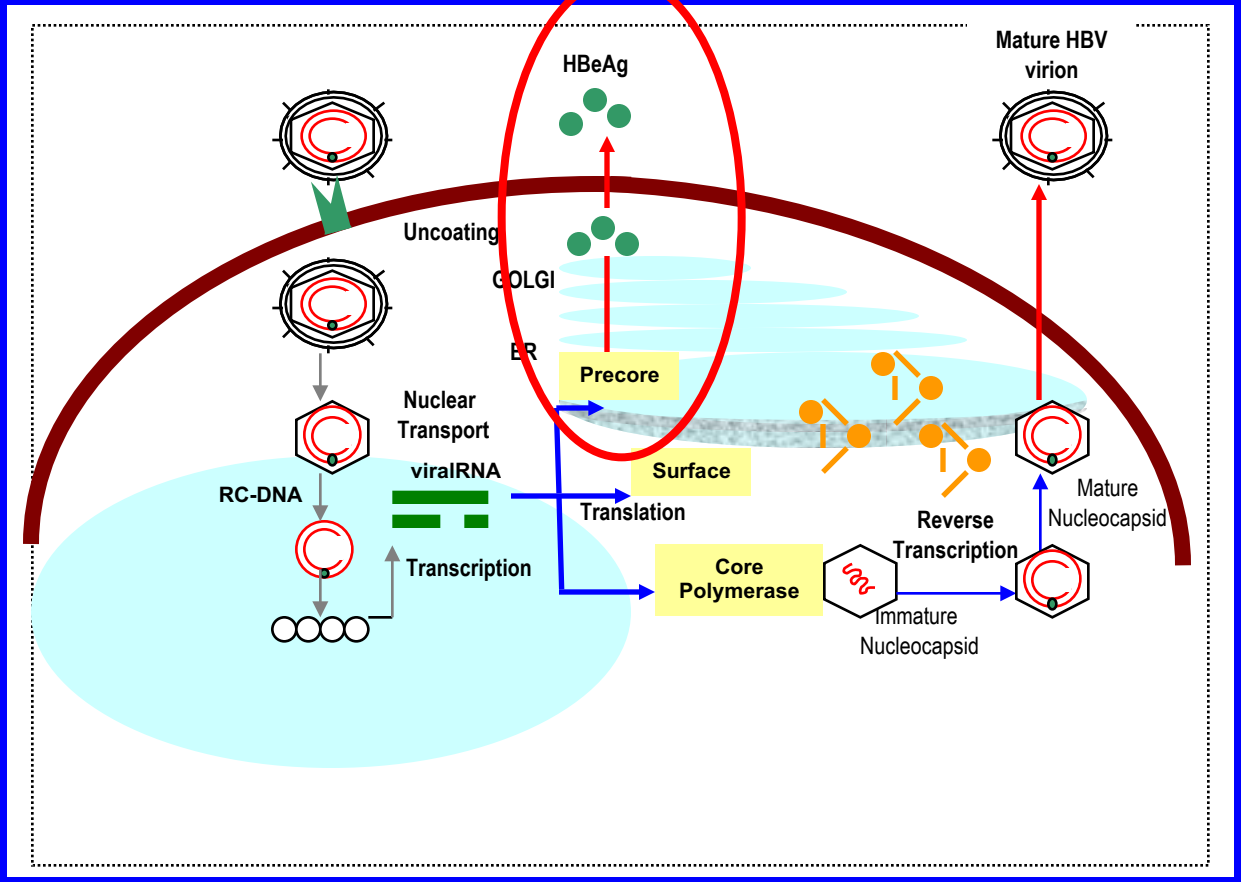


Most patients have a titre <100 PE IU/ml



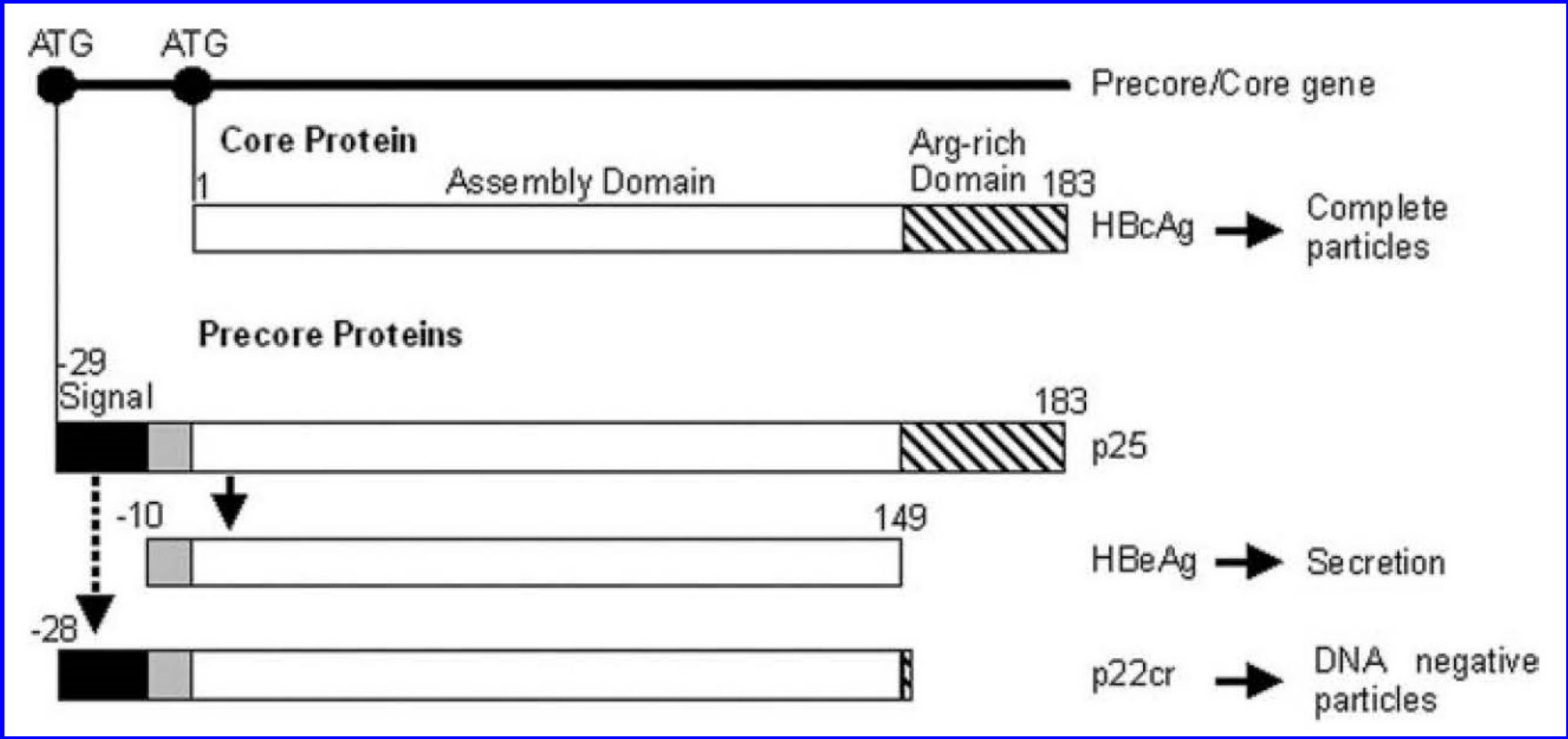
Thompson A, et al. AASLD 2008

HBV Replication: HBeAg (Secretory) Pathway



- pre-core mRNA Derived [full length HBV DNA]
- made from cccDNA templates only

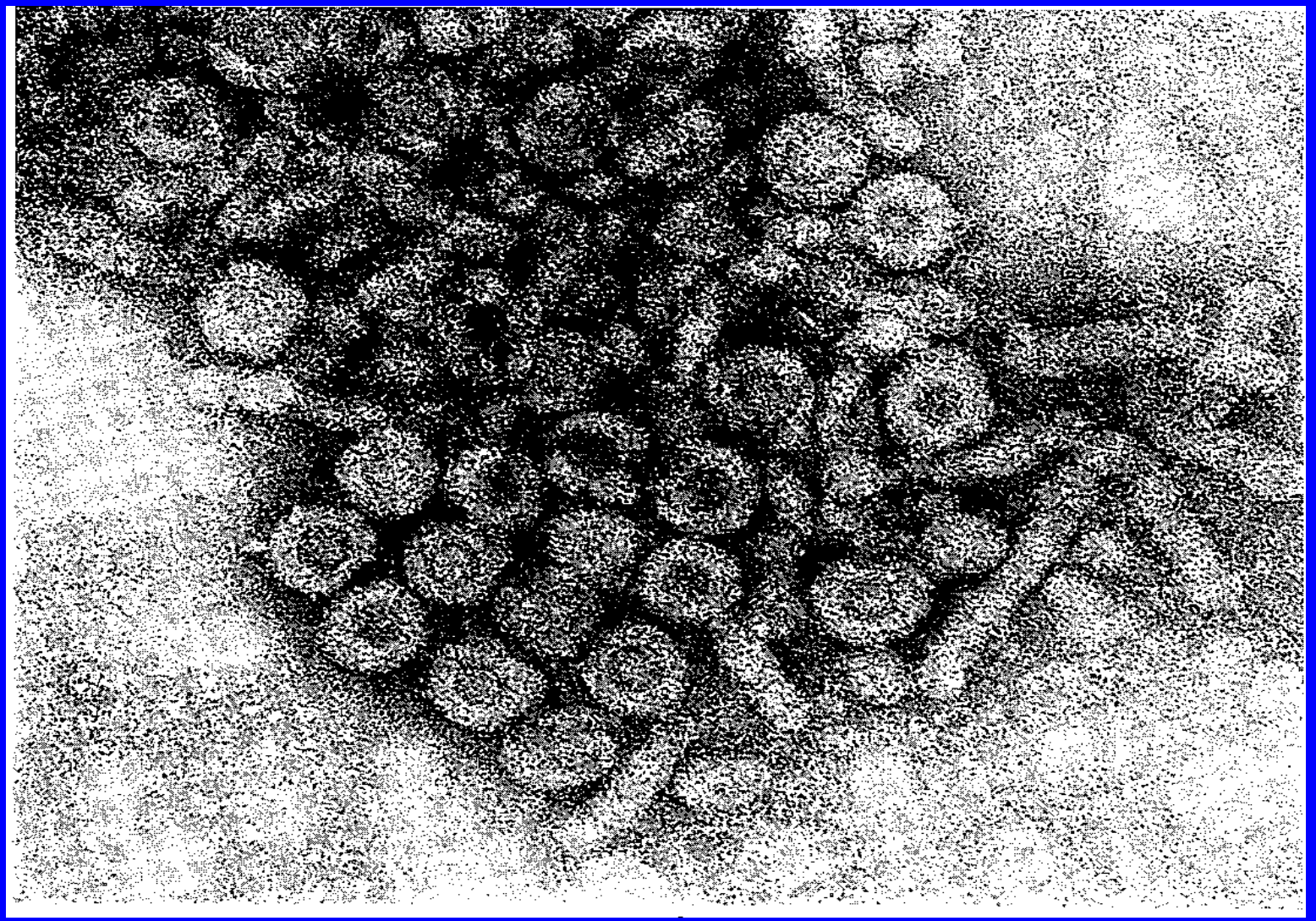
HEPATITIS B CORE-RELATED ANTIGEN (HBcrAg) PRECORE/CORE GENE PRODUCTS AND THEIR PROCESSING



HBcrAg = combined measure of HBcAg, HBeAg and p22cr

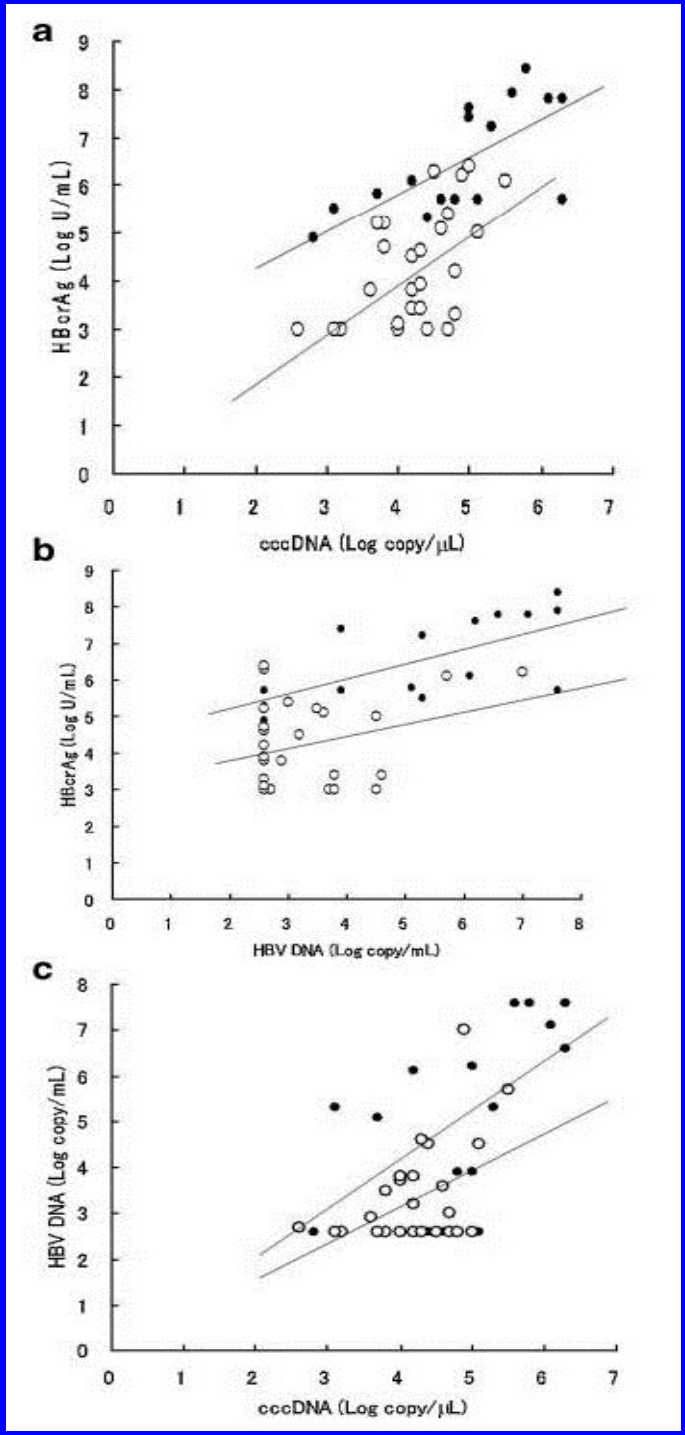
Kimura, T et al 2005. J Biol Chem;280:21713-21719.
Kimura, T et al 2002. J Clin Microbiol;40:436

Electron Microscopy: HBV in Serum



Empty particles: p22cr Capsids

Correlation Between HBcrAg, cccDNA and HBV DNA



In HBeAg-neg CHB, what is being measured??

Suzuki, F et al 2008. J Med Virol;81:27-33

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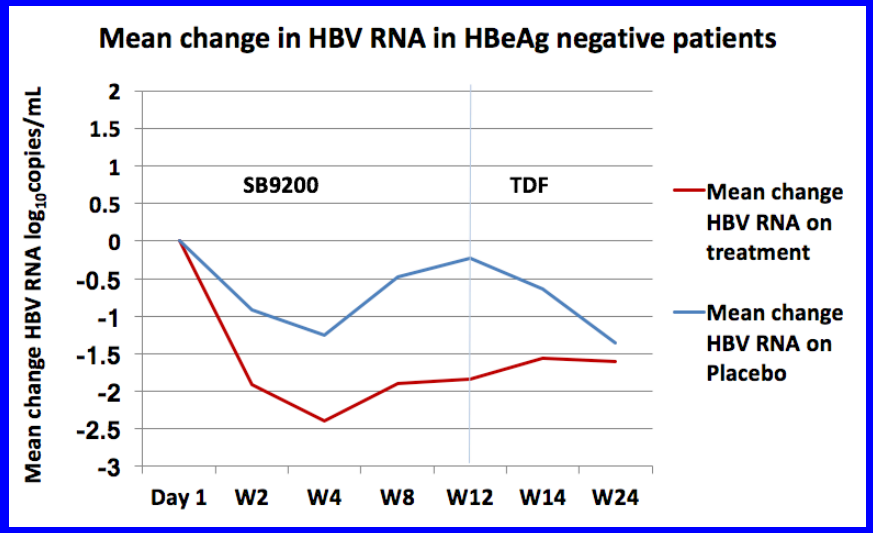

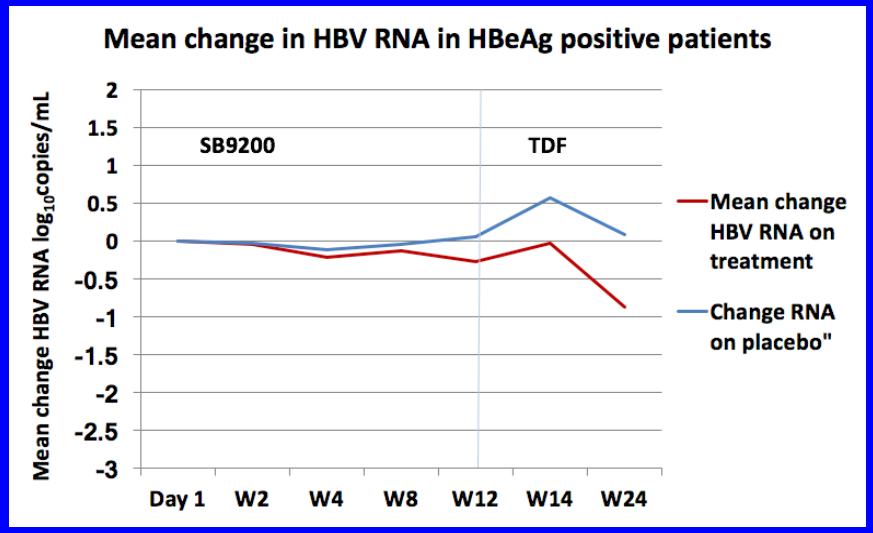

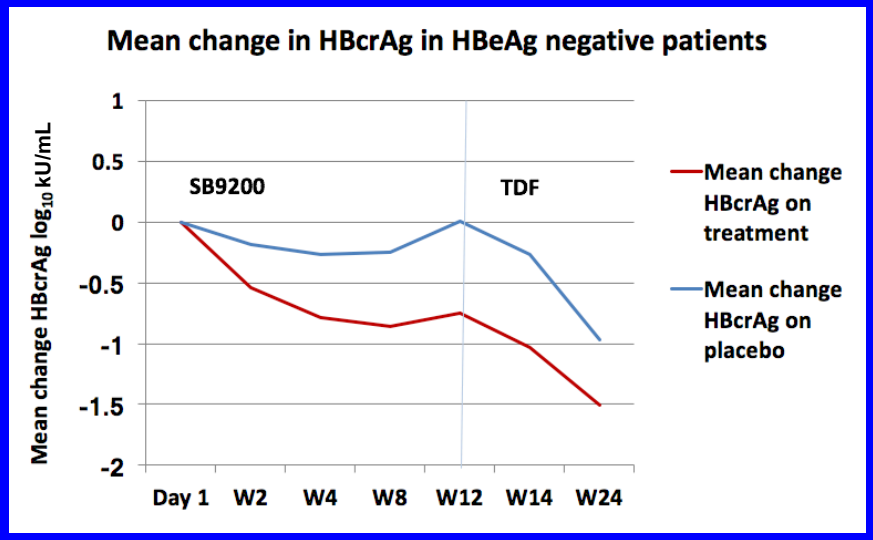

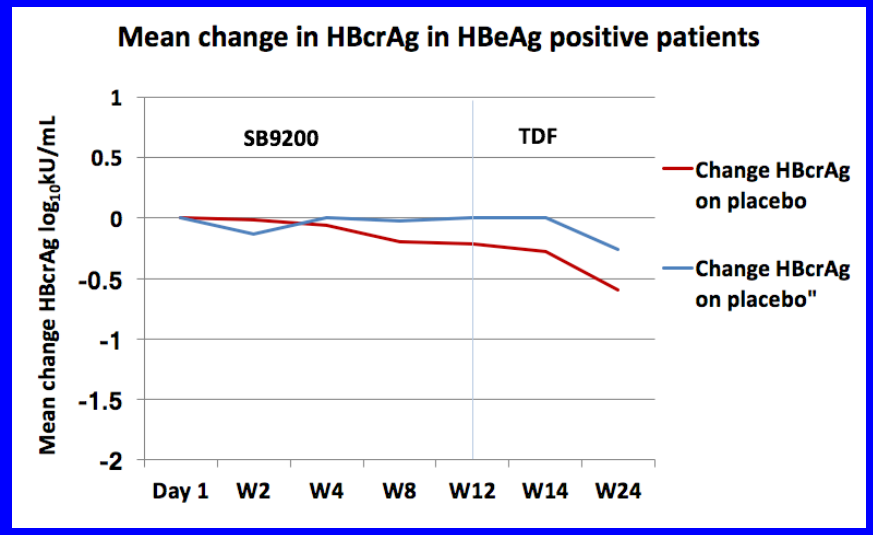

In HBeAg-NEG group: 3 log rapid decline HBV RNA whilst 1 log gradual decline HBV DNA

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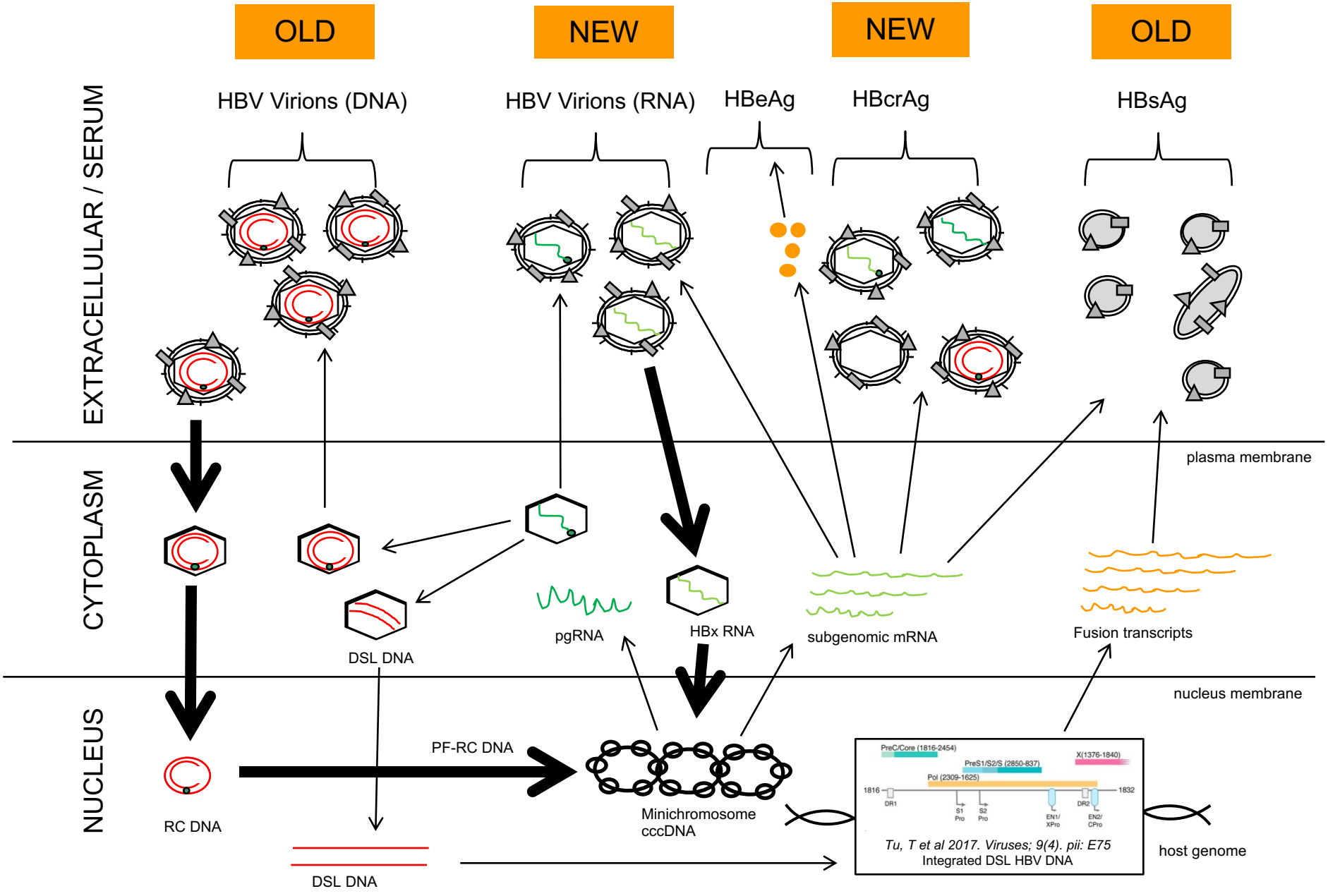
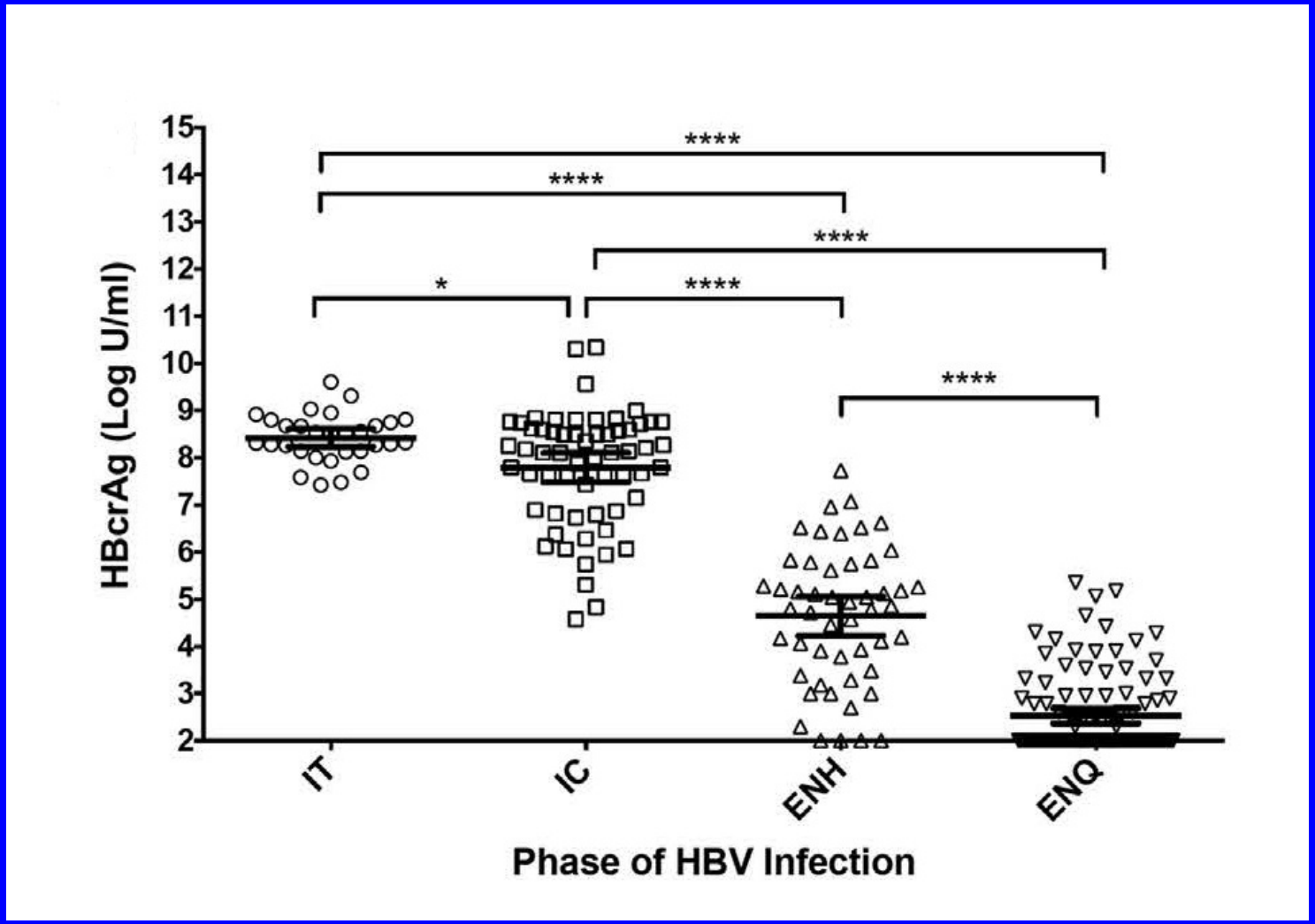
Biomarkers and MOA of DAAs

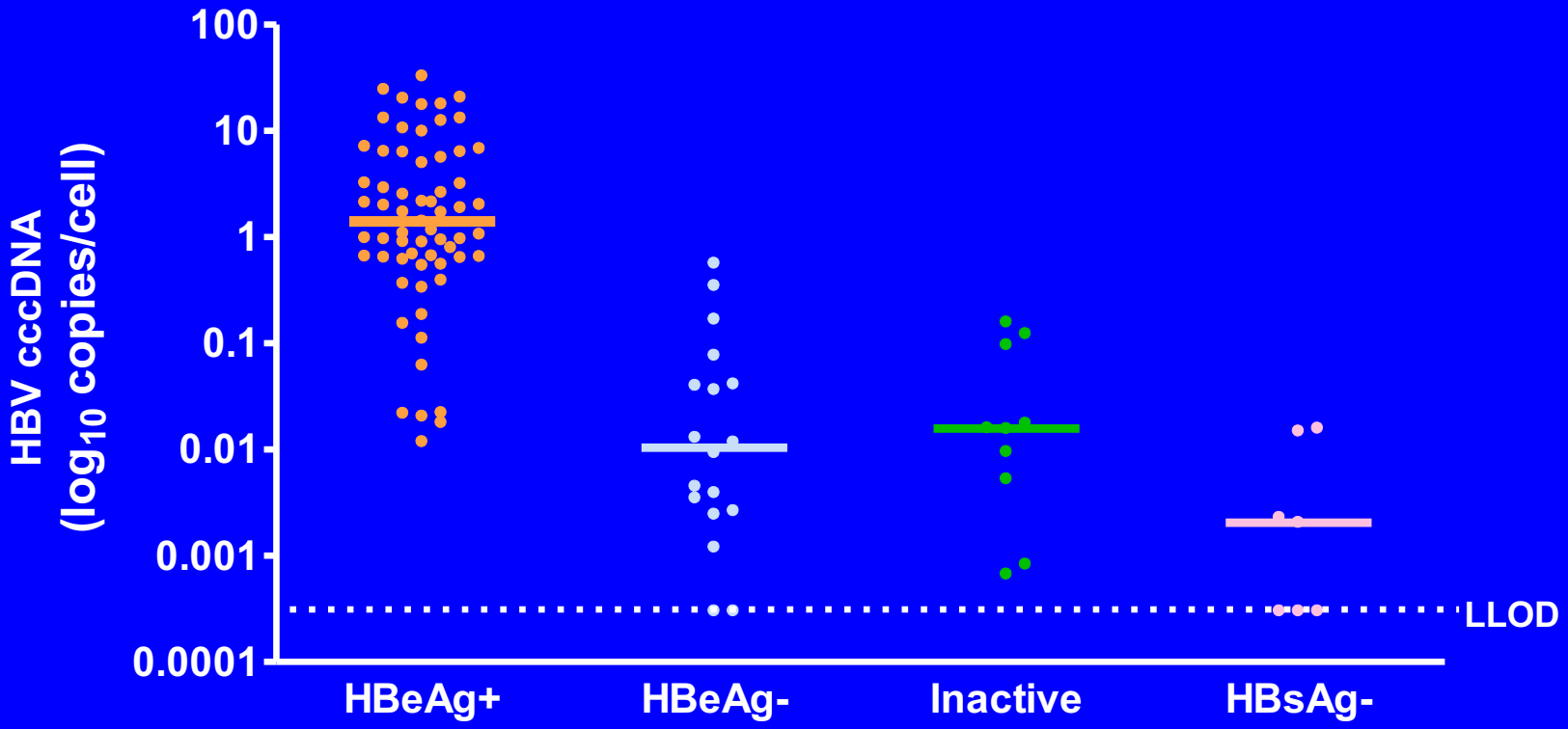


HBcrAg Across Phases of CHB



Maasoumy, B 2015. Clin Microbiol Infec;21: 606.e1–606.e10

Hepatic HBV cccDNA Levels in Different Patient Populations



- cccDNA persists through all phases of the natural history of chronic hepatitis B
- PCR Measures **Level** of cccDNA NOT Activity
- **Copy number 0.1-10 cccDNA/hepatocyte**

(Werle-Lapostolle, B. et al 2004. Gastroenterology;126:1750

Natural History: HBsAg Levels are Lowest in the Immune Control Phase

	HBeAg-positive		HBeAg-negative		P value
	Immune tolerance	Immune clearance	Immune clearance/ Reactivated	Immune control	
ASIA	**4.53**	**4.03**	**3.35**	**2.86**	**0.001**
N	32	55	83	50	
EUROPE	**4.96**	**4.37**	**3.89**	**3.09**	**<0.001**
N	30	48	68	68	

Reliable identification of inactive carriers through a combination of HBV DNA <2000 IU/mL and HBsAg <1000 IU/mL

Nguyen T, et al. J Hepatol 2010
Jaroszewicz J, et al. J Hepatol 2010

Serum qHBsAg Predict HBsAg Loss in HBeAg Seroconverters

- 390 patients who spontaneously underwent HBeAg SC (genotype B/C)
- Low serum levels of HBsAg (alone or in association with HBV DNA levels*) 1.0 year after HBeAg SC can predict HBsAg loss:

	PPV HBsAg Loss Within 6 years
HBsAg < 100 IU/ml	46%
HBsAg 100-999 IU/ml	29%
HBsAg > 1000 IU/ml	< 10%

* HBV DNA < 200 IU/ml

CONCEPT: VIROLOGICAL SET POINTS

Tseng, T-C et al 2011. Gastroenterology;141:577

Response Guided Therapy for Peg-Interferon in the Treatment of Hepatitis B

Week 12 and 24 are the Key

HBeAg - Positive	HBeAg - Negative
Week 12 – Define Possible Non-Responders	
Criteria: 1) Absence of HBsAg decline OR 2) HBsAg > 20,000 IU/ml	Criteria: 1) Absence of HBsAg decline AND 2) HBV DNA reduction < 2 log
Week 24 – Define the Level of Treatment Response	
High: HBsAg < 1,500 IU/ml Mid: HBsAg 1,500 to 20,000 IU/ml Low: HBsAg > 20,000 IU/ml	High: HBsAg decline >10% Low: HBsAg decline < 10%

Chan, HLY et al 2011. J Hepatol;55:1121

INTEGRATE WITH HBV RNA, HBcrAg, and qHBeAg TO IMPROVE PPV

Viral Biomarker Scenarios: DAAs and Cytokines

Serum Marker	Possible Interpretation*
HBV DNA	• priming RT [ETV vs TDF] • RT [first-strand] • DNA polymerase [second-strand] • priming RT • Pol-5´-ε binding/encapsidation
HBV RNA: •pgRNA [full length]	• Pol-5´ε binding • priming RT • encapsidation inhibition • nucleocapsid assembly inhibition • cccDNA dependent
Other [Truncated]	• ? splice HBV RNAs • ? chimeric HBV RNAs
HBeAg	• precore mRNA [cccDNA dependent]
HBcrAg [HBcAg; HBeAg; p22cr]	• pregenomic RNA [cccDNA dependent] • precore mRNA [cccDNA dependent] • cccDNA “activity”
HBsAg	• phase of CHB [set-points] • episomal HBV (ccc)DNA [HBeAg-POS] • integrated HBV DNA [HBeAg-NEG]

*** Substantial Overlap**

Conclusion: Key Serum Biomarkers

- Phase of CHB
  - * HBV DNA
  - * qHBsAg
  - * qual HBeAg/anti-HBe
    [transition vs flip-flop]
  - * HBV RNA
  - * HBcrAg
- Interpret ALL available serum markers in context of CHB Natural History in order to define both known and new viral targets [packaging vs core assembly inhibitors]
- View HBV Lifecycle in full context for insight into mechanism(s) of action of DAA and cytokines [identification of regulatory pathways: virus replication eg: cccDNA $\leftrightarrow$ envelope protein]