HBV Virology - Novel Targets

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What Would HBV Elimination Look Like?

In the blood: HBV DNA/HBsAg negative
anti-HBs positive

In the liver: no HBV cccDNA
no HBV RC/DSL DNA
HBcAg staining negative
± HBsAg (occasional)*

*[reflecting integrated HBV DNA]

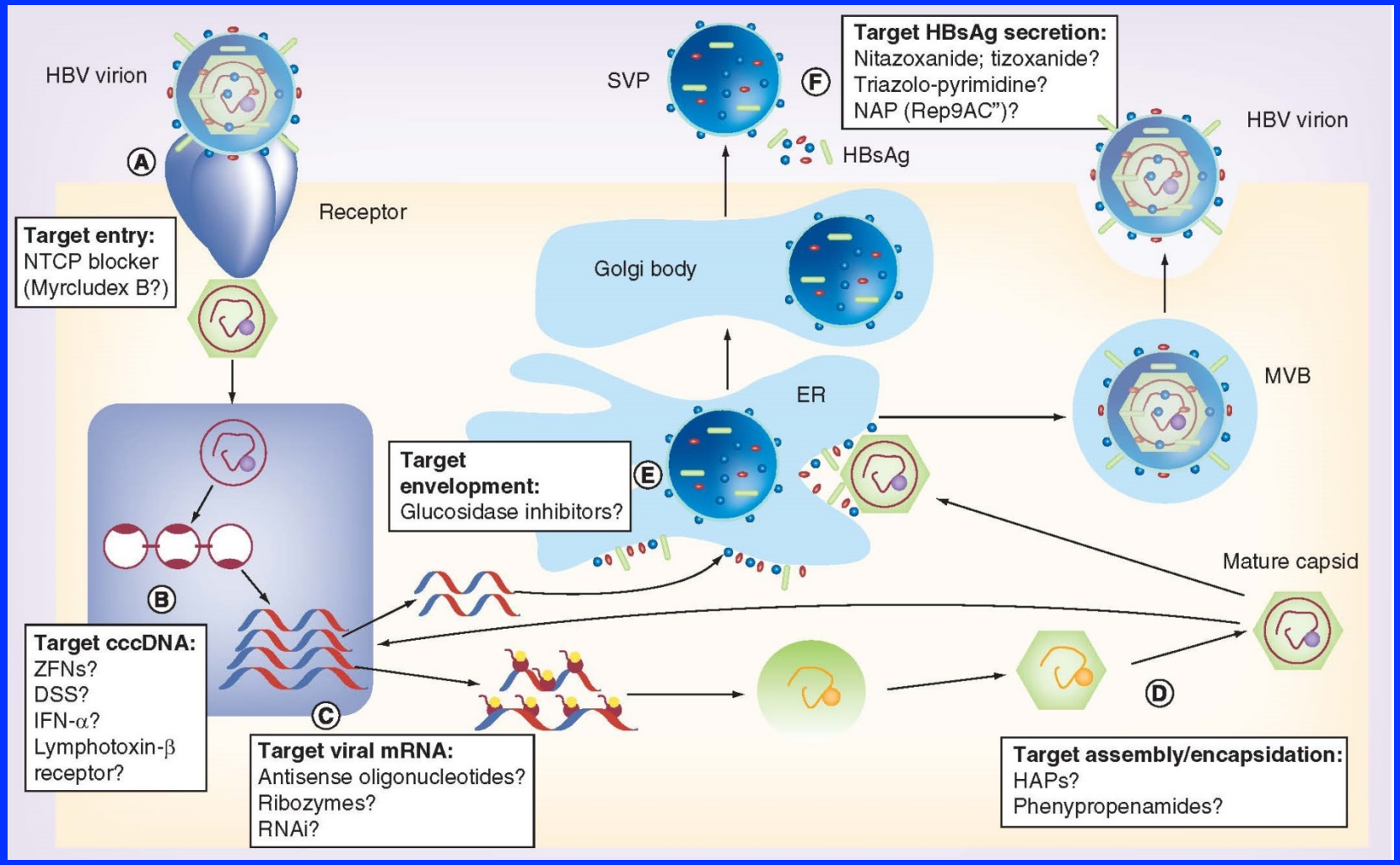
Functional Cure: HBsAg loss/Seroconversion: Maintenance of undetectable serum HBV DNA off-treatment

Absolute or Complete Cure: No cccDNA or HBV DNA anywhere!

New Viral Targets

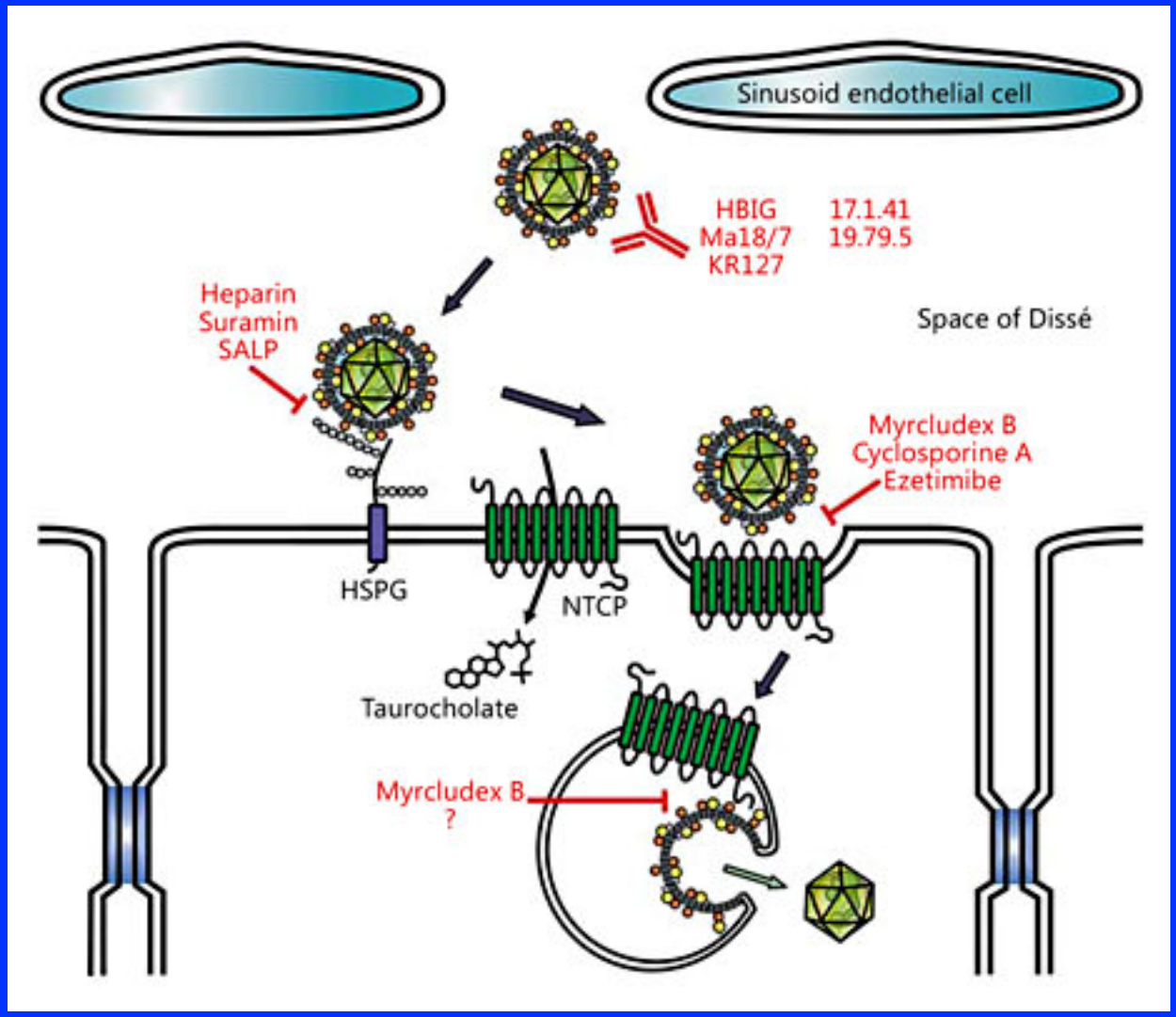
- Attachment and Entry
- cccDNA Generation & Processing (HBcAg and HBx)
- Reverse Transcription
- HBV Nucleocapsid Assembly (HBcAg)
- Packaging Inhibitors
- Molecular Based Therapies (RNAi)
- Combination Therapy

HBV Lifecycle Showing Novel Approaches for Viral Targets



Kapoor R & Kottilil S. 2014. Future Virol;9:565-585

Inhibitors of HBV Attachment and Entry

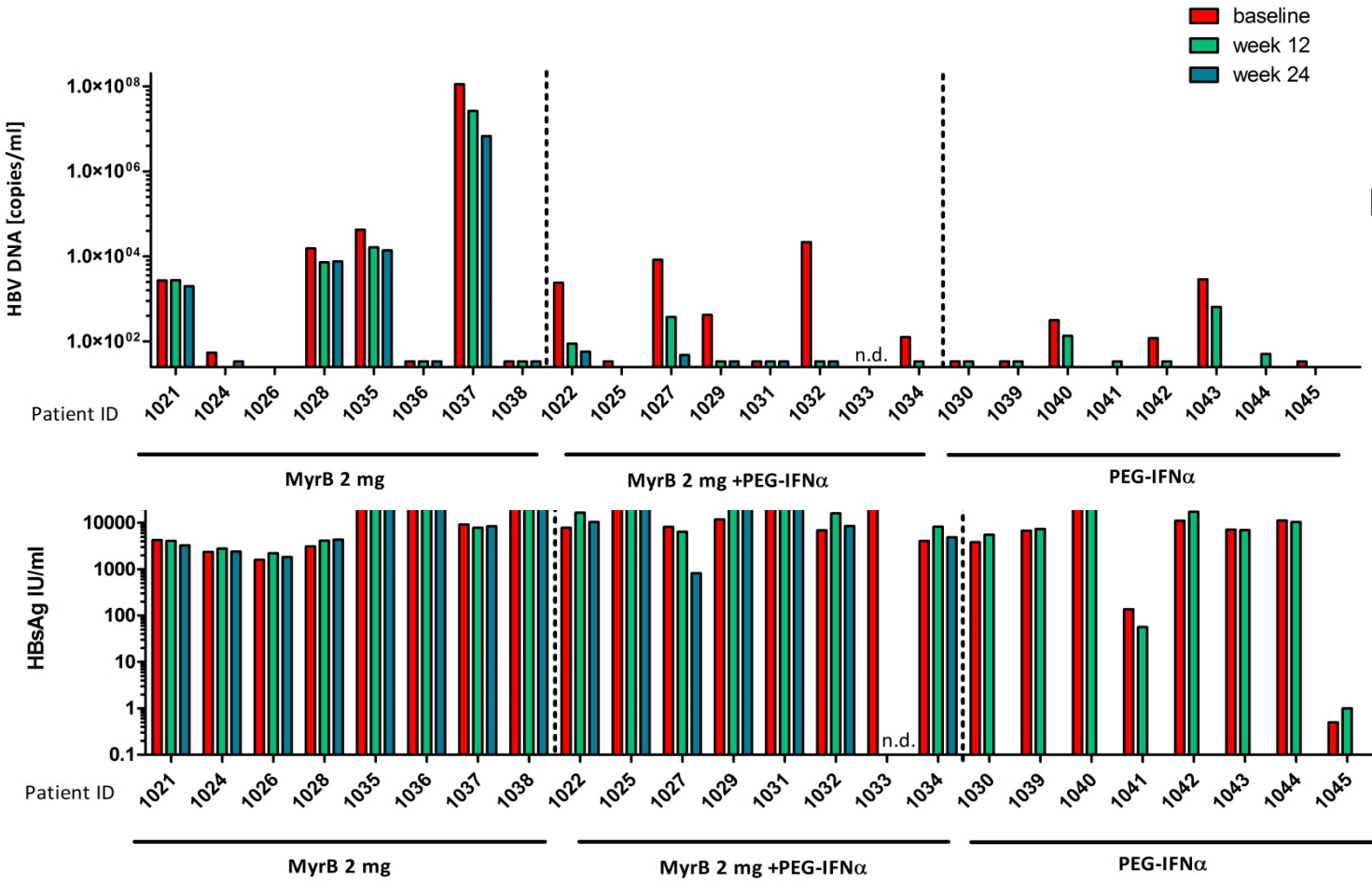


Sodium taurocholate cotransporting polypeptide (NTCP) identified as HBV and HDV receptor in 2012

Myrcludex in phase 2 trials in chronic HBV and chronic HDV decrease in HBV DNA and HDV RNA

Yan H, Elife 2012; 1:e00049
Lempp RA, Urban S. Intervirol 2014'; 57: 151

(B) HBV Serum DNA- and HBsAg Levels During Myr B and Myr B/IFNa Treatment



HBV DNA

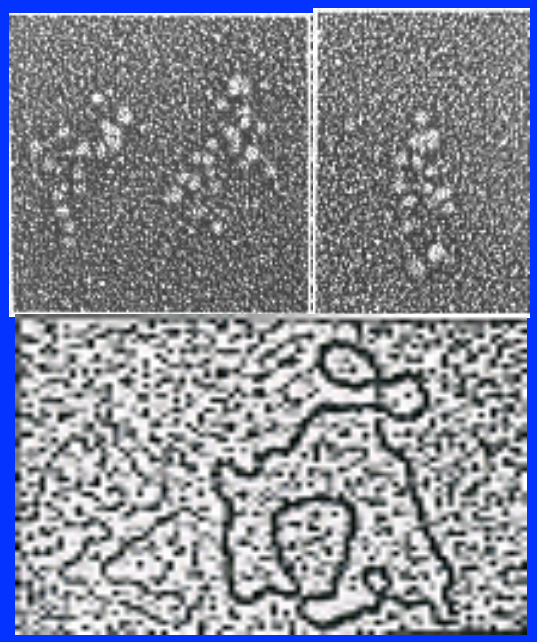
HBsAg

⇒ HBV DNA levels decline during Myrcludex B treatment in 4/8 patients (consistent with HBV trial).

⇒ More pronounced decline of HBV DNA in the Myrcludex B/PEG-IFNα group (5/8 patients).

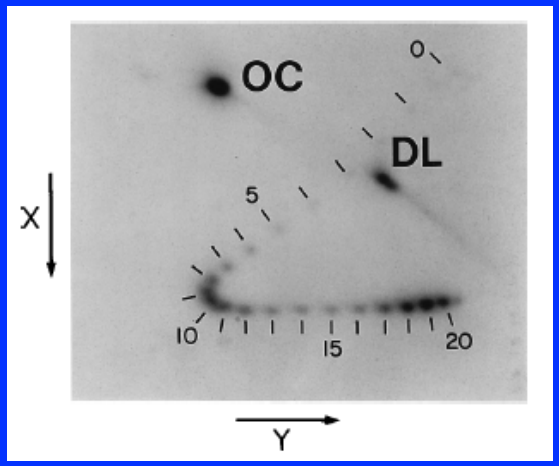
⇒ No significant changes (except patient 1027) in HBsAg levels .

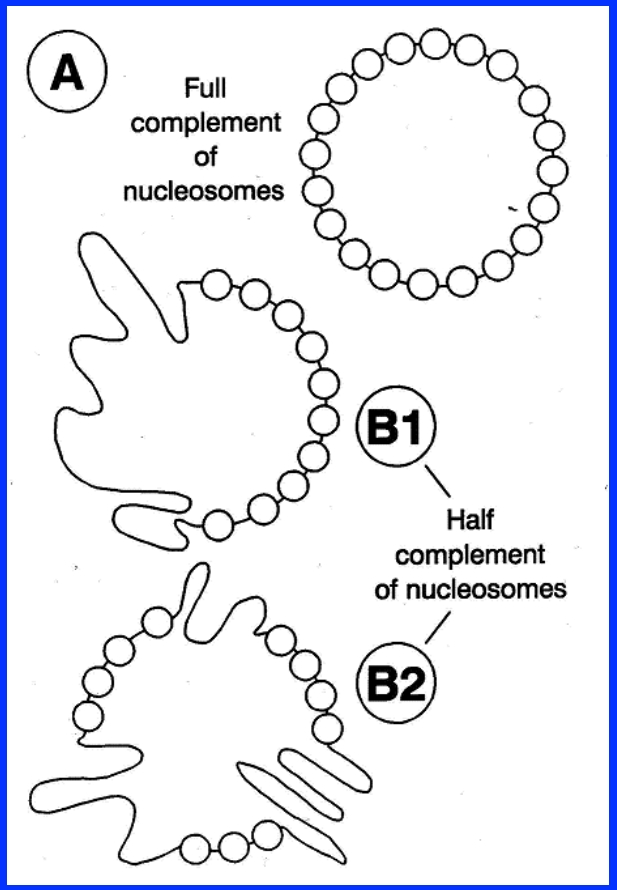
cccDNA Generation and Processing: cccDNA is a Minichromosome



Bock, T. et al 1994. Virus Genes;8:215

Bock, T. et al 2001. JMB;307:183





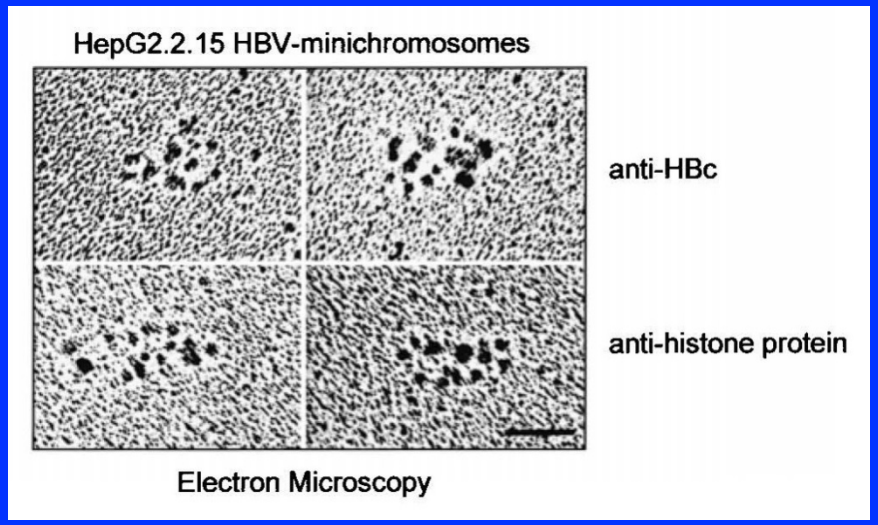
Low Replication Phenotype
Quiescent or active
Medium to Low Viraemia

High Replication Phenotype
Transcriptionally Active
High Viraemia

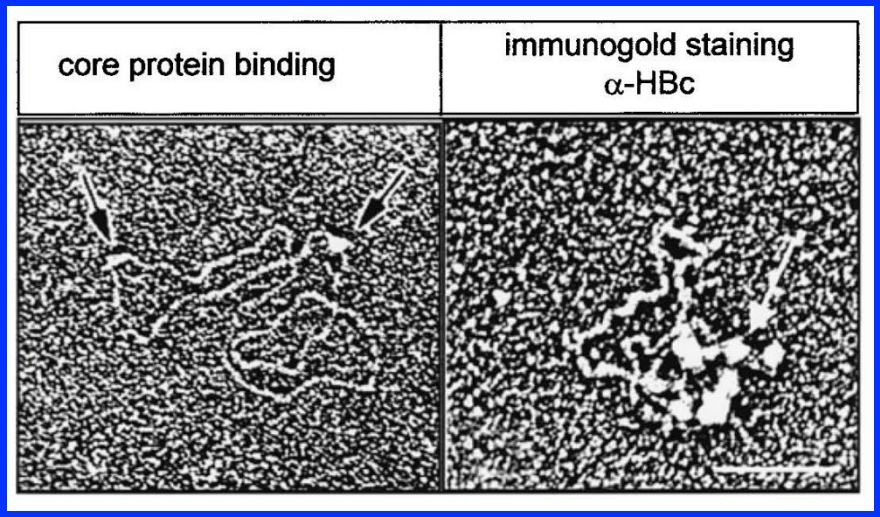
Newbold, J and Locarnini, S 1995. J. Virol;69:3350

Preferential Binding of HBcAg to HBV Minichromosome

A



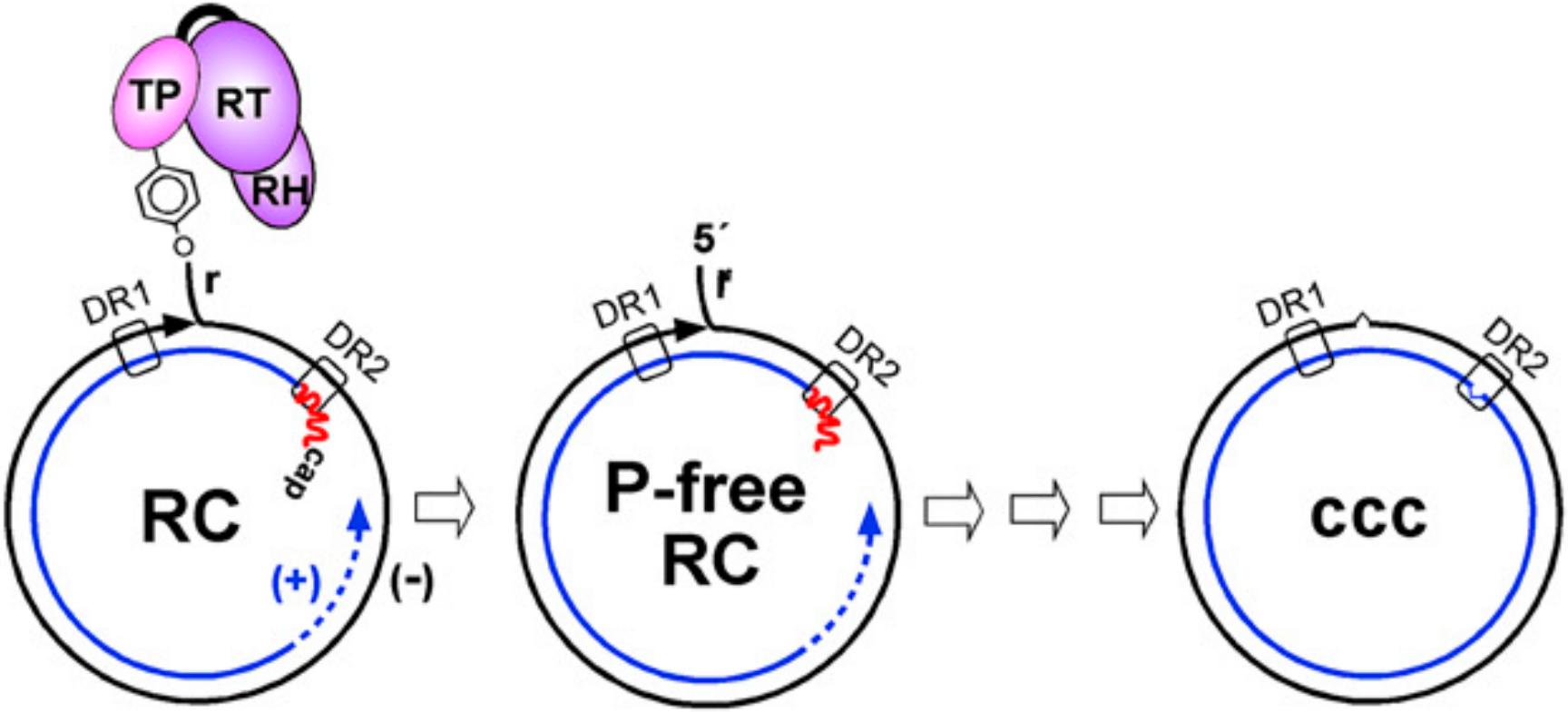
B



- HBcAg binds to CpG Island II (Guo, YH et al 2011. Epigenetics;6:720)
- in the presence of HBcAg peak number of nucleosomes increased from 15 to 16, resulting in a 20bp decrease in nucelosomal spacing

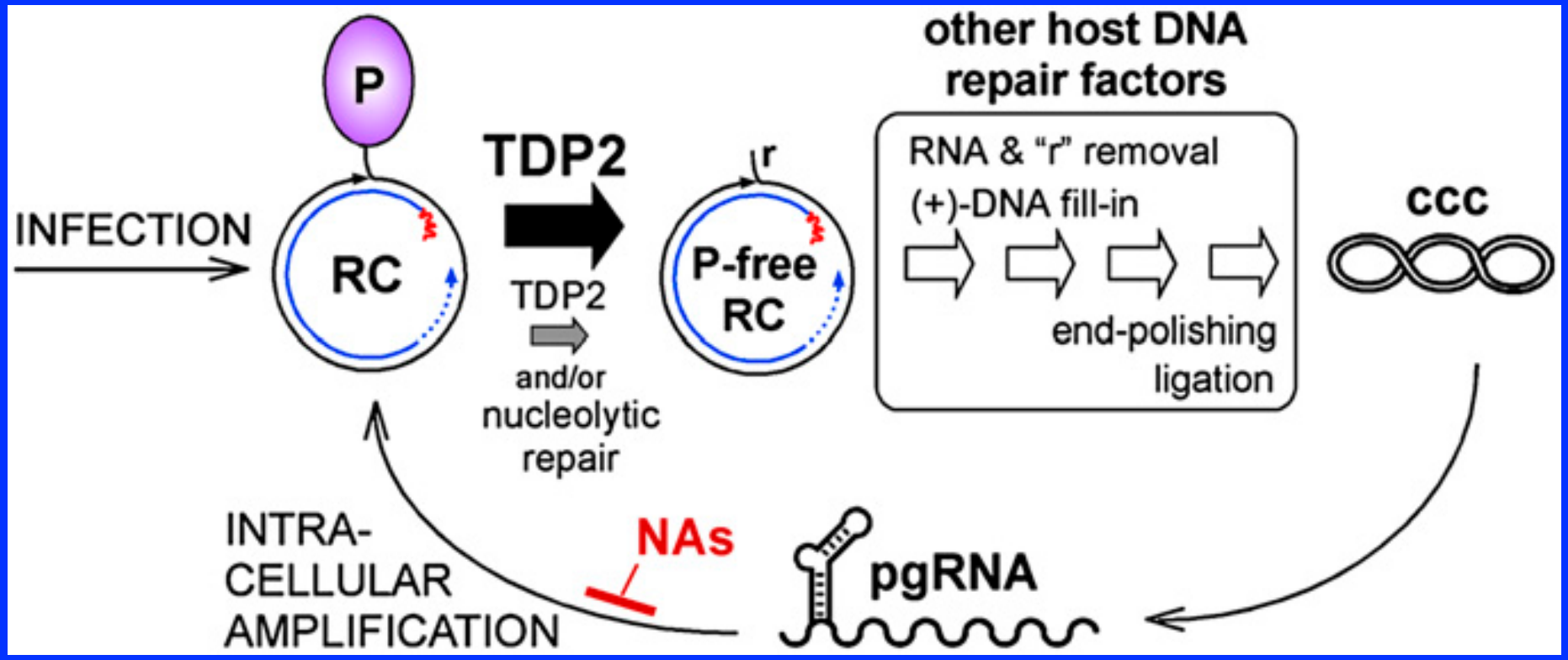
Bock, CT et al 2001. J Mol Biol;307:183

Conversion of RC DNA to cccDNA



Koniger, C et al 2014. PNAS;111(40):E4244-53

Role of Tyrosyl-DNA-Phosphodiesterases



Koniger, C et al 2014. PNAS;111(40):E4244-53

Preferential Binding of HBcAg to HBV Minichromosome

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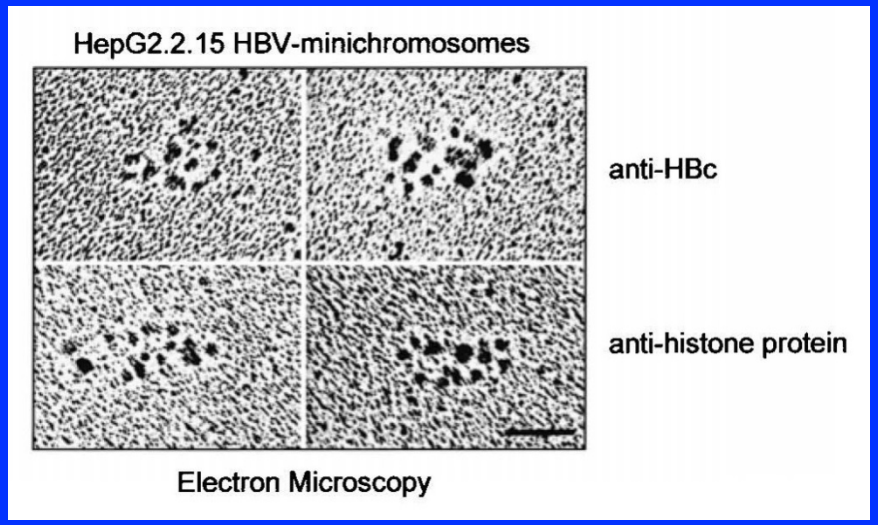

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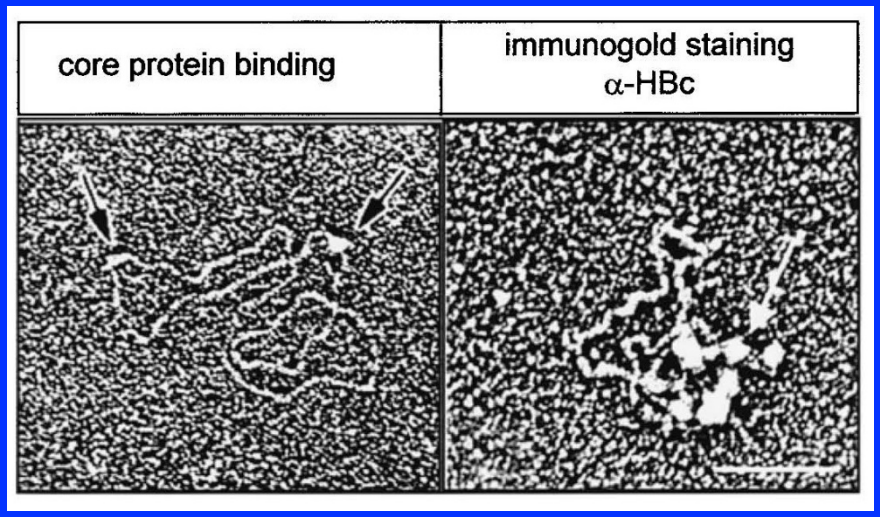

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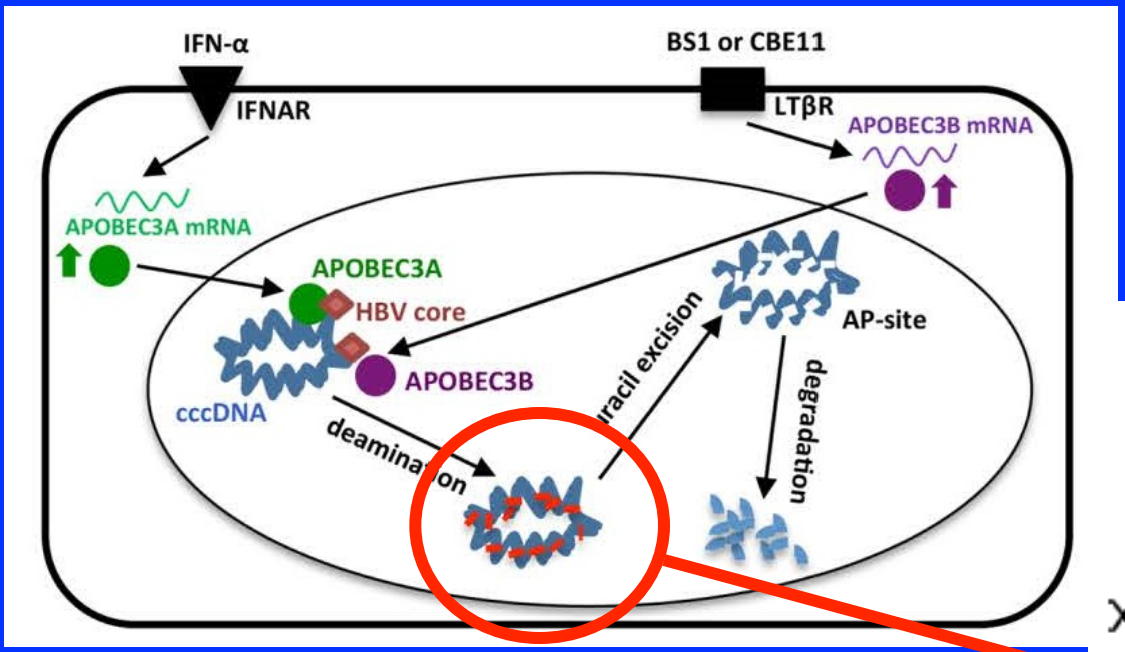
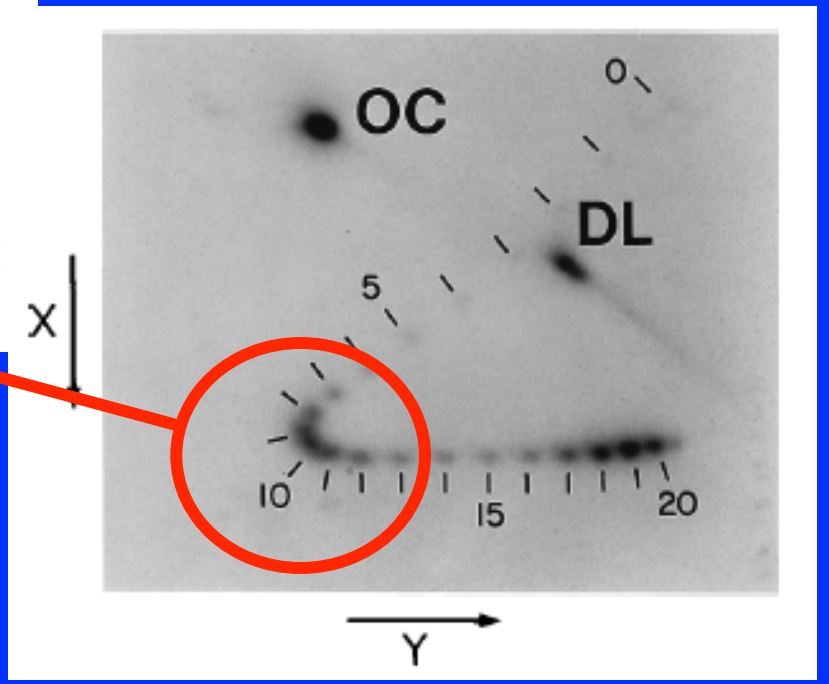
Bock, CT et al 2001. J Mol Biol;307:183

Interaction of APOBEC 3A/3B, HBV Core Protein (HBc) and cccDNA



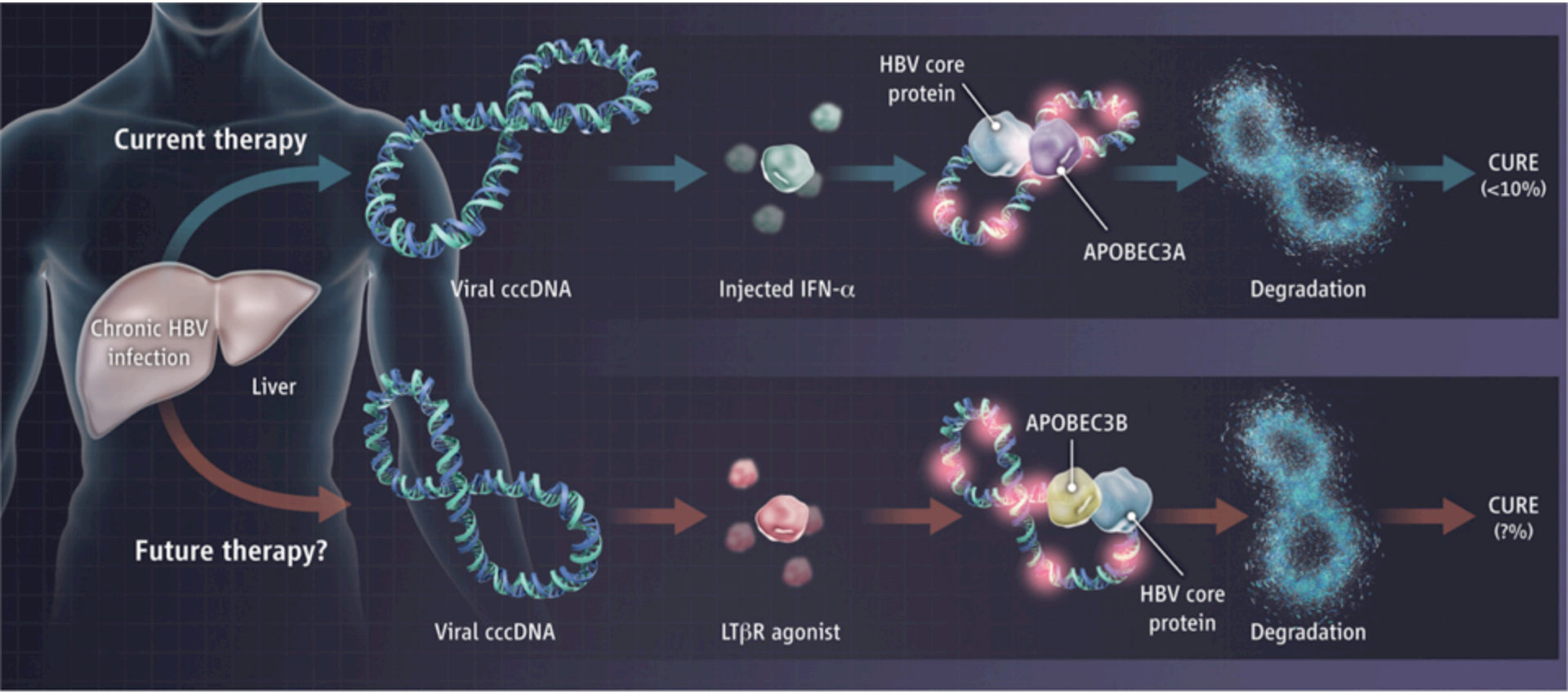


Modified from Lucifora, J et al 2014. Science;343(6176):1221-8

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

Model for cccDNA Degradation

IFNalpha /Lymphotoxin beta can induce **APOBEC3A/B dependent degradation** of HBV cccDNA



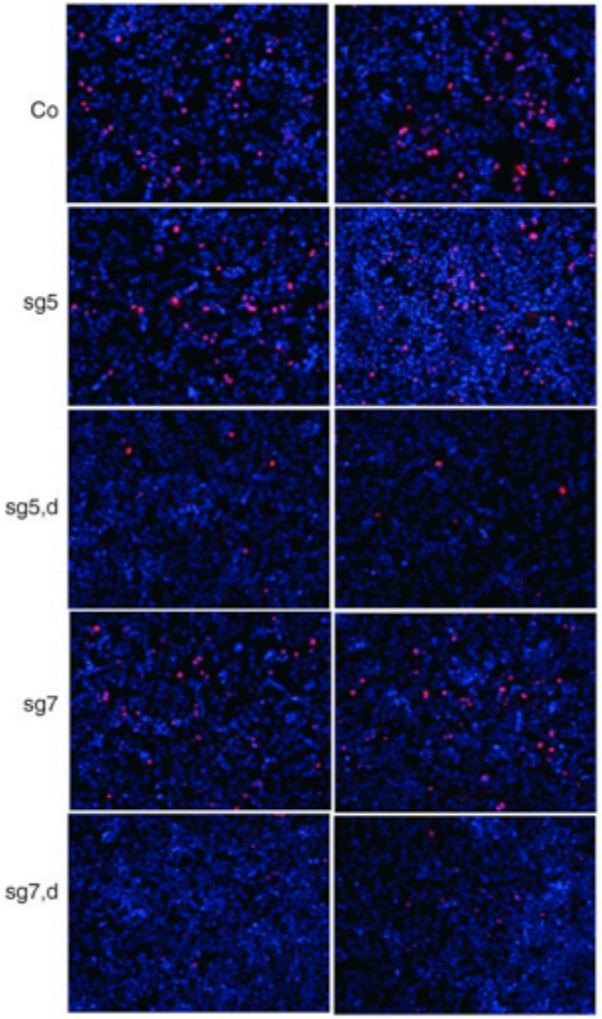
Lucifora et al, Science 2014; Shlomai & Rice, Science 2014

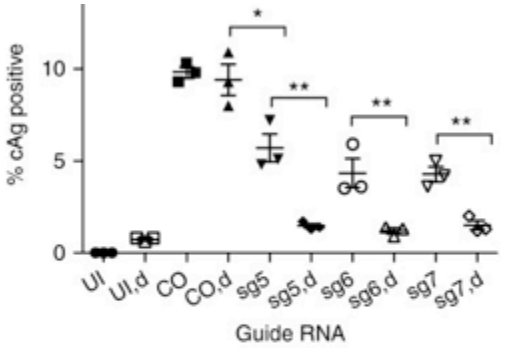
Similar observation with IFNγ and TNFα – *Xia et al, Gastroenterology 2015*

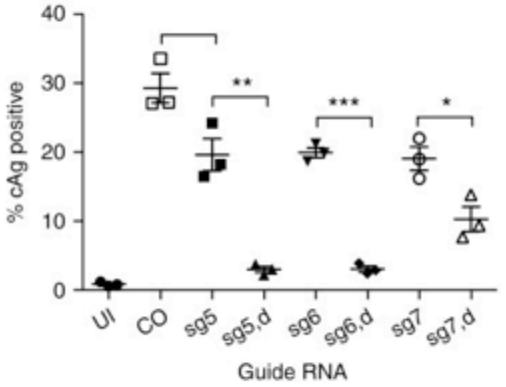
Targeting Hepatitis B Virus with CRISPR/Cas9

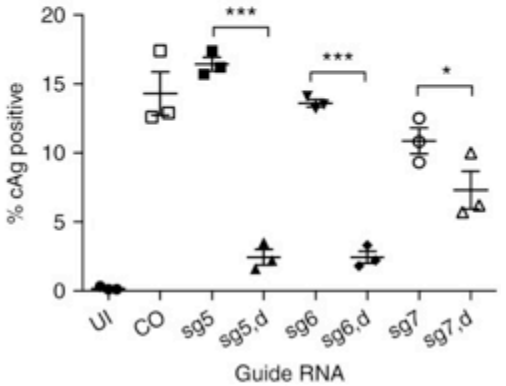
Induction of deletions in cccDNA

Decreased number of cells expressing viral antigens









Mol Ther Nucleic Acids. 2014; Seeger et al

HBV Minichromosomes and Chromatin Modelling

- Relaxed Chromatin

  Activation of Gene Expression : Histone Acetylase (HAT)

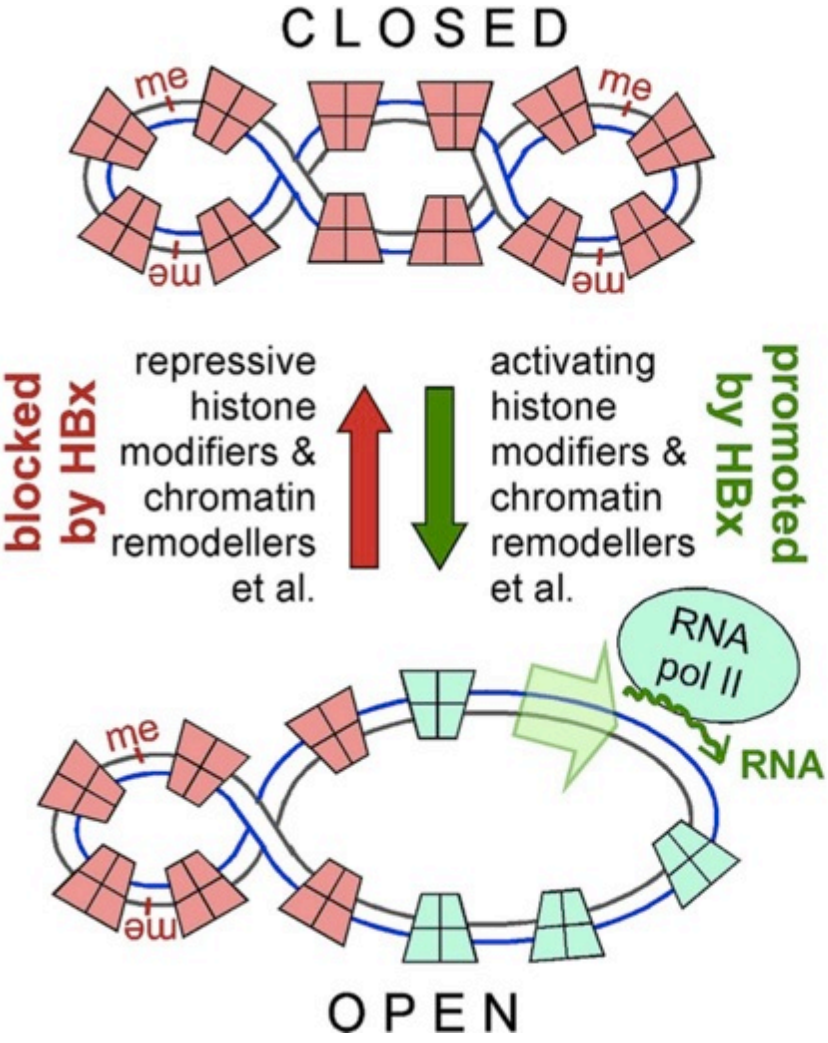
  - Transcription activation complex containing HATs
  - HATs acetylate lysine residues of the histone tails
- Compacted Chromatin

  Repression of Gene Expression : Histone Deacetylases (HDAC)

  - Transcription repression complex containing HDAC
  - HDACs deacetylate histone lysine tails
- Conclusion
  - Acetylation status of HBV minichromosome (cccDNA-bound H3 & H4 histones) regulates HBV transcription/replication and is reflected in viral load

Pollicino, T. et al 2006. Gastroenterology;130:823

Epigenetics of covalently closed circular (ccc)DNA
Regulation by viral proteins (HBc and HBx)



me, repressive DNA methy-lation

repressive nucleosome spacing

nucleosomes with repressive histone PTMs

nucleosomes with activating histone PTMs

activating nucleosome spacing

Silencing
Epigenome modifiers
Interferon alpha,
Capsid inhibitors,
Inhibition of HBx function

Pollicino et al. Gastroenteroplogy 2006
Levrero et al. J Hepatol, 2009
Lucifora et al, J Hepatol 2012
Belloni et al, PNAS 20O9
Belloni et al, J Clin Invest 2012
Decorsière et al, Nature 2016

Nassal, M 2015. GUT; 64(12):1972-1984

haematologica
the hematology journal

Haematologica 2009 [Epub ahead of print]

doi:10.3324/haematol.2009.008607

BRIEF REPORTS

Reactivation of DNA viruses in association with histone deacetylase inhibitor (HDI) therapy: a case series report

David Ritchie,[1] Richard L. Piekarz,[2] Piers Blombery,[1] Laszlo J. Karai,[3] Stefania Pittaluga,[3] Elaine S. Jaffe,[3] Mark Raffeld,[3] John E. Janik,[4] H Miles Prince,[1] and Susan E. Bates[2]

[1]Department of Haematology and Medical Oncology, Peter MacCallum Cancer Centre and University of Melbourne, Melbourne, Victoria, AUSTRALIA; [2]Medical Oncology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA; [3]Laboratory of Pathology, Hematopathology Section, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, and [4]Metabolism Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

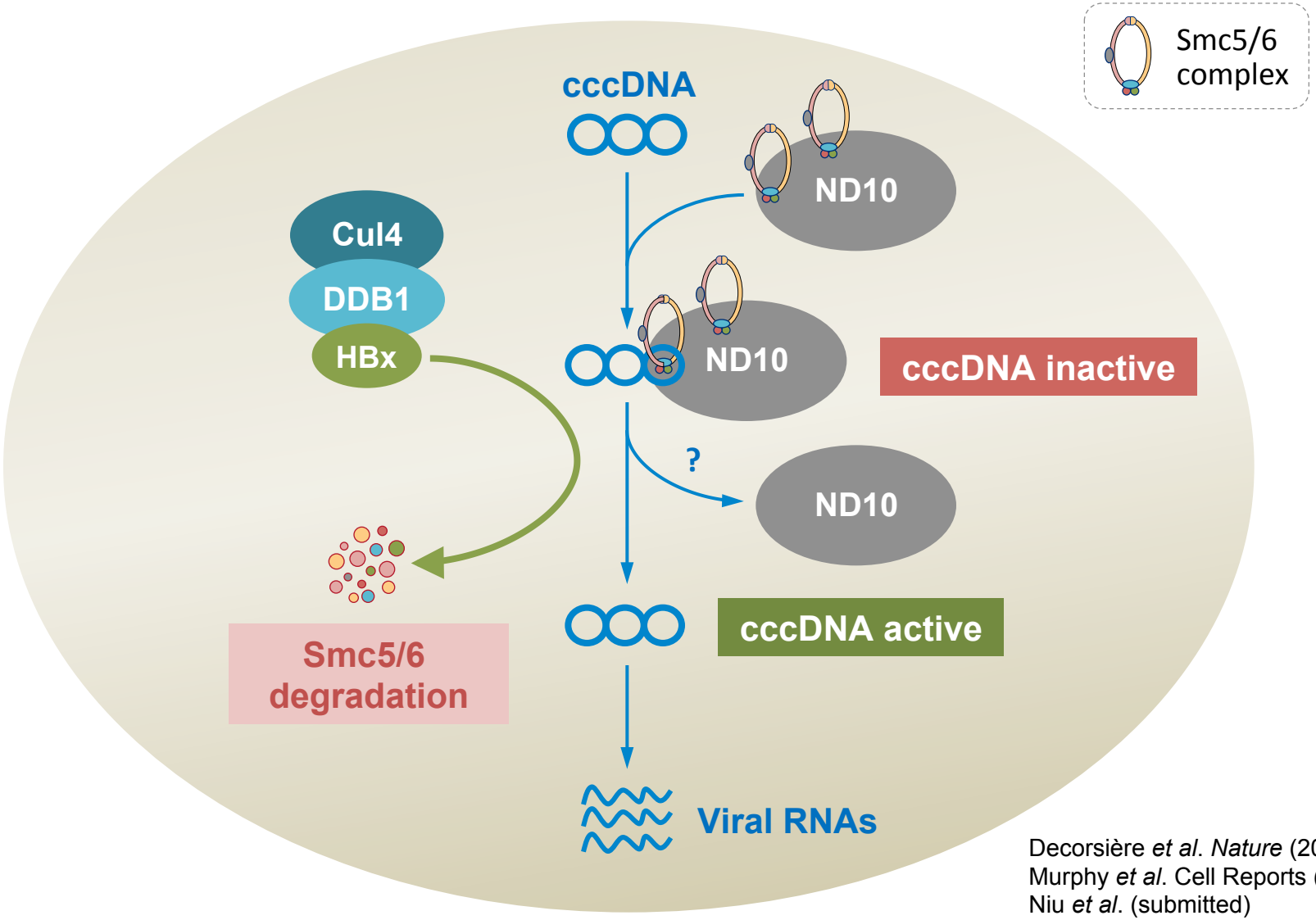
HBx Induces Degradation of the Structural Maintenance of Chromosomes (SMC) Complex

Hepatitis B virus X protein identifies the Smc5/6 complex as a host restriction factor

Adrien Decorsière[1]*, Henrik Mueller[1]†*, Pieter C. van Breugel[1]†*, Fabien Abdul[1]*, Laetitia Gerossier[2], Rudolf K. Beran[3], Christine M. Livingston[3], Congrong Niu[3], Simon P. Fletcher[3], Olivier Hantz[2] & Michel Strubin[1]

2016. Nature;531:386-389

HBx Promotes the Degradation of the Smc5/6 Complex to Prevent Silencing of cccDNA



Decorsière *et al*. *Nature* (2016)
Murphy *et al*. Cell Reports (2016)
Niu *et al*. (submitted)

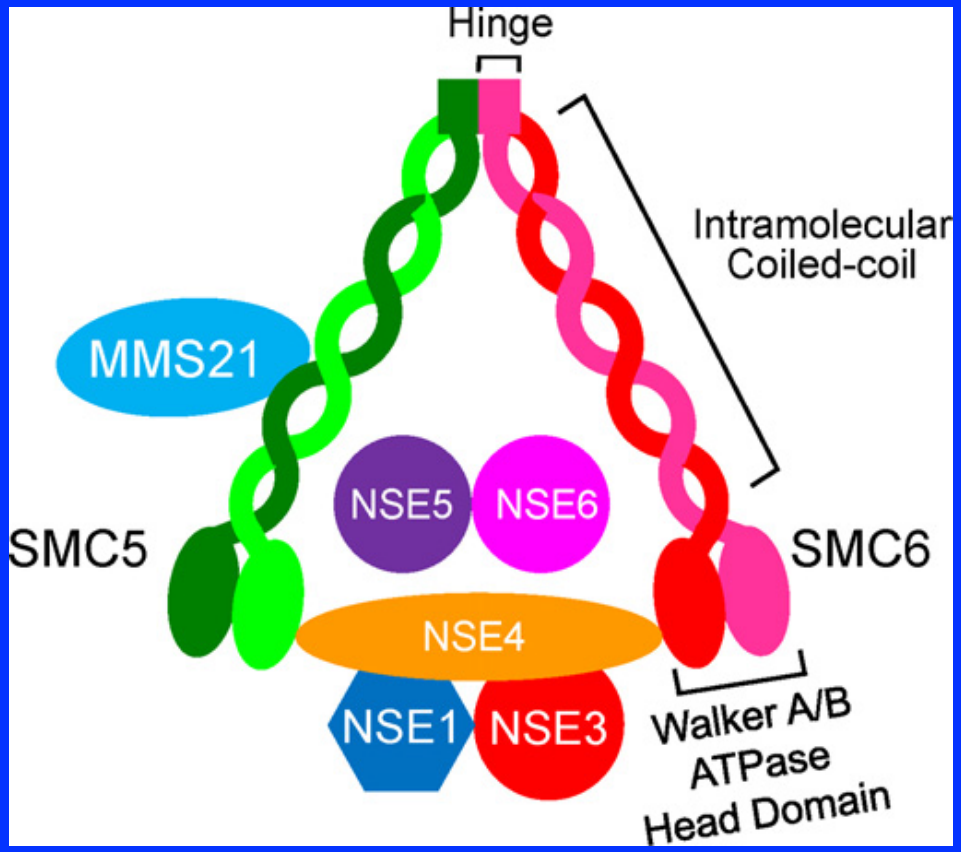
The Smc5/6 Complex

Structural Maintenance of Chromosomes (Smc) Complexes

- Condensin
- Cohesin
- Smc5/6 complex

Smc5/6

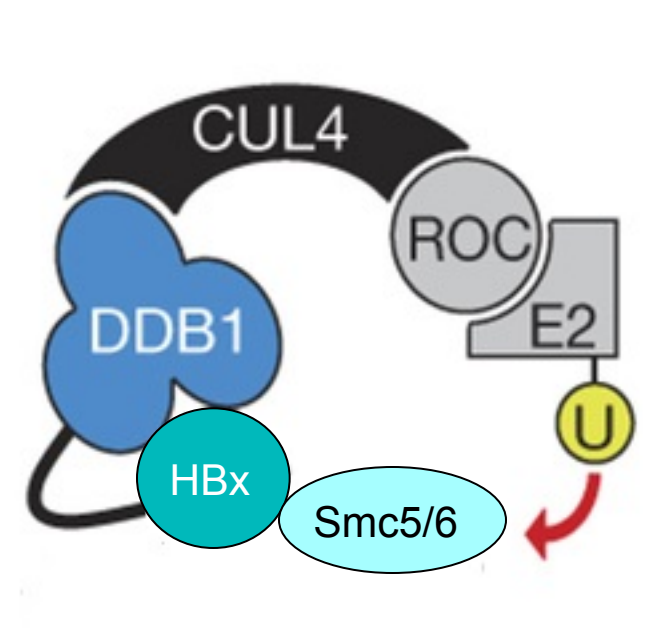
- Nuclear complex
- DNA repair
- Chromosome topology and organization

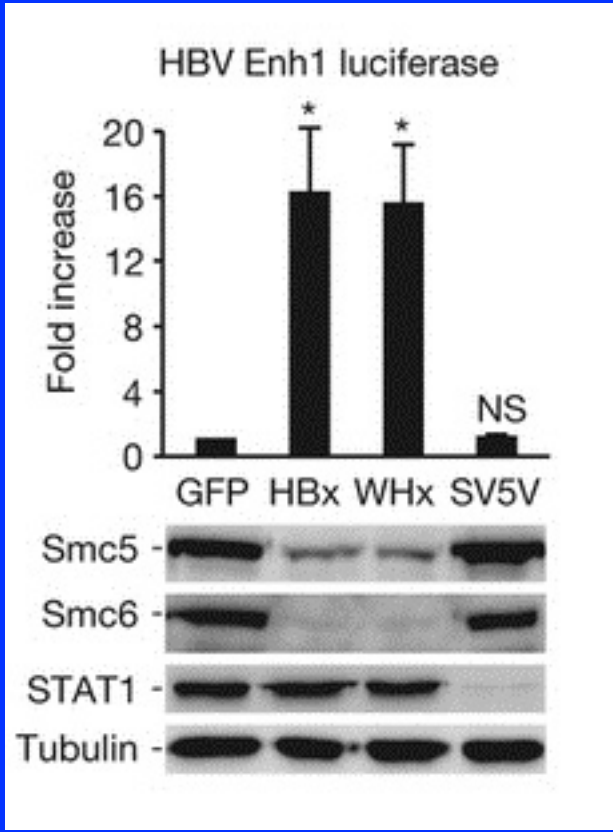


Potts, PR. 2009. DNA Repair;8:499

- Depletion of any subunit results in destabilization of the complex *(Taylor, 2008)*

HBx Induces Degradation of Smc5/6

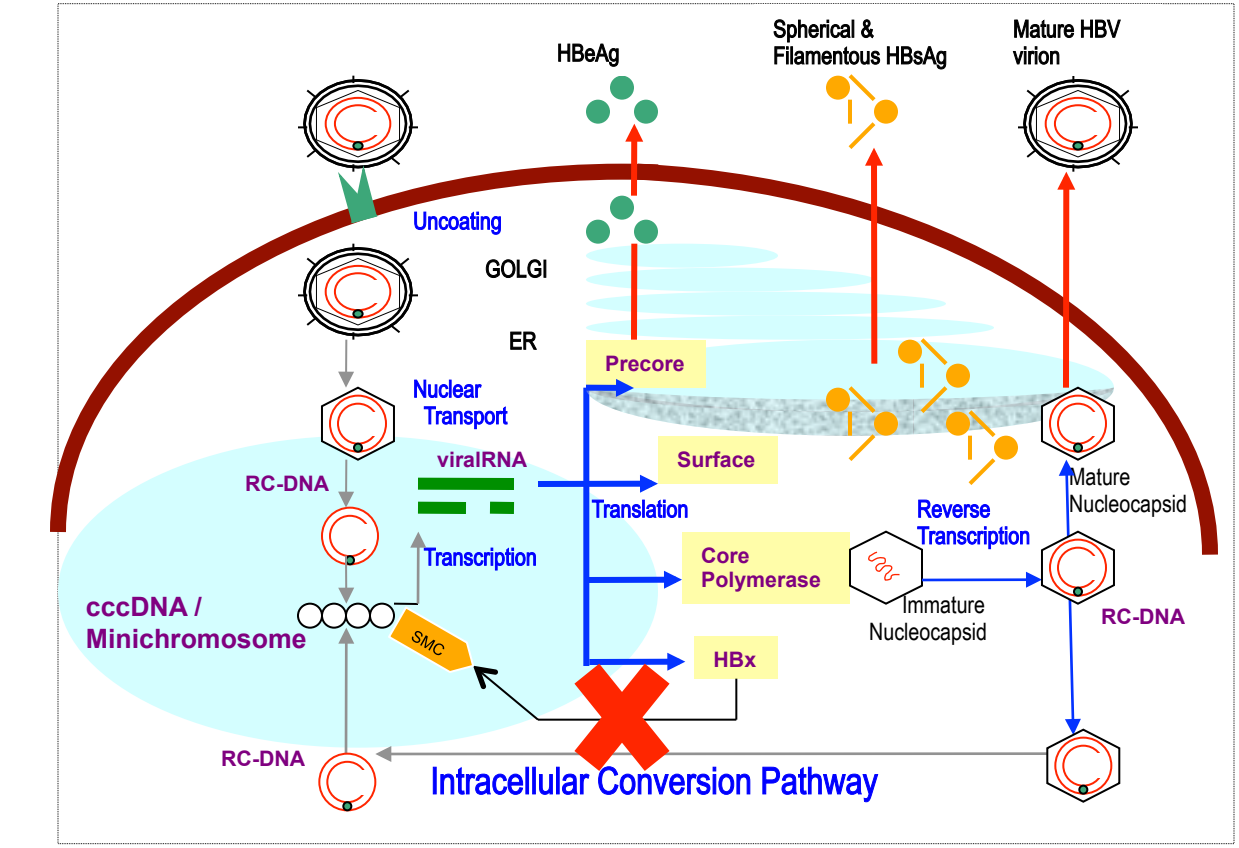




- The effect is rapid
- Blocked by proteasome inhibitors
- Blocked by E3 ligase inhibitors
- No changes in Smc5/6 mRNA levels

Decorsiere, A et al 2016. Nature;531:386-389

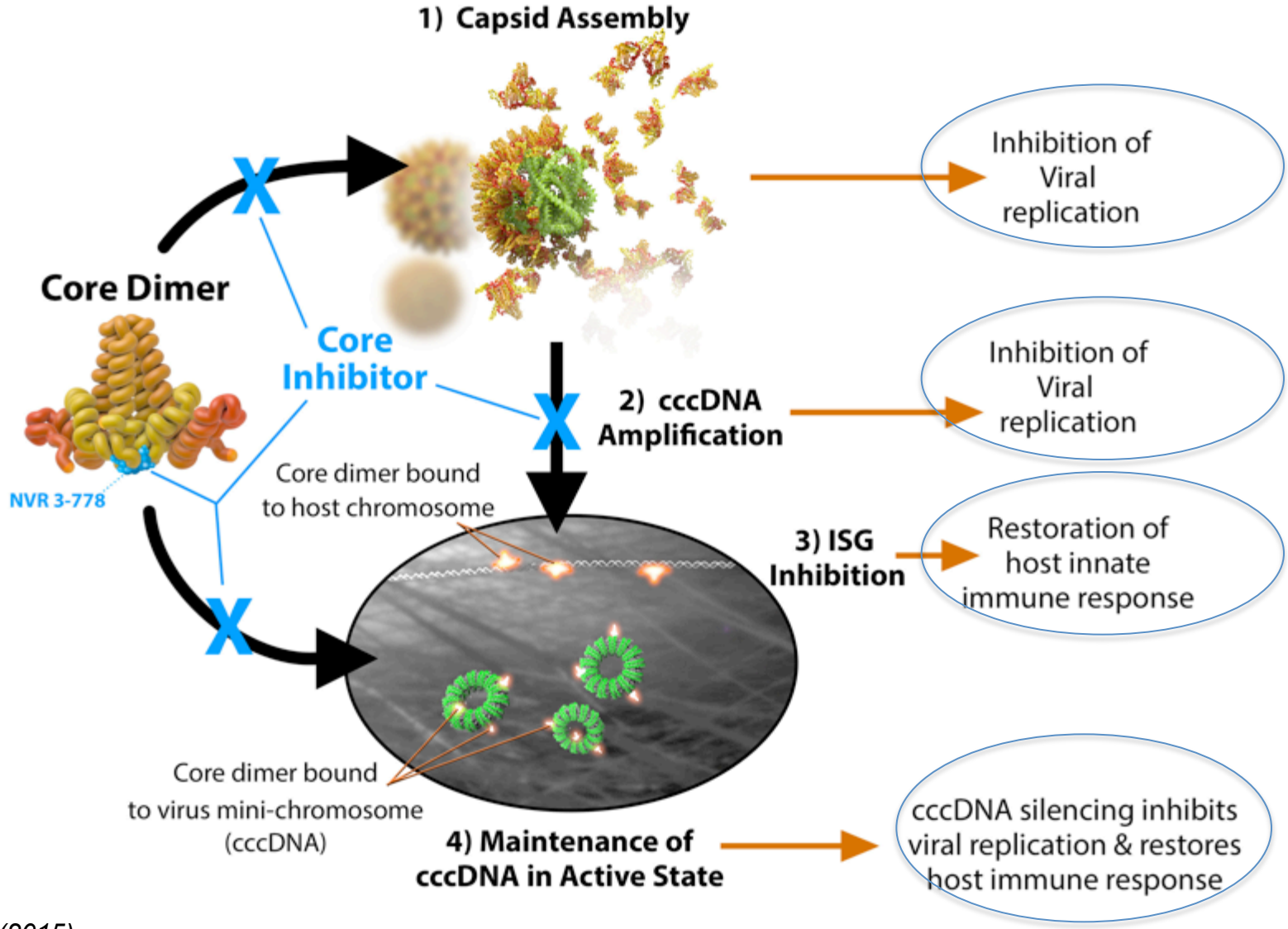
HBV Replication: cccDNA Pathway



1. RC DNA → cccDNA
   - DNA repair
   - TDP-2
2. HBeAg (early protein)
   - synthesised from precore mRNA

Koniger, C et al 2014. Proc Natl Acad Sci;111:4244

Targeting the HBV Nucleocapsid



Zoulim, F (2015)

Targeting HBV Nucleocapsids

Heteroaryldihydropyrimidines

Destabilization of nucleocapsids

Deres et al, Science 2003
Klumpp et al, PNAS 2015

Phenylpropenamide derivatives

Prevent pgRNA encapsidation

Feld, J et al 2007. AVR; 76:168-177

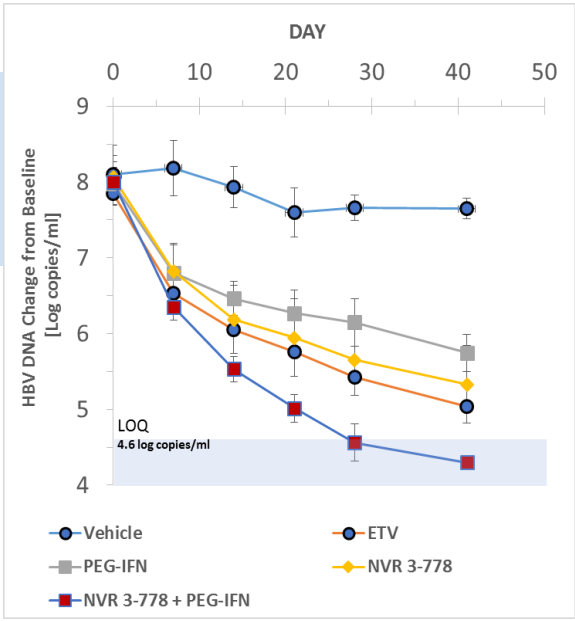
Antimicrob Agents Chemother. 2002.

Novel classes of capsid inhibitors based on the 3D structure of HBc
Novira, Assembly Biosciences, Janssen, Roche, and others
Phase 1 studies with Novira completed

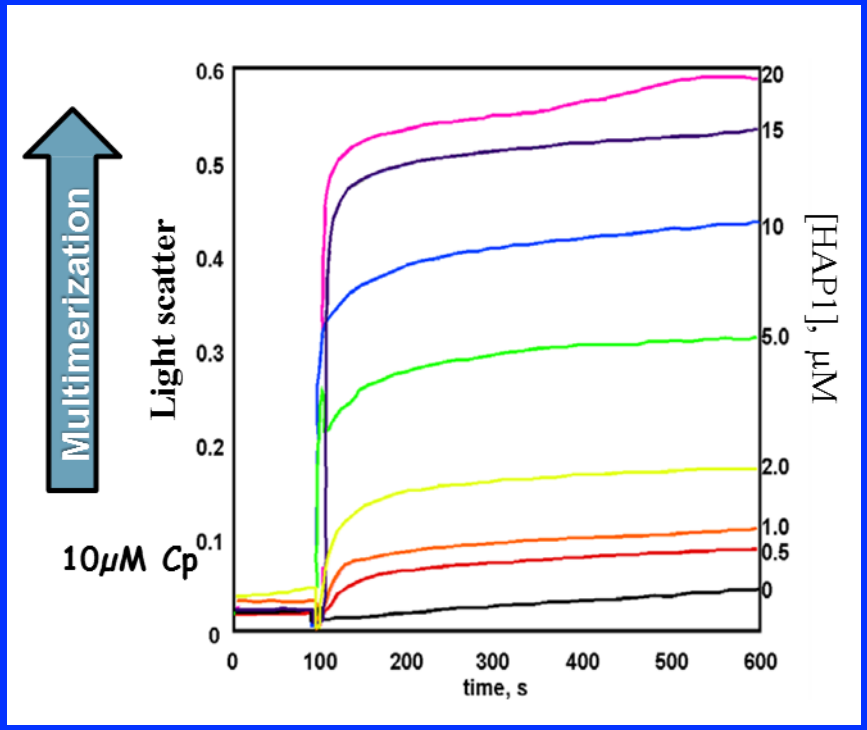
Lam A, et al. AASLD 2015, San Francisco. #33

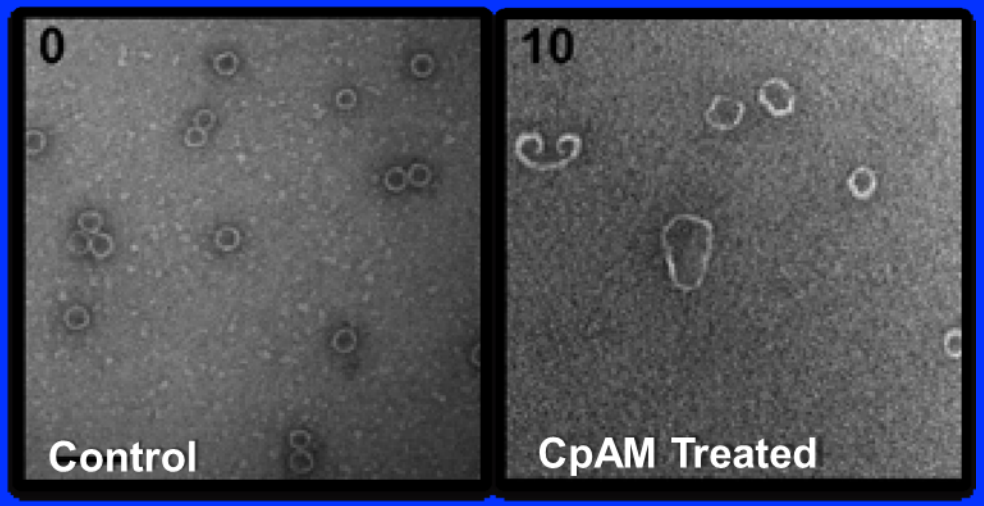
[sulphonamide/sulfamoyl benzimide derivatives]

CpAM: Core Protein Allosteric Modifier



CpAMs Accelerate/Promote Capsid Assembly

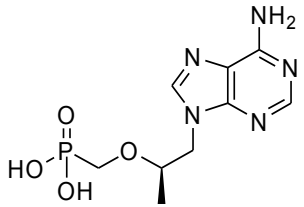


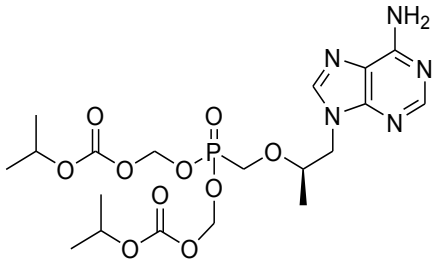


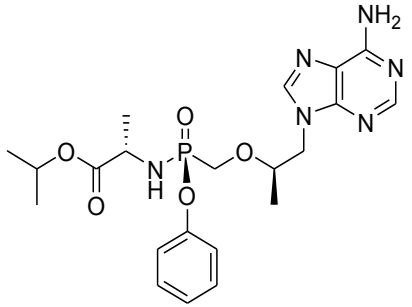
Stray et al 2005. PNAS102, 8138-43

Reverse Transcription: Improved Potency of NA Tenofovir Alafenamide (TAF)

- TAF = orally bioavailable phoshonoamidate prodrug of tenofovir (TDF
- In comparison with tenofovir, TAF enables enhanced delivery of the parent nucleotide and its active diphosphate metabolite into lymphoid cells and hepatocytes.
- This is attributed to an improved plasma stability and differential intracellular activation mechanism for TAF relative to TDF

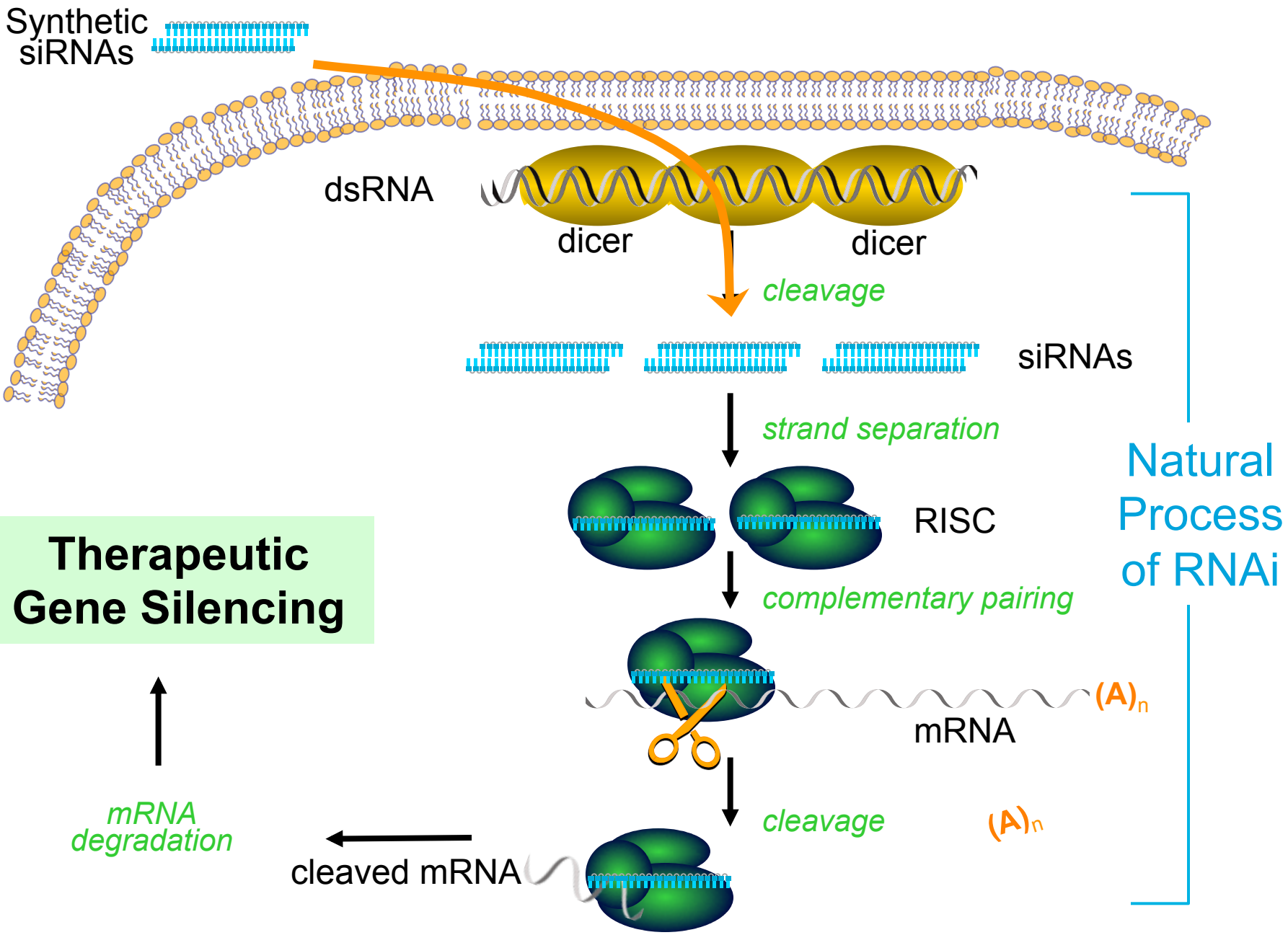




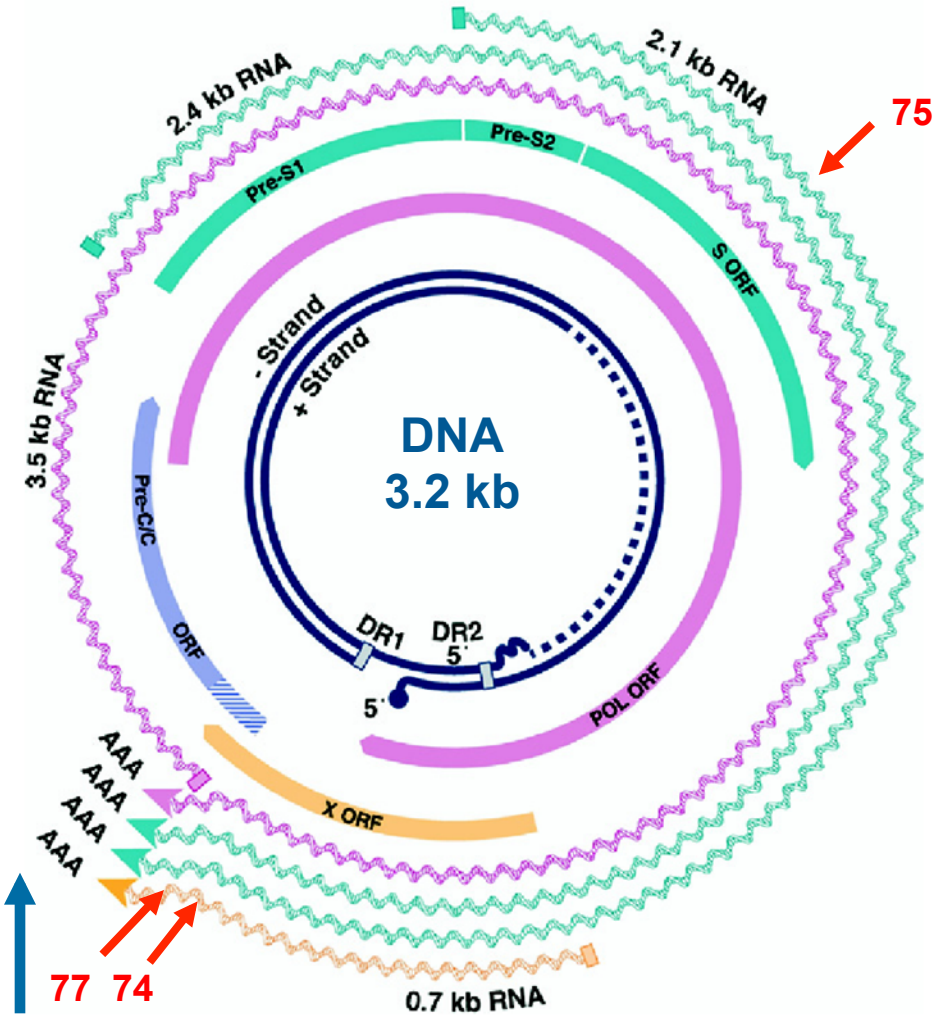


EC_{50} HIV-1 (PBMCs)	Tenofovir	Tenofovir Disoproxil	TAF
	1.2 µM	0.015 µM	0.003 µM

Mechanism of RNA Interference (RNAi)



HBV genome and siRNA target sites



Same polyadenylation signal for all mRNAs

- **HBV mRNA**
  - 3.5 kb pre-genomic RNA
  - 3.5 kb pre-core mRNA
  - 2.4 kb pre-S1 mRNA
  - 2.1 kb pre-S2/S mRNA
  - 0.7 kb X mRNA
- **HBV proteins**
  - Polymerase (with reverse transcriptase function)
  - Core (HBcAg), forms capsid
  - E antigen (HBeAg), also called pre-core, a secreted protein
  - Large, middle and small surface proteins (HBsAg), form envelope
  - X protein (Transactivator)

Ghany & Liang (2007), *Gastroenterology* **132**: 1574-1585

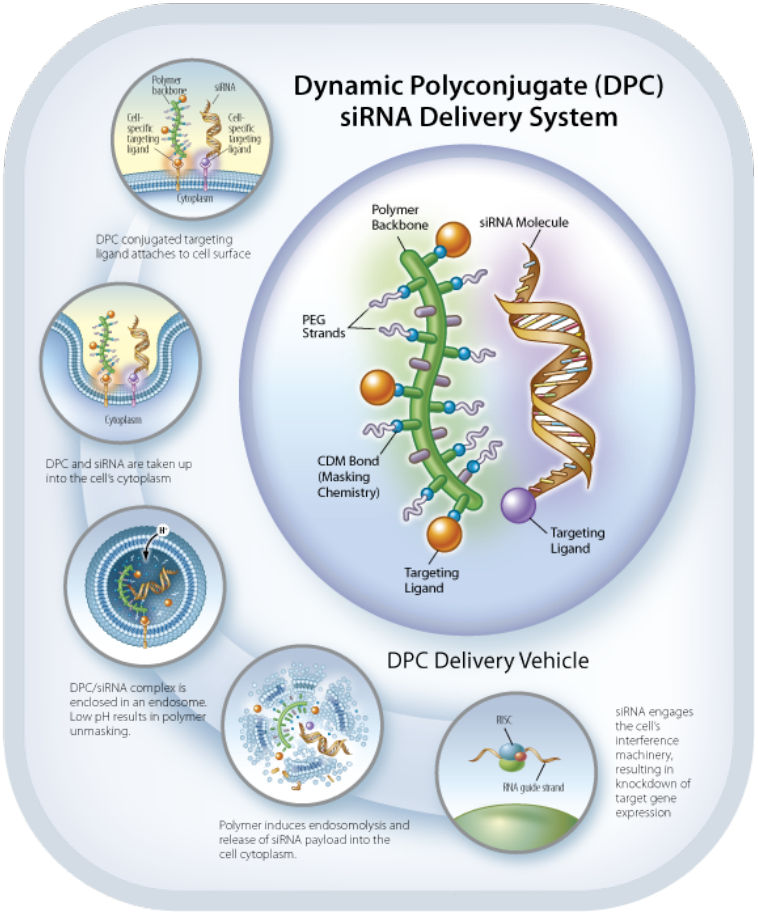
Groups Involved in RNAi Therapy and HBV

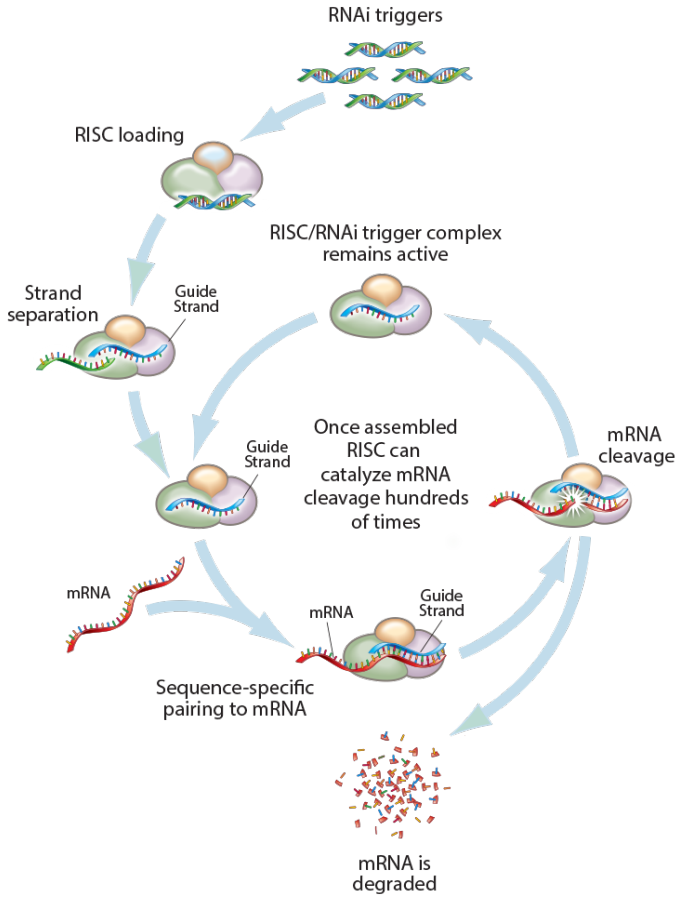
- Arrowhead Pharmaceuticals
  - ARC-520 (phase 2)
  - ARC-521 (phase 1/2)
- Arbutus Biopharma
  - ARB-1467 (phase 1/2)
- Alnylam Pharmaceuticals
  - ALN-HBV (phase 1)

ARC-520 RNAi delivery technology

DPC polymer composition: amphipathic peptide with reversibly "masked" amines

- Ligand-driven cellular uptake of (N-acetyl galactosamine for hepatocytes)
- Liver tropic siRNA attachment by lipophilic ligand (e.g. cholesterol)
- ↓ pH in endosomes unmasks peptide to disrupt endosomal membrane
- siRNA released to cytoplasm

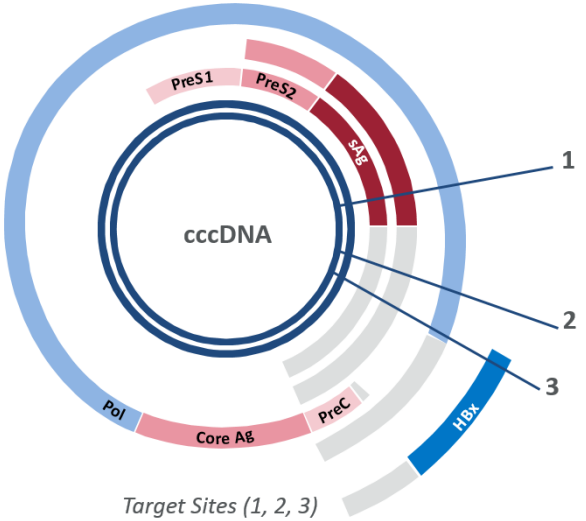




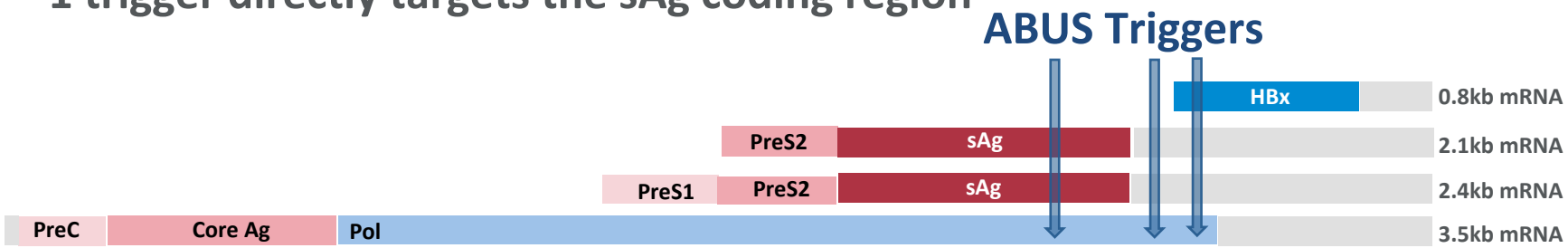
From: ArrowheadPharma.com/science

ARB-1467 Targets Multiple HBV Genomic Sites

- Primary viral target is HBsAg
- Target sites are regions of high conservation in HBV viral genomes
- Advantages of the **3-trigger combo:**
  - Increased potency
  - Coverage extension to 99.8% of HBV genotypes
  - Targets all HBV transcripts and prevents production of all antigens
  - 1 trigger directly targets the sAg coding region



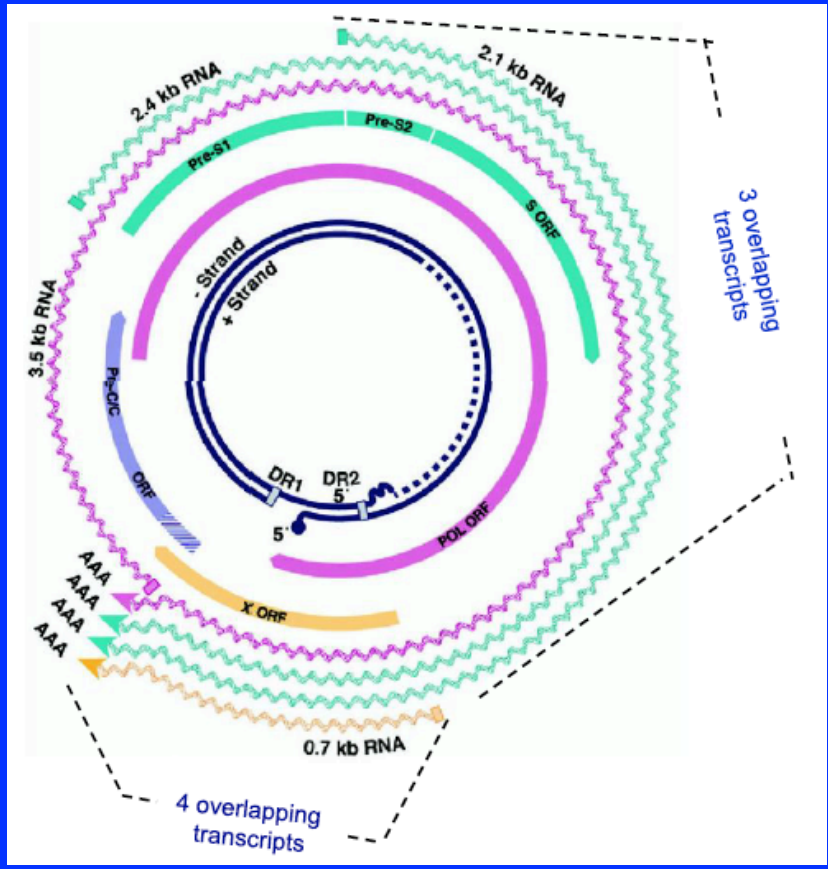
Target Sites (1, 2, 3)



Kindly provided by Dr Mike Sofia

Alnylam RNAi (Preclinical)

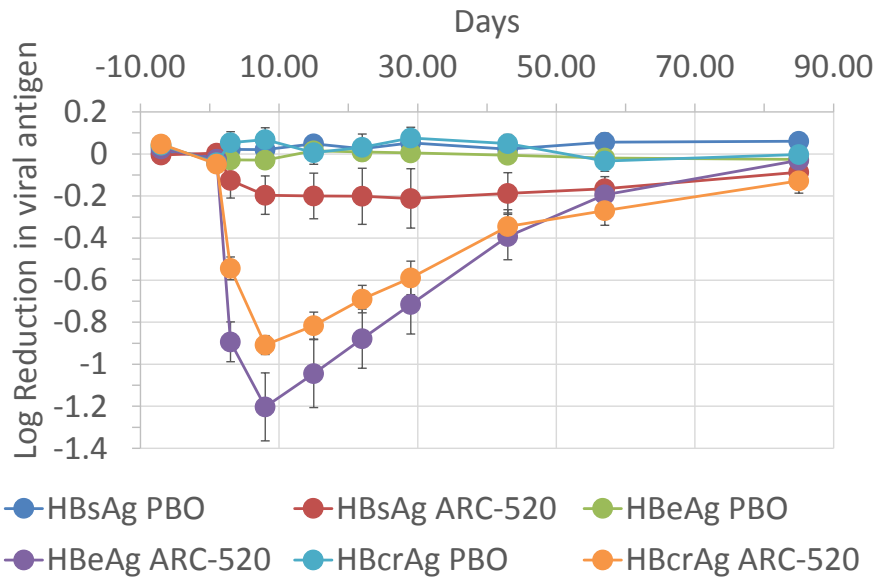
- Delivery: **Multi-component lipid nanoparticles** for delivery to the liver via LDL receptor
- Triantennary Gal/Nac conjugated to 3' end of sense strand of siRNA
- Two target regions:
  - 0.7 kb region overlapping across all 4 HBV transcripts.
  - 1.4 kb region overlapping across 3 transcripts
    - Inhibits replication, assembly and secretion of virus as well as subviral antigens that overlaps across 3 HBV transcripts



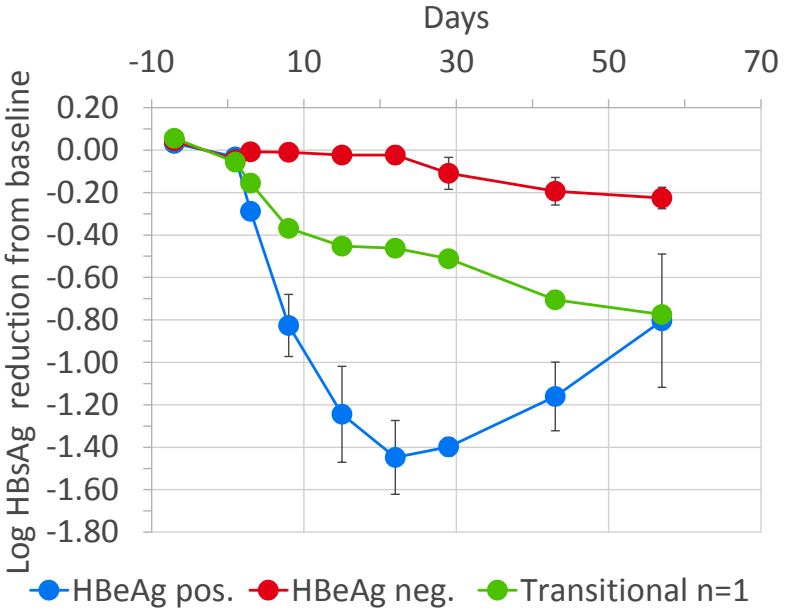
http://www.alnylam.com/

ARC-520 Produces Deep and Durable Knockdown of Viral Antigens and DNA in a Phase II Study in Patients with Chronic Hepatitis B

HBV antigen reduction in ETV experienced HBeAg-positive patients with a single 4 mg dose (cohort 5)



HBsAg reduction in ETV naïve patients with a single 4 mg dose (cohort 7)



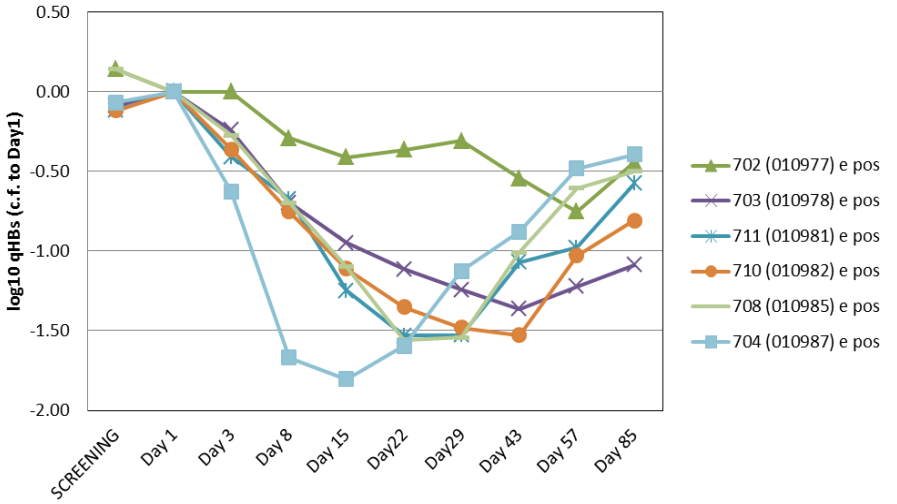
Direct antiviral effect lasted up to 57 days after a single dose of ARC-520, delayed response duration >85 days

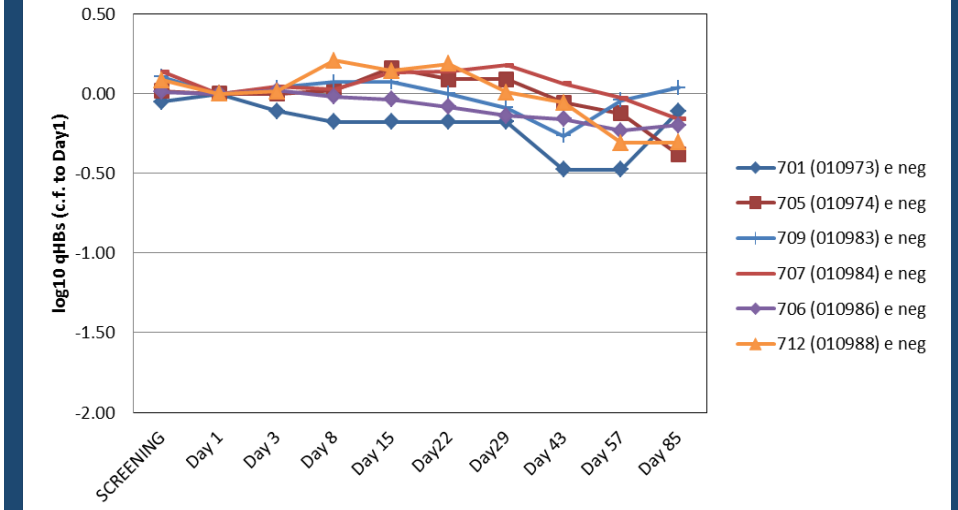
- Small dose-related reduction in HBsAg
- Maximum effective dose not reached
- HBV DNA results pending in ETV naïve patients

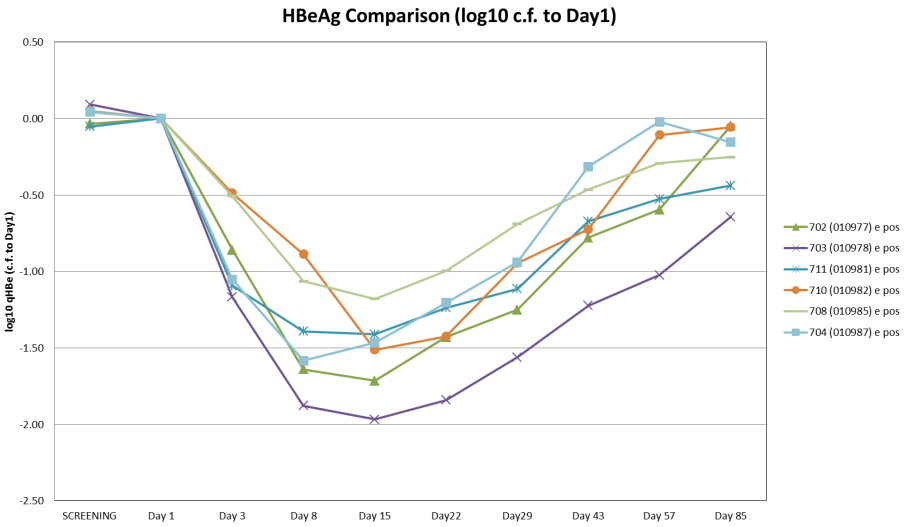
Yuen M-F, et al. AASLD 2015, San Francisco. #LB-9

ARC-520 RNAi: clinical responses

NUC naïve cohort (n=12): 50% HBeAg positive, 1x 4mg dose







HBsAg:

- > 1log drop in HBsAg achieved by all HBe pos subjects

(excluding 702; transitional HBe <0.1 PE IU/mL at BL)

HBeAg:

- > 1log decline in HBeAg achieved

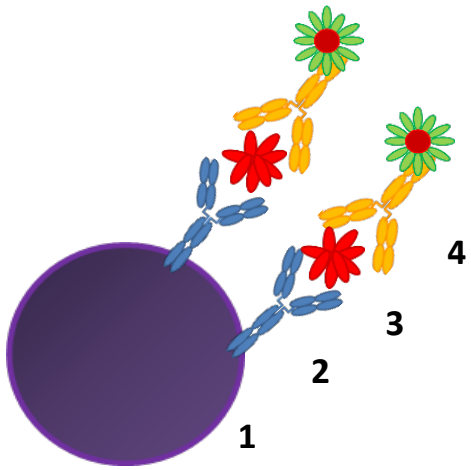
Two Predictive Biomarkers of Functional HBV cure

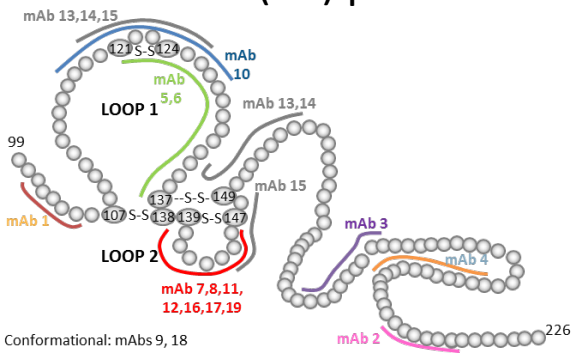
HBsAg epitope mapping 19plex immunoassay to identify a Clearance Profile (CP) predictive of HBsAg clearance

ASSAY 1

HBsAg Epitope Mapping

19 mAbs





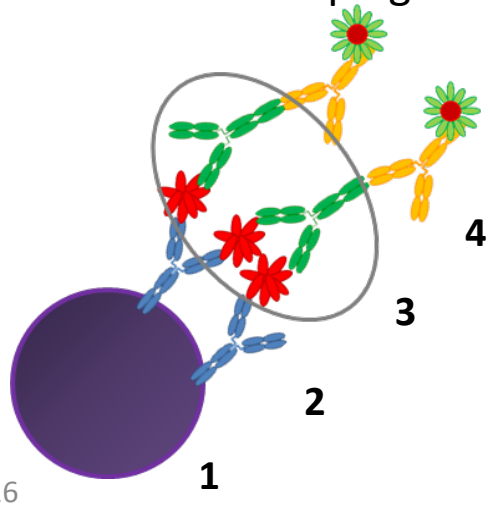
1. Magnetic bead
2. Capture Ab: 19plex mouse anti-HBs mAbs to HBsAg 'a' determinant
3. Patient HBsAg sample
4. Reporter Ab: PE conjugated polyclonal anti-HBs

Walsh R, et al. AASLD 2015

Immuno-detection of the developing anti-HBs response (complexed to HBsAg)

ASSAY 2

Complexed Anti-HBs



1. Magnetic bead
2. Capture Ab: mouse anti-HBs mAbs to control epitopes (C-term, Combo Loop1/2)
3. HBsAg/anti-HBs complexed (*patient sample)*
4. Reporter Ab: HRP conjugated Goat anti-Human IgG Fc

Complexed HBsAg/anti-HBs must be present in the tested sample to get reporter binding (absorbance)

Walsh R, et al. EASL 2016

Assays validated against G103 cohort, TDF registration (Marcellin, P. et al. 2008. NEJM 359, 2442)

In a Treatment Naïve Cohort of Genotype A Chronic Hepatitis B (CHB) Patients Receiving Tenofovir Disoproxil Fumarate (TDF) Therapy (TF103 Trial):

HBsAg clearance profile (CP)

HBsAg epitope pressure (reduced recognition) at *both* loop 1 **AND** loop 2 epitopes

- associated with HBsAg response/decline (>1log) and potentially HBsAg loss/ seroconversion

HBsAg non-clearance (or escape) profile (NCP)

No change in HBsAg epitope profile, OR reduced epitope binding at *only* one loop

- associated with no HBsAg response/decline (<1log)

Conclusion/Findings

Significant association ($p < 0.02$) between the development of a HBsAg CP and HBsAg Loss/Seroconversion [PPV 83%] by 48 weeks of treatment

Walsh, R and Locarnini, S (2015), AASLD presentation

Synopsis: ARC-520 effect on HBsAg CP

Identification of an HBsAg CP during the ARC-520 treatment cohorts trials 1-5:

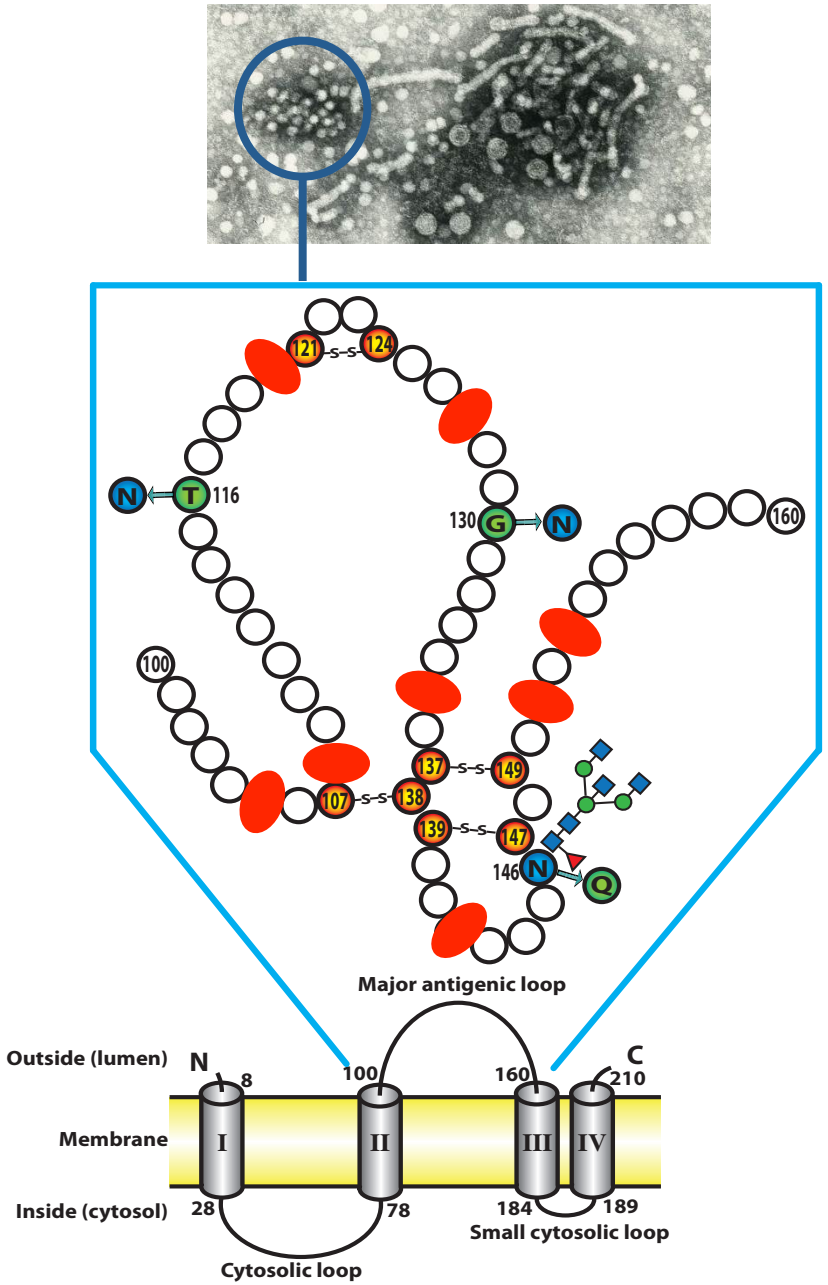
Cohort	HBeAg	BL (pre-treat)	W1	W2	W3	W4	W6	W8	W12
1 (ARC, n=6)	neg	4	1	1	1	1	2	nt	0
1 (placebo, n=2)	neg	2	0	0	0	0	0	nt	0
2 (ARC, n=6)	neg	3	1	3	3	3	1	0	0
2 (placebo, n=2)	neg	1	0	0	0	0	0	0	0
3 (ARC, n=6)	neg	3	3	5	4	4	4	1	1
3 (placebo, n=2)	neg	2	0	0	0	1	0	0	1
4 (ARC, n=6)	neg	2	4	2	4	4	4	2	2
4 (placebo, n=2)	neg	0	0	0	0	0	0	1	0
5 (ARC, n=6)	pos	1	2	1	4	3	3	2	2
5 (placebo, n=2)	pos	0	0	0	0	1	0	0	0

Cohorts 1-5	BL (pre-treat)	W1	W2	W3	W4	W6	W8	W12
ARC (n=30)	13	11	12	16	15	14	5 (of 24)	5
placebo (n=10)	5	0	0	0	2	0	1 (of 8)	1
p-value	0.730	**0.038**	**0.019**	**0.003**	0.145	**0.007**	1.000	1.000

There is a significant association between development of a HBsAg CP and ARC-520 RNAi treatment at multiple timepoints

Walsh, R et al 2016. J Hepatol;64:S602 [FRI-144])

Immune Regulation by HBsAg



- HBsAg secreted in vast excess over virions (>10^3 fold)
- Circulate in blood 100-400 μg/ml (1% of total serum protein)
- Unique conformational structure (8 cysteines ● and 8 prolines ⬮)
- Associated with increased risk of HCC *(Yuen, MF. et al 2008. Gastro;135:1192–1199)*
- Plays a key role in HBV persistence
- Suppress both innate (TLR-2, TLR-9 and IFN-α) as well as adaptive (mDC) responses to infection

Wang, S et al 2013. J Immunol;190:5142.; Xu, Y et al 2009. Mol Immunol;46:2640.; Op den Brouw, ML et al 2009. Immunol;126:280.

Importance of HBsAg Clearance/ Seroconversion

- ↓ Hepatic decompensation
- ↓ HCC
- ↑ Survival
- ↓ Levels of cccDNA
- **As close to cure as we can expect to achieve in chronic hepatitis B**

Fattovich G, et al. Am J Gastro 1998; 93:896-900. Werle-Lapostolle B, et al. Gastroenterology 2004; 126(7):1750-1758. Perrillo R. Hepatology 2009; 49:1063-1065

OPEN ACCESS Freely available online

PLoS one

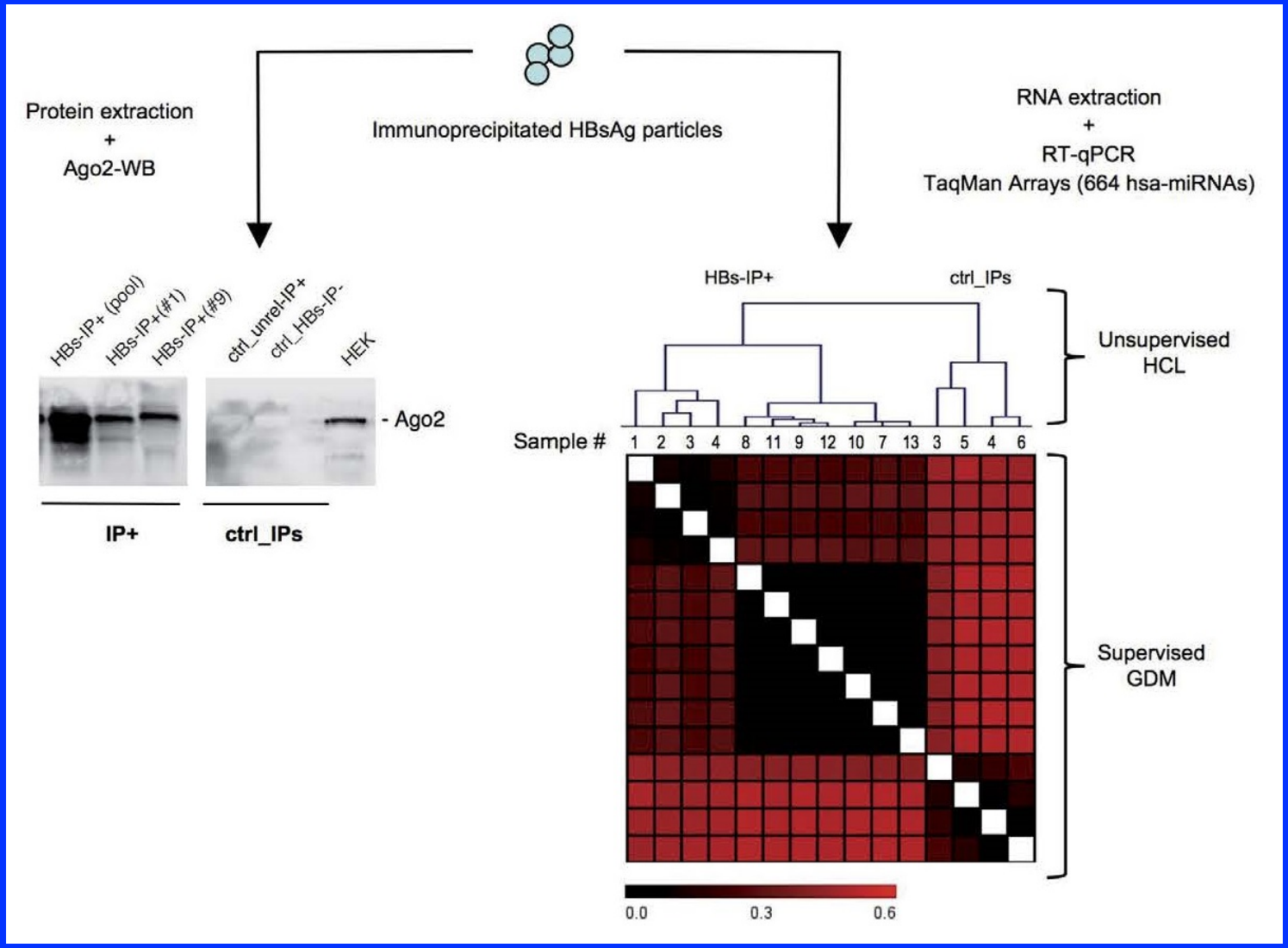
Circulating Hepatitis B Surface Antigen Particles Carry Hepatocellular microRNAs

Luisa Novellino[1], Riccardo L. Rossi[2], Ferruccio Bonino[3]*, Daniela Cavallone[1], Sergio Abrignani[2], Massimiliano Pagani[2], Maurizia R. Brunetto[1]

1 Hepatology Unit and Liver Physiopathology Laboratory, University Hospital of Pisa, Pisa, Italy, **2** Department of Integrative Biology, Istituto Nazionale di Genetica Molecolare (INGM), Milan, Italy, **3** General Medicine Unit 2, Department of Internal Medicine, University Hospital of Pisa, Pisa, Italy

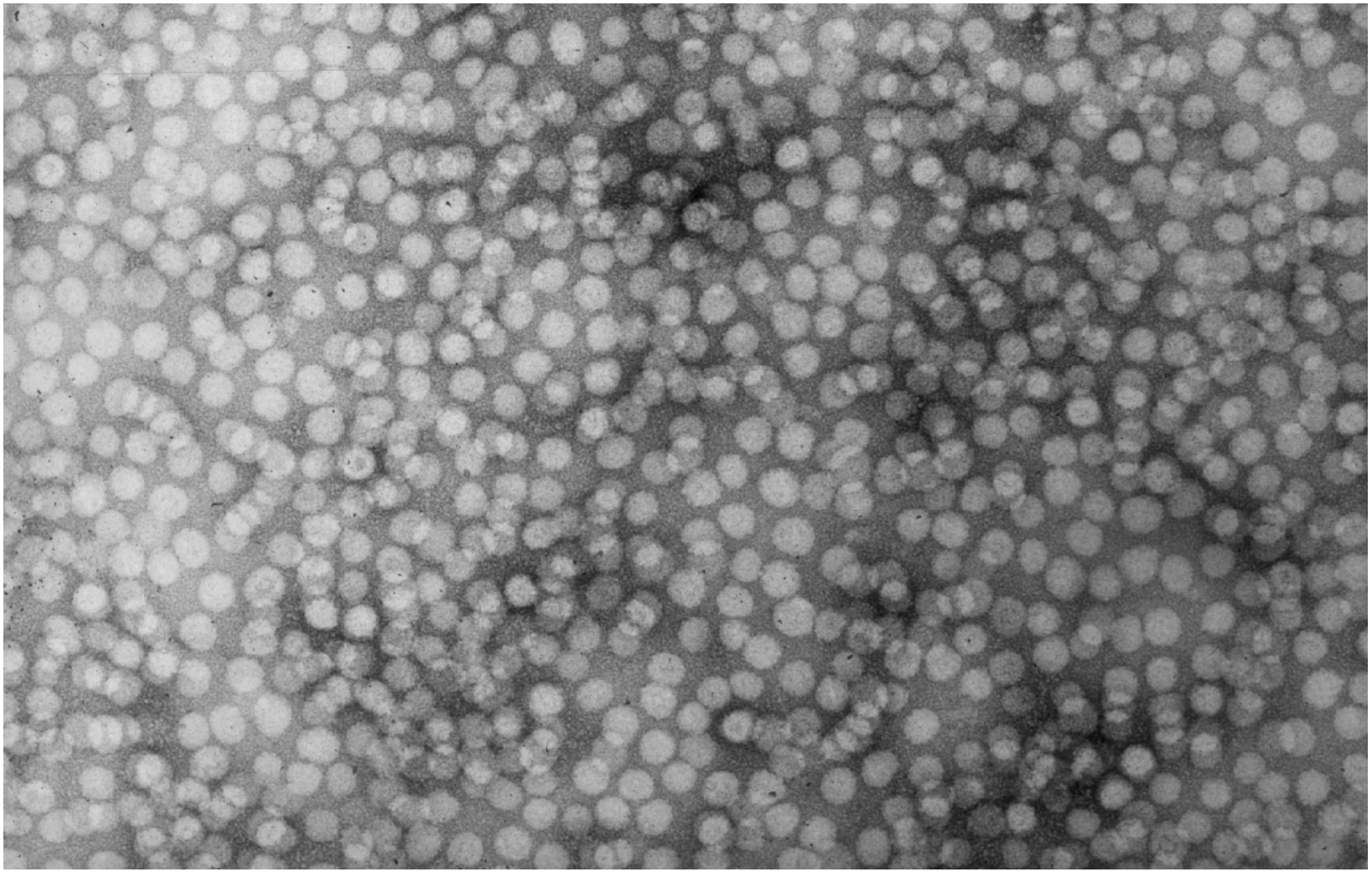
March 2012. Volume 7, Issue 3: e31952

Differences in Western Blotting and Human miRNA Analyses Between Immunoprecipitated HBsAg Particles and Control Immunoprecipitations



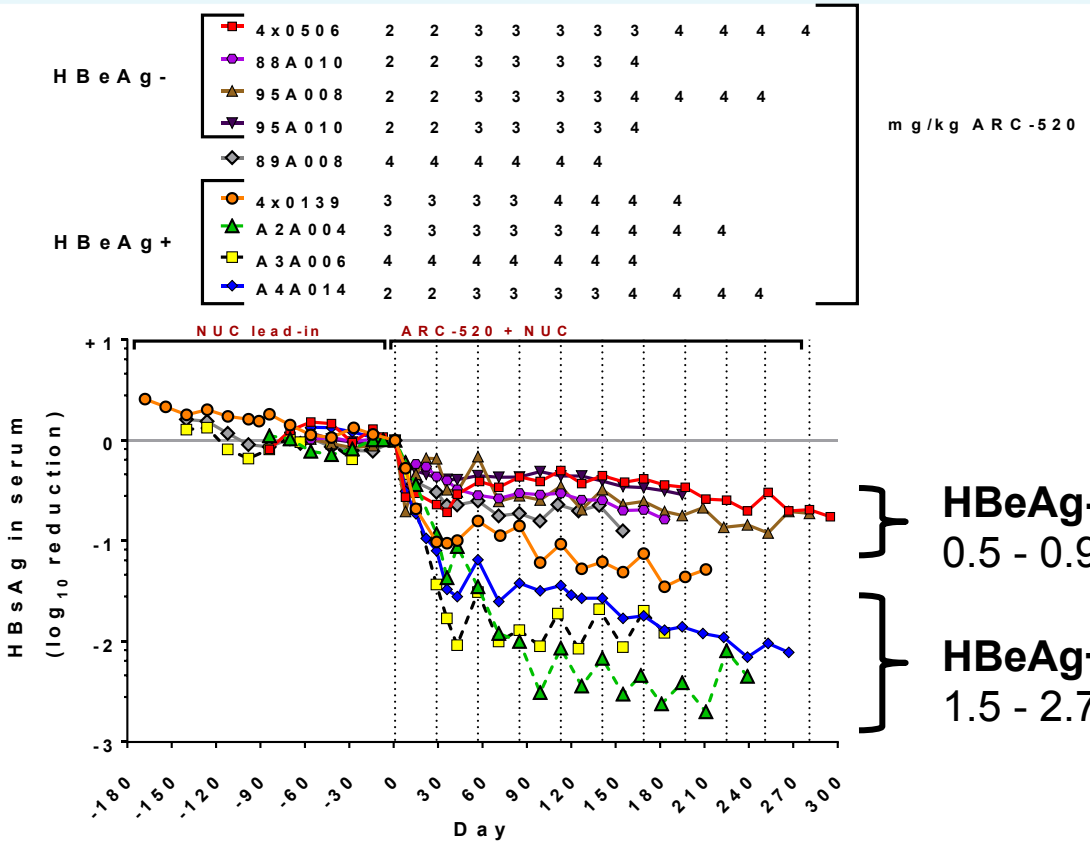
Novellino, L et al 2012. PLoS ONE;7(3):e31952

20 nm-Particles associated with Australia-Antigen



W. Gerlich, 1971

HBsAg reduction correlated with HBeAg status



HBeAg-
0.5 - 0.9 $\log_{10}$ reduction at nadir

HBeAg+
1.5 - 2.7 $\log_{10}$ reduction at nadir

- Similar phenomenon was observed in human HBV patients
- *What accounts for the difference in response between HBeAg positives vs. negatives?*

Wooddell C, Schluep T, Given B. With permission, 2016

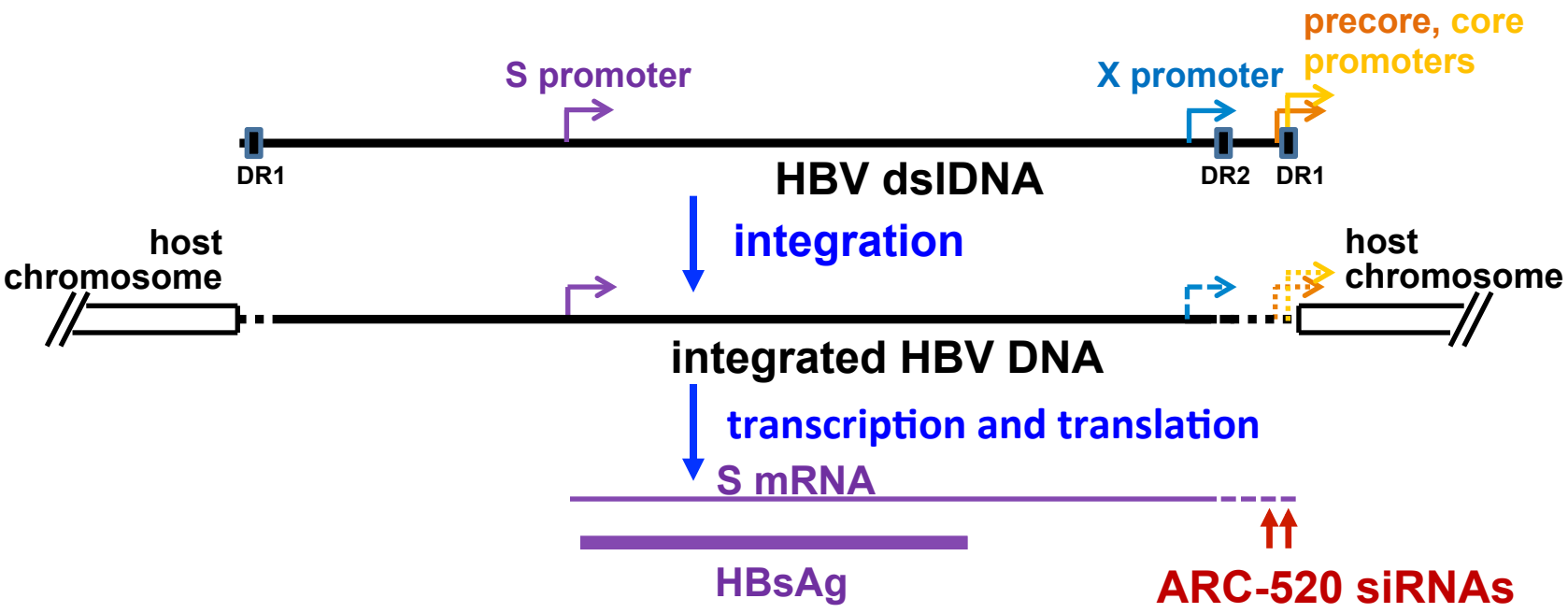
Novel finding: Predominant liver HBV DNA differs in HBeAg neg and HBeAg pos chimps

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, Schluep T, Given B. With permission 2016

HBV integration into the host genome



1. HBV DNA integrates into host chromosome, during which regions between DR2 and DR1 can be randomly deleted (not new!)

2. Significant HBsAg mRNA can be produced from integrated HBV DNA
   - These S transcripts contain complete HBsAg CDS
   - Expected loss of ARC-520 target sites in many

Wooddell C, Schluep T, Given B. With permission, 2016

HBV transcripts in HBeAg+ vs. HBeAg- chimps prior to ARC-520 treatment

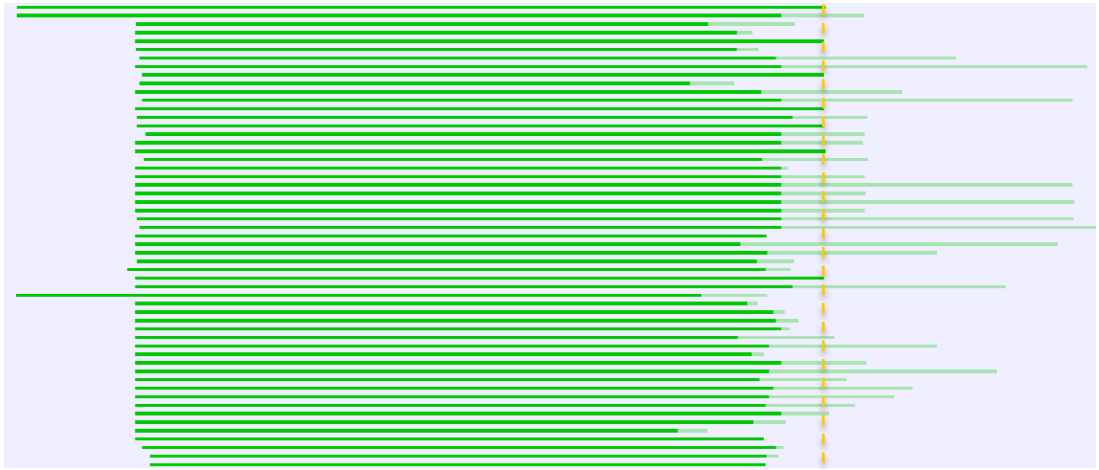
PacBio Single Molecule Real-Time (SMRT) Sequencing

A2A004
HBeAg+

HBeAg+

- Mostly non-fusion transcripts terminating near HBV poly(A) signal as expected

88A010
HBeAg-



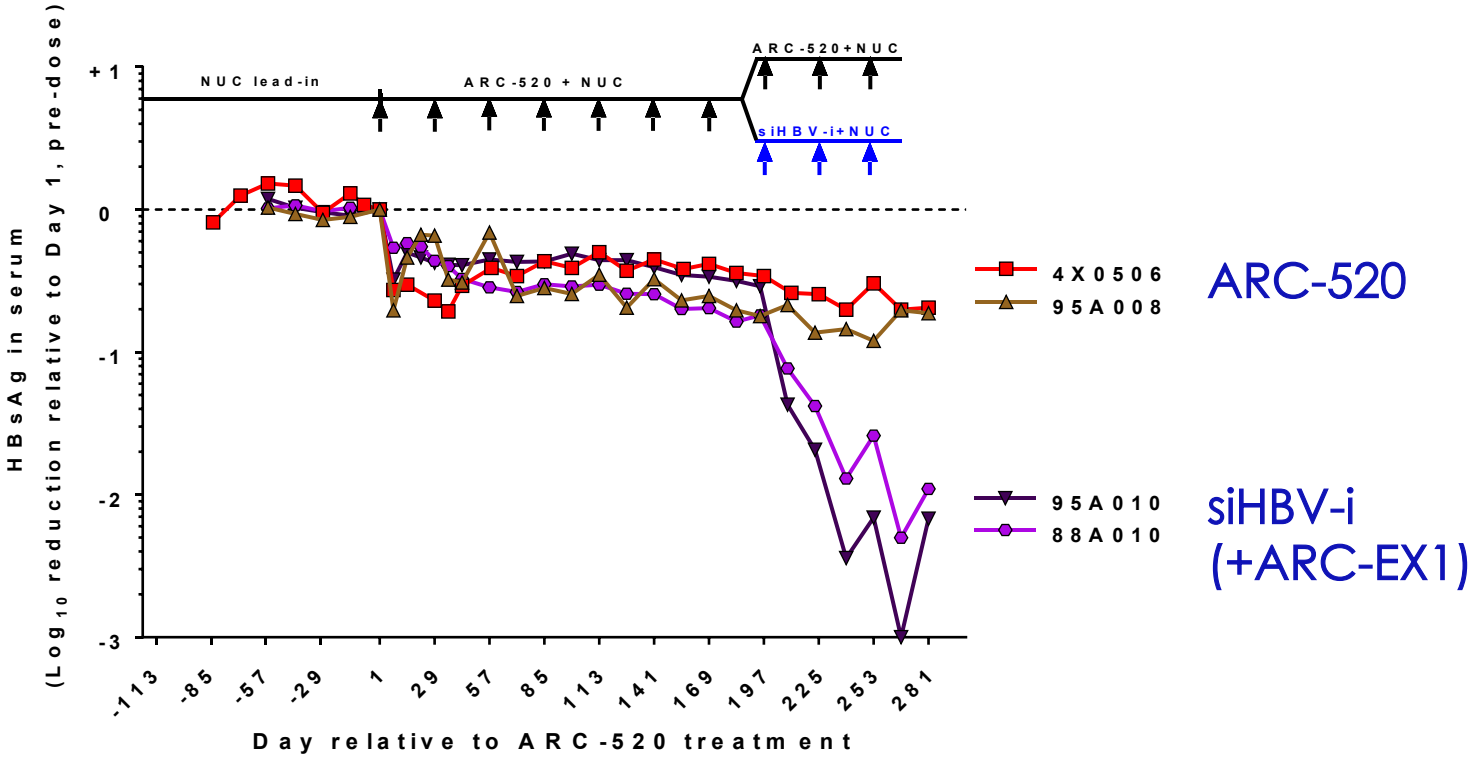
HBeAg-

- Mostly fusion transcripts encoding HBsAg with cryptic poly(A) signal at 3' end
- Fusion points typically between DR2 and DR1 as expected if transcripts arose from integrated dslDNA

HBV-aligning
Non-aligning

Wooddell C, Schluep T, Given B. With permission, 2016

siRNA designed to target RNA derived from HBV integration products in HBeAg- chimps



- siHBV-i targets HBV RNA even if expressed from integrated HBV DNA
- siHBV-i gave deep reductions in HBsAg in HBeAg- chimps, similar to those observed using ARC-520 in HBeAg+ chimps

Wooddell C, Schluep T, Given B. With permission, 2016

Summary

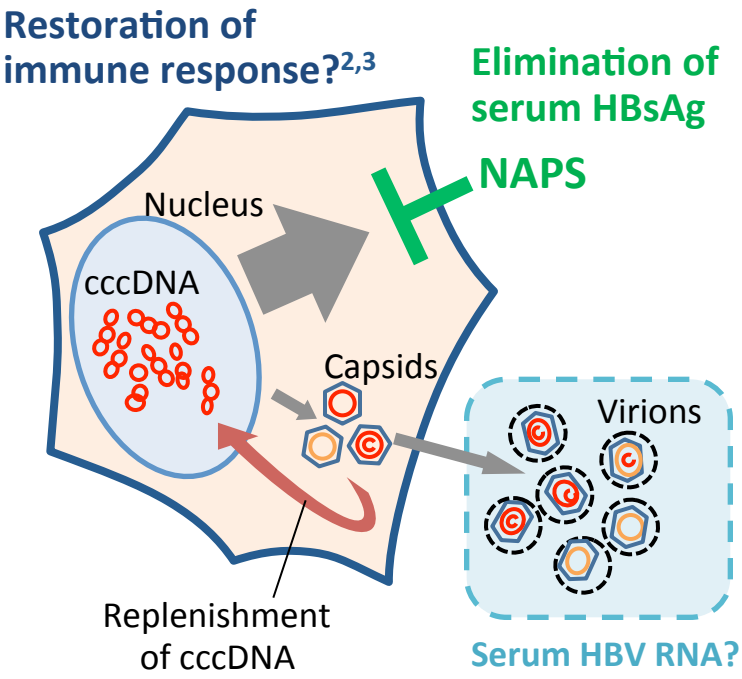
Key Virological Findings for ARC-520

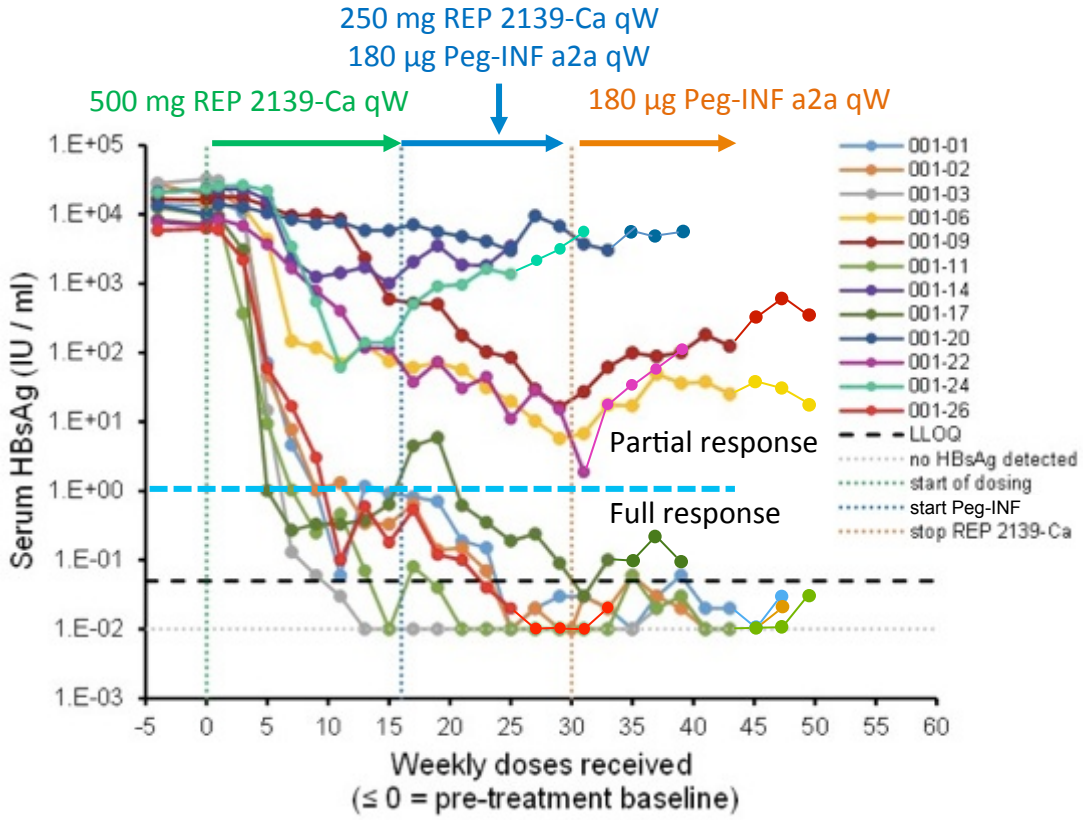
- Direct antiviral effect on serum HBsAg, HBeAg, and HBcrAg levels which are substantial
- HBeAg-Pos CHB and HBeAg-Neg CHB have very different viral patho-physiologies
- This has important therapeutic and prognostic significance

Nucleic Acid Polymers (NAP): Virion Assembly and HBsAg Egress
Update on the Safety and Efficacy of REP 2139 Monotherapy and Subsequent Combination Therapy with Pegylated Interferon Alpha-2a in Chronic HBV/HDV Co-Infection in Caucasian Patients

MOA of Nucleic Acid Polymers (NAP)

- NAPs have entry and post-entry antiviral effects in HBV infection *in vitro*[1]





Bazinet M, et al. AASLD 2015, San Francisco. #31.

1. Noordeen, F et al. AAC. 2013; 2. Wu et al. Hepatology. 2009; 3. Boni et al. Gastroenterology. 2012

Stopping Treatment

APASL Recommendation to Stop Antiviral Treatment

(Liaw, Y-F et al 2008. Hepatol Int;2:263)

In HBeAg-positive patients: when HBeAg seroconversion has developed > 6 months

In HBeAg-negative patients: when HBV DNA remaining undetectable for three separate occasions 6 months apart

- Outcomes
  - 25-50% develop viral relapse with hepatitis
  - up to 40% remain treatment free (SVR)
  - half of these lose HBsAg
- Factors
  - HBV DNA undetectable at stop
  - HBsAg < 100 IU/ml [low]
  - duration of AV therapy (4-5 years)

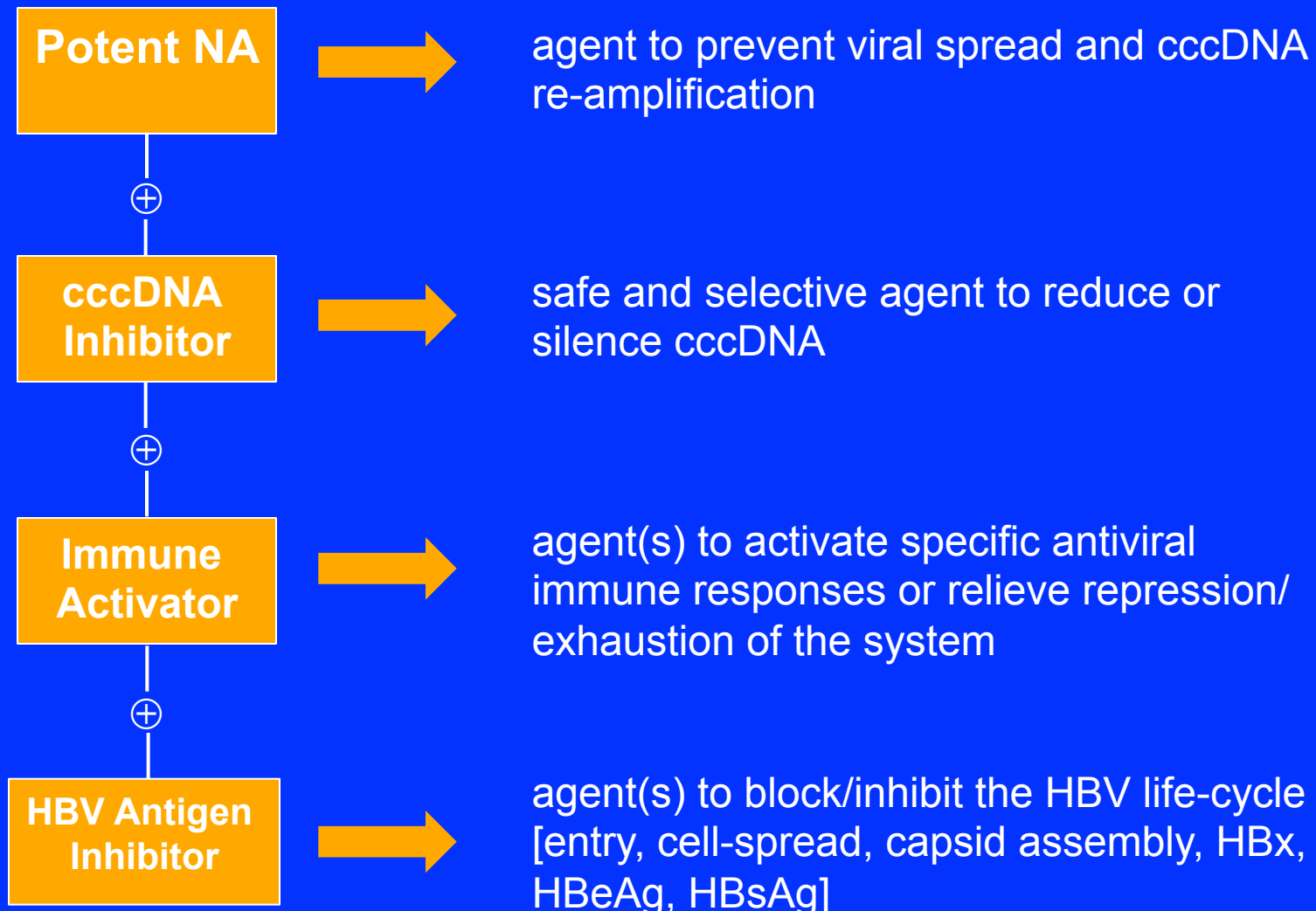
Hadziyannis, S et al 2012. Gastro;143:629.
Liang, Y et al 2011. Aliment Pharacol Ther;34:344.
Patwardham, N et al 2014. Aliment Pharmacol Ther;40:804.
He, D et al 2013. BMC Infec Dis;13:458.
Jeng, W-J et al 2013. Hepatol;58:1888.

Future Directions and Challenges

- The goalposts are shifting
- The medium-term aim for the field is to achieve functional “cure”
  - HBsAg seroconversion; HBV DNA undetectability
  - An immunomodulator is likely to be required
- New and Novel agents for CHB are starting to emerge
  - Identification of the HBV-R (NTCP) a major breakthrough
  - Improved delivery to the liver for molecular therapeutics
  - Molecular therapies blocking HBV protein/production antigen (HBsAg; HBeAg; HBx)

PALPABLE OPTIMISM

What Might a HBV Curative Regimen Look Like?



The Concept of Combination Therapy