Future Therapies for HBV

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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]

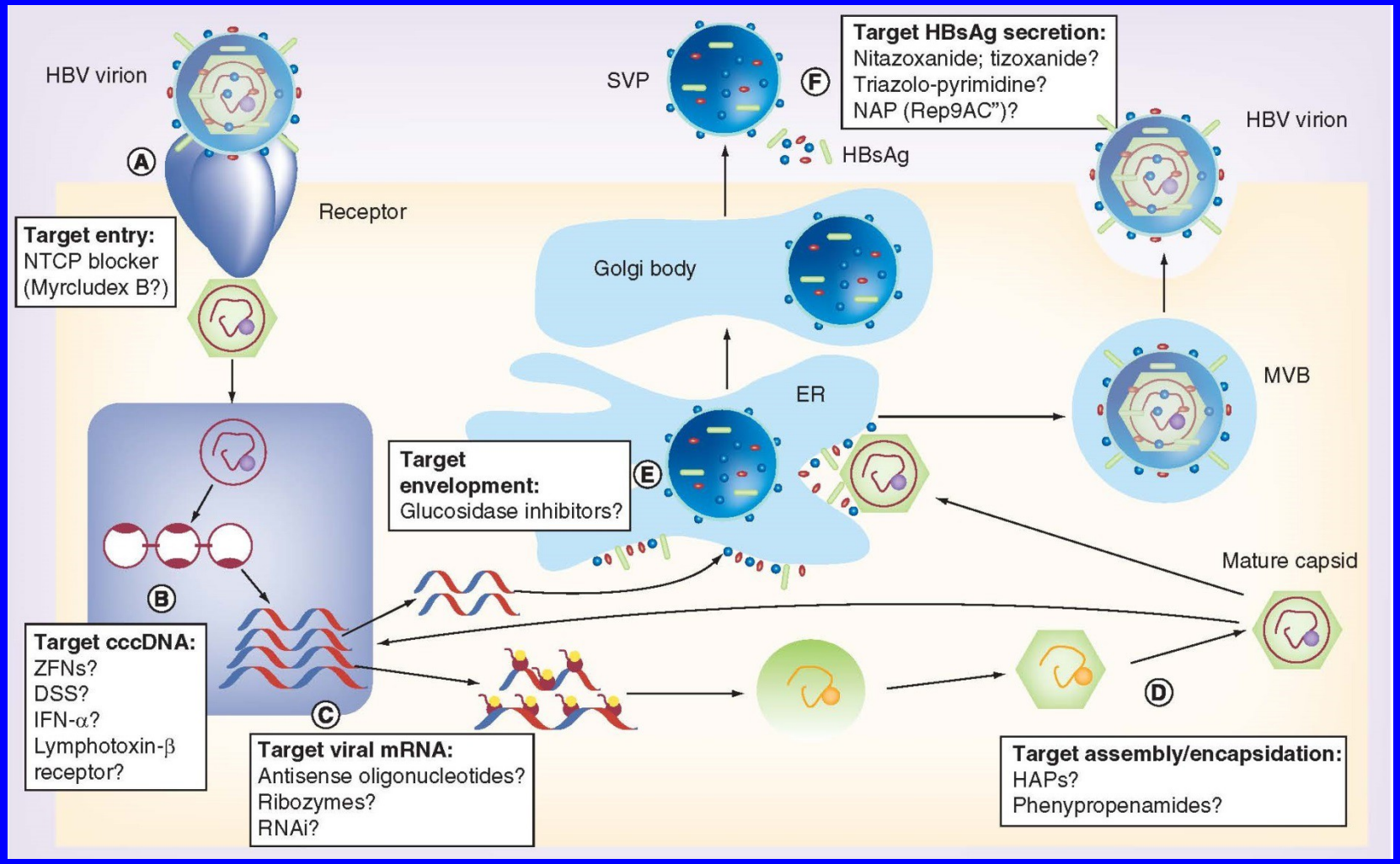
Functional Cure: HBsAg loss/Seroconversion: Maintenance of undetectable serum HBV DNA off-treatment

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

New Viral Targets

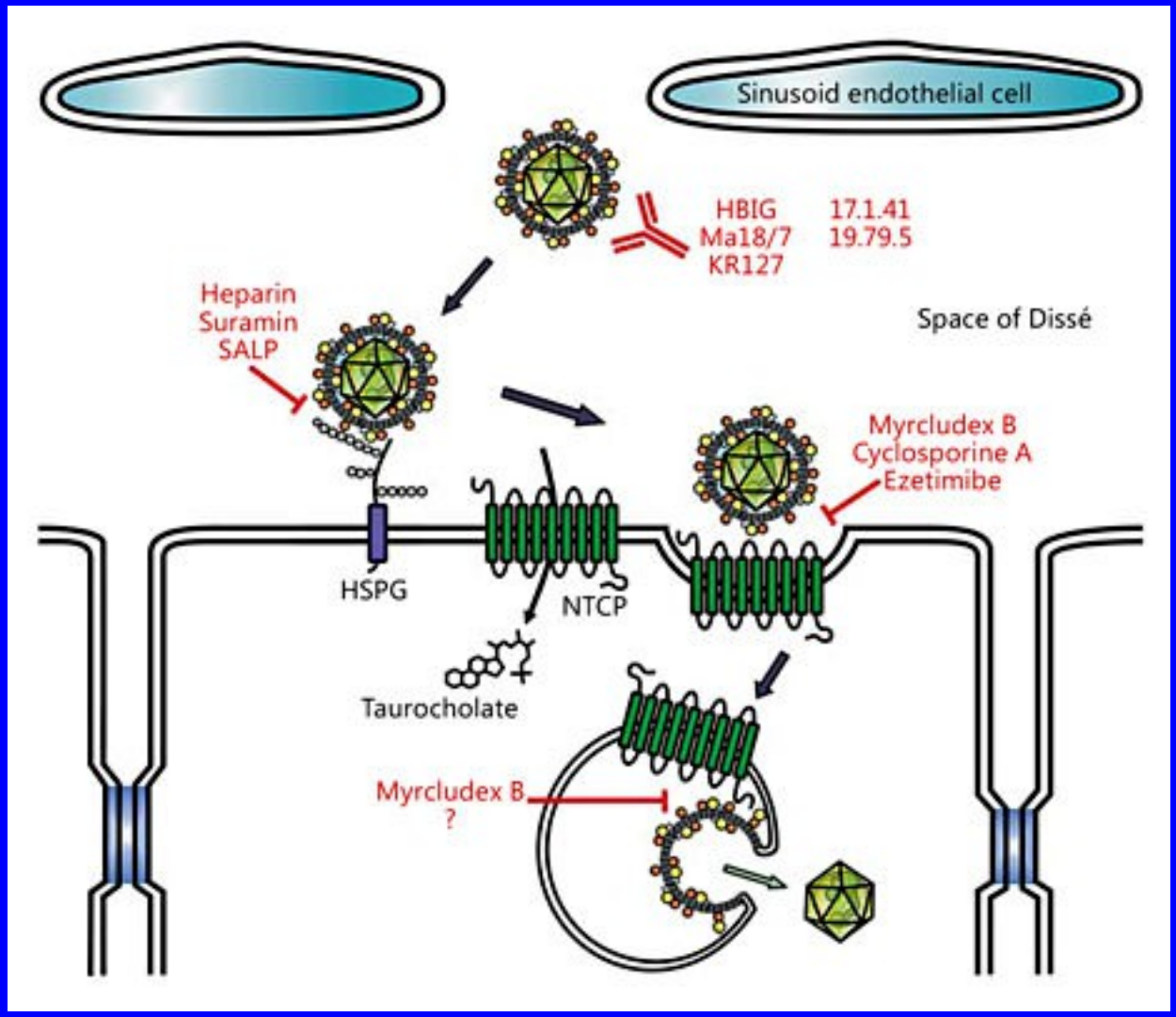
- Attachment and Entry
- cccDNA Generation & Processing (HBcAg and HBx)
- Reverse Transcription
- HBV Nucleocapsid Assembly (HBcAg)
- Packaging Inhibitors
- Molecular Based Therapies (RNAi)
- Combination Therapy

HBV Lifecycle Showing Novel Approaches for Viral Targets



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

Inhibitors of HBV Attachment and Entry

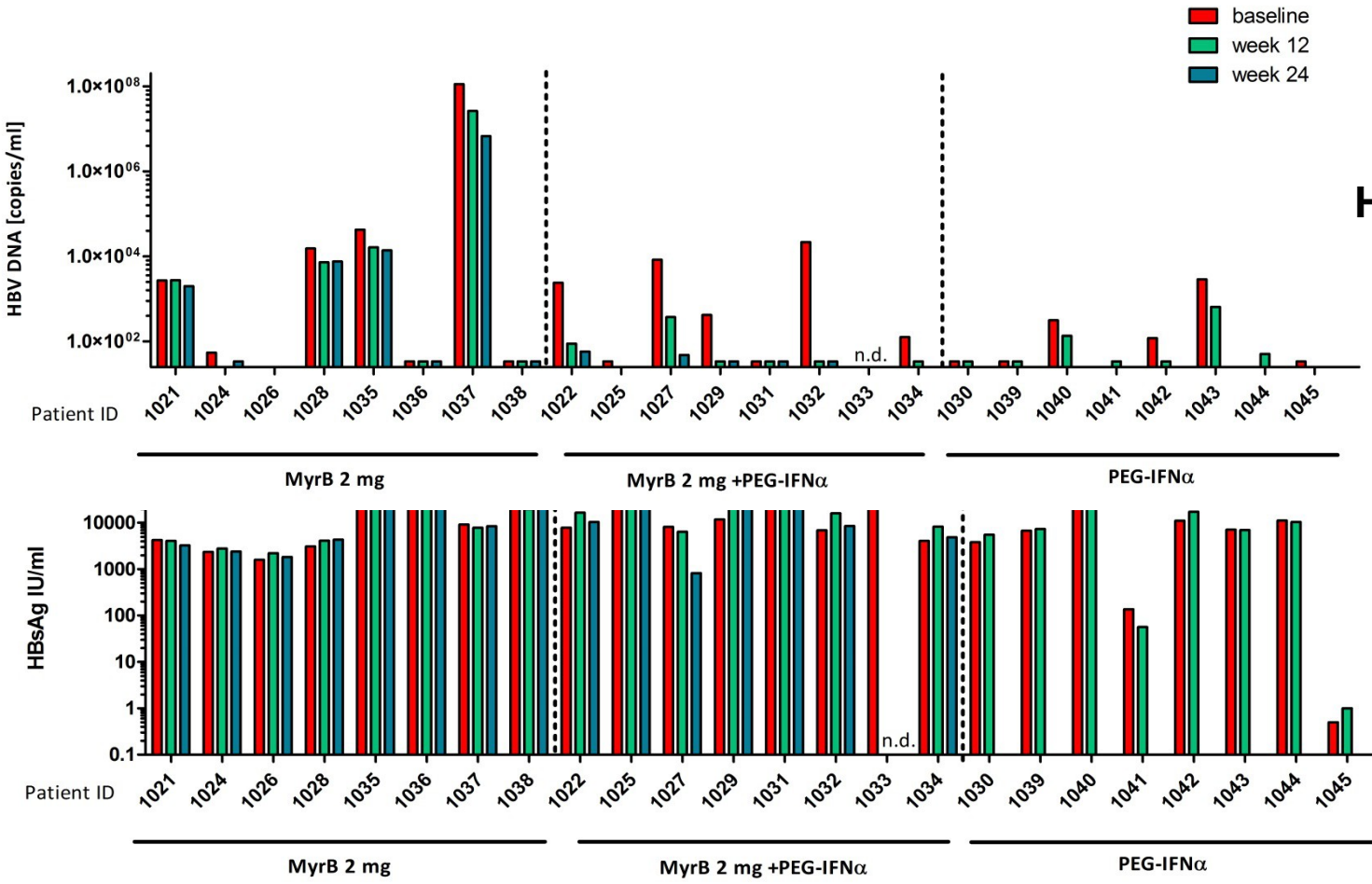


Sodium taurocholate cotransporting polypeptide (NTCP) identified as HBV and HDV receptor in 2012

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

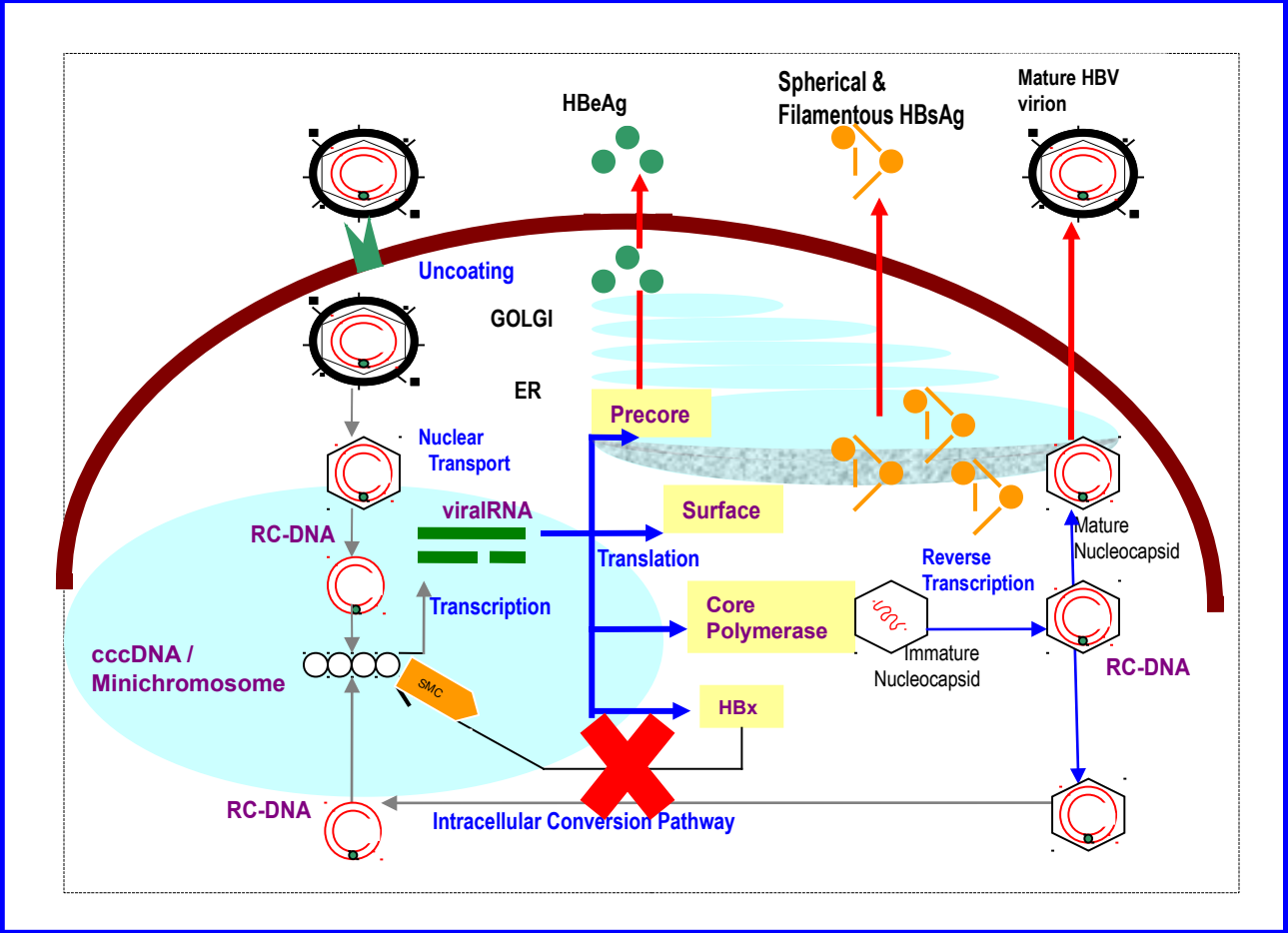


⇒ HBV DNA levels decline during Myrcludex B treatment in 4/8 patients (consistent with HBV trial).

⇒ 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 .

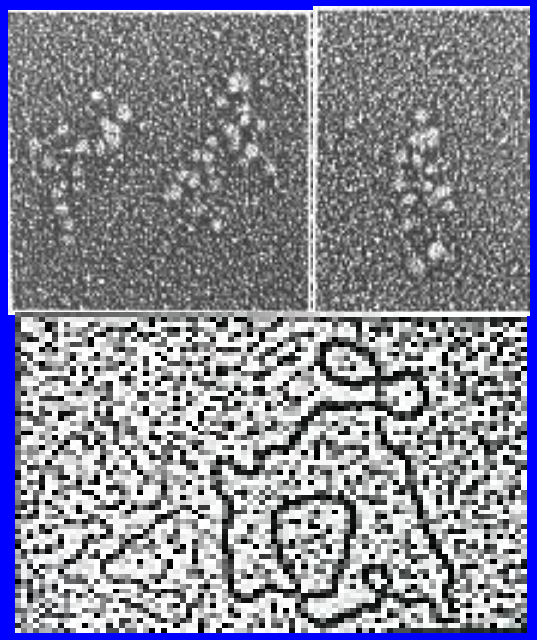
HBV Replication: cccDNA Pathway



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

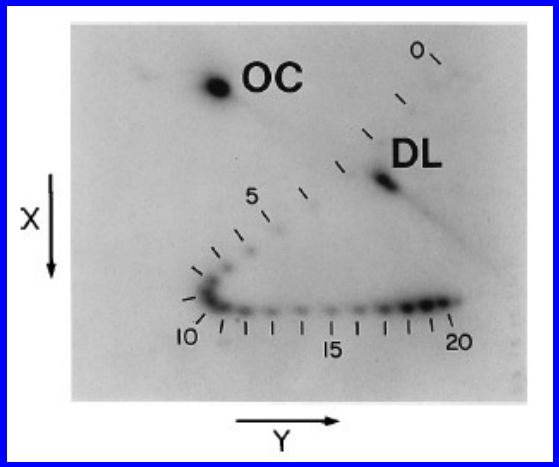
Koniger, C et al 2014. Proc Natl Acad Sci;111:4244

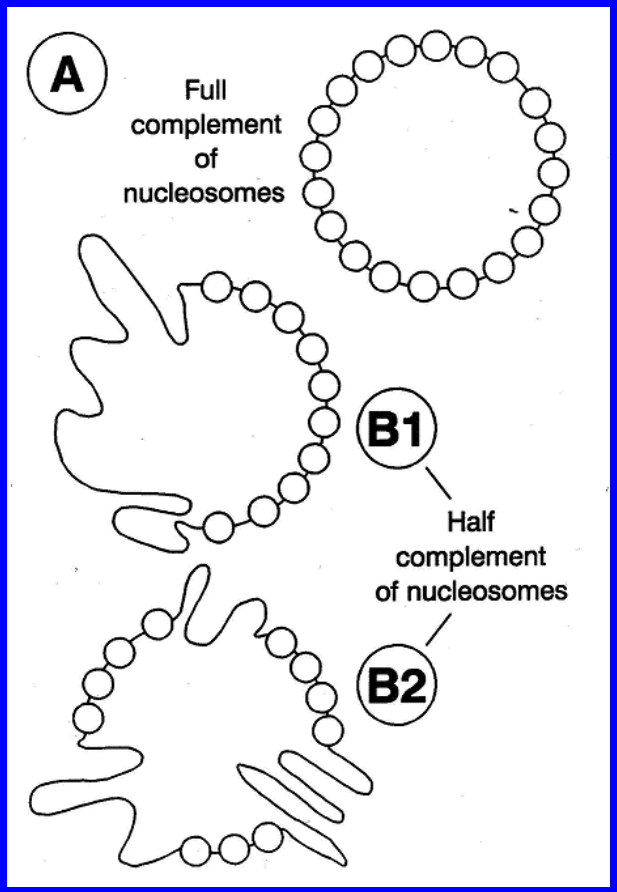
cccDNA Generation and Processing: cccDNA is a Minichromosome



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

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



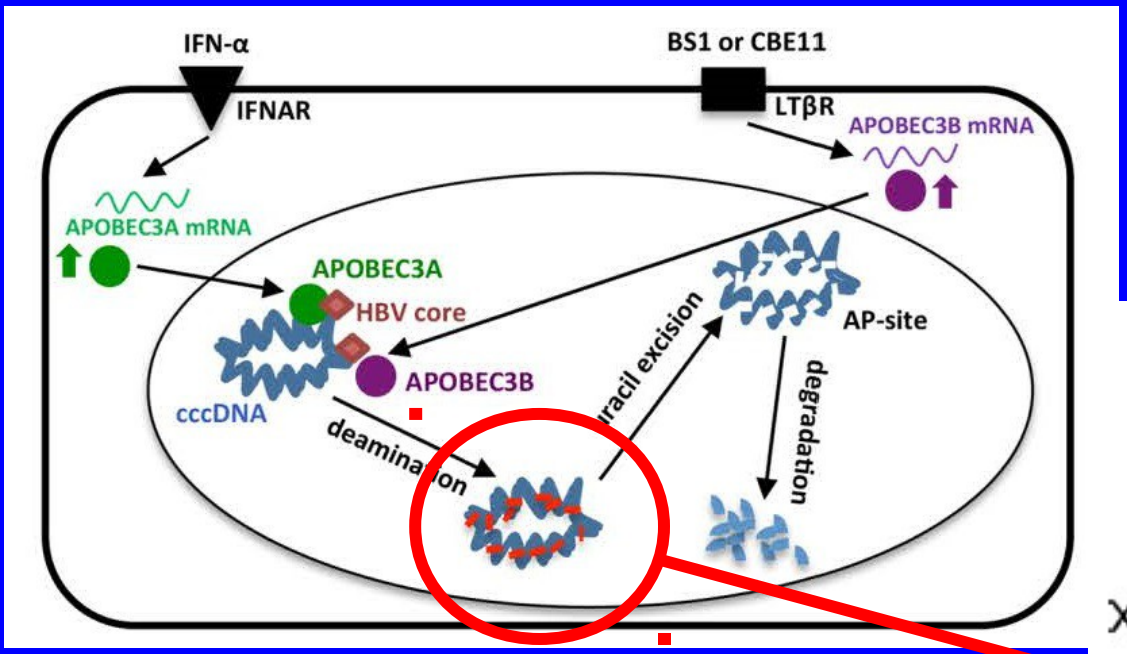


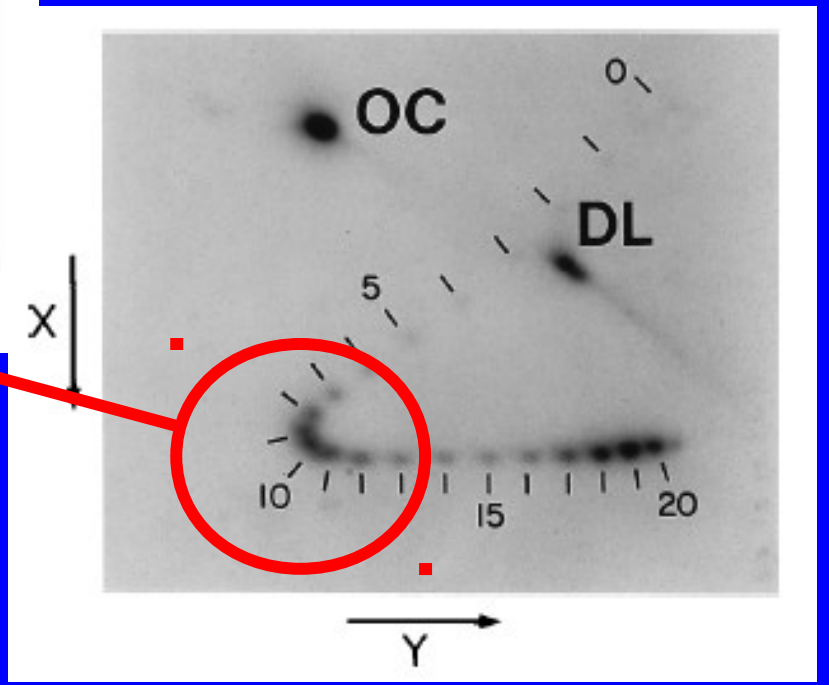
Low Replication Phenotype
Quiescent or active
Medium to Low Viraemia

High Replication Phenotype
Transcriptionally Active
High Viraemia

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

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



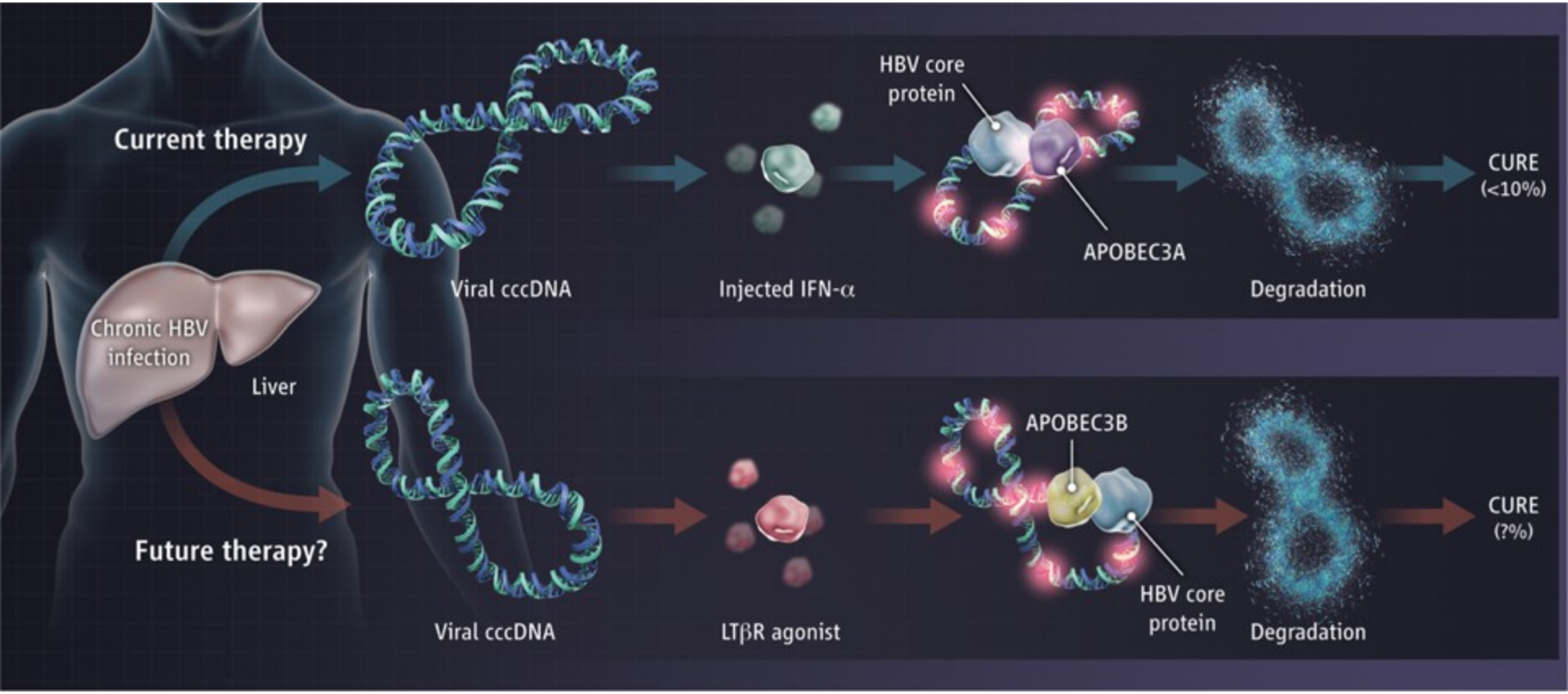


Modified from Lucifora, J et al 2014. Science;343(6176):1221-8

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*

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

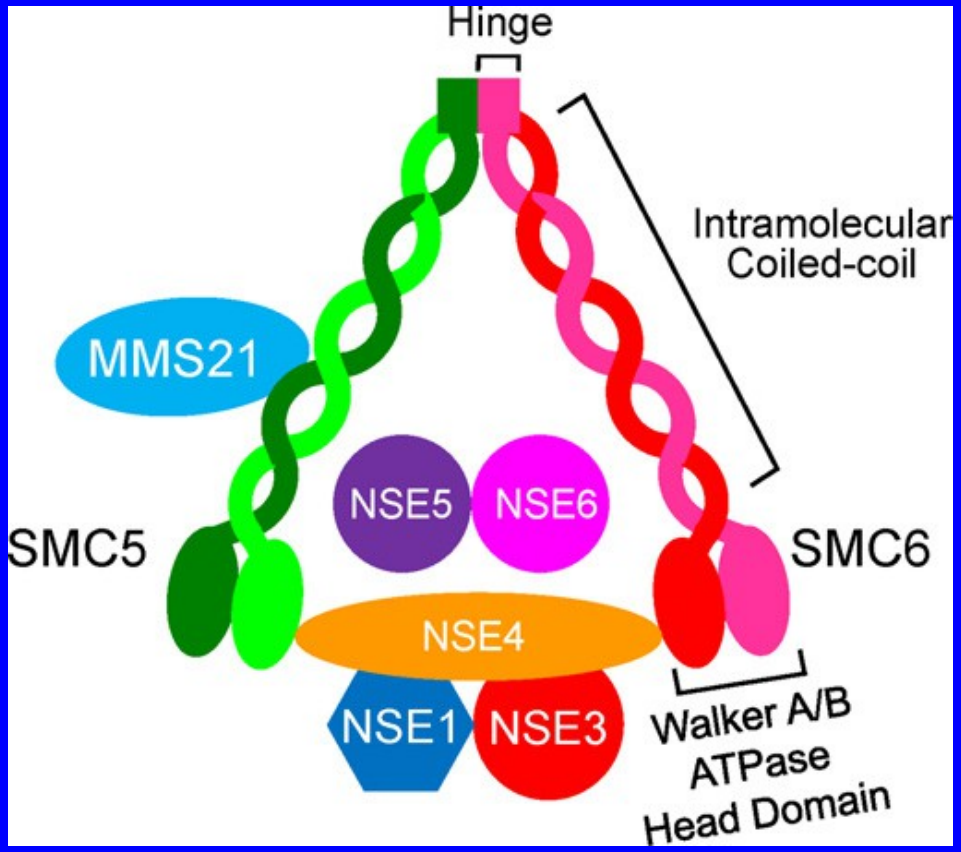
The Smc5/6 Complex

Structural Maintenance of Chromosomes (Smc) Complexes

- Condensin
- Cohesin
- Smc5/6 complex

Smc5/6

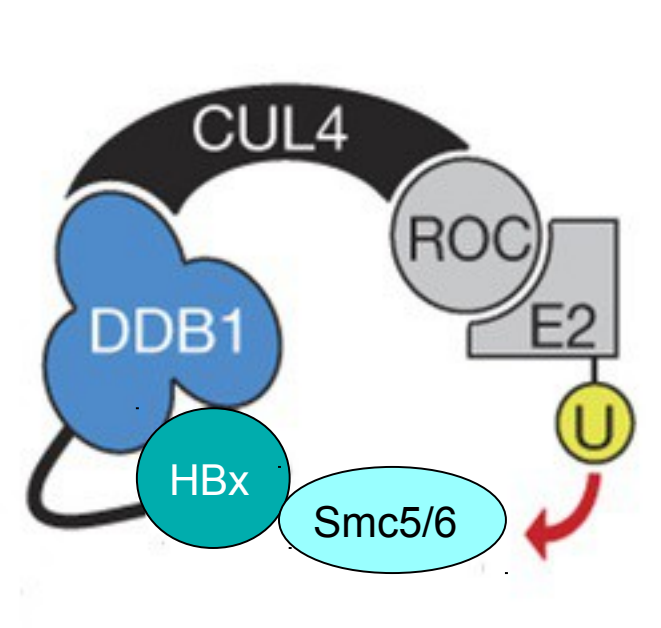
- Nuclear complex
- DNA repair
- Chromosome topology and organization

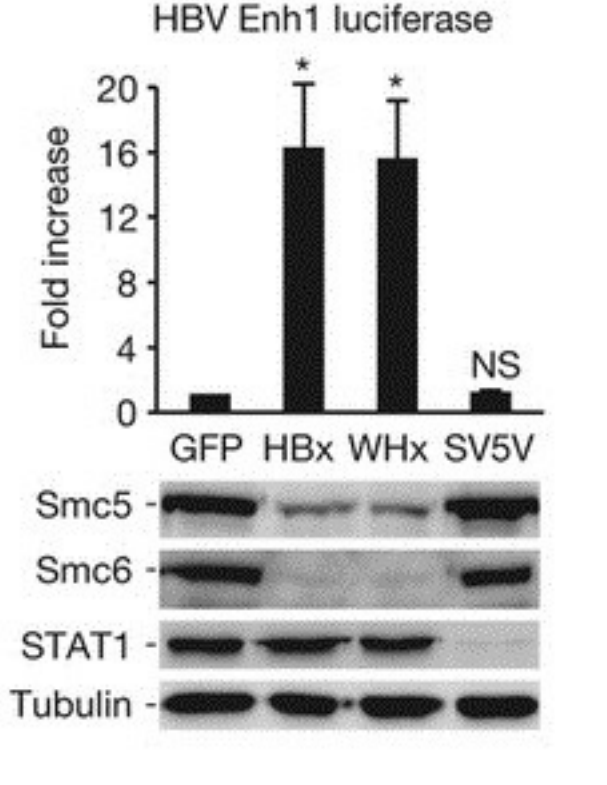


Potts, PR. 2009. DNA Repair;8:499

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

HBx Induces Degradation of Smc5/6

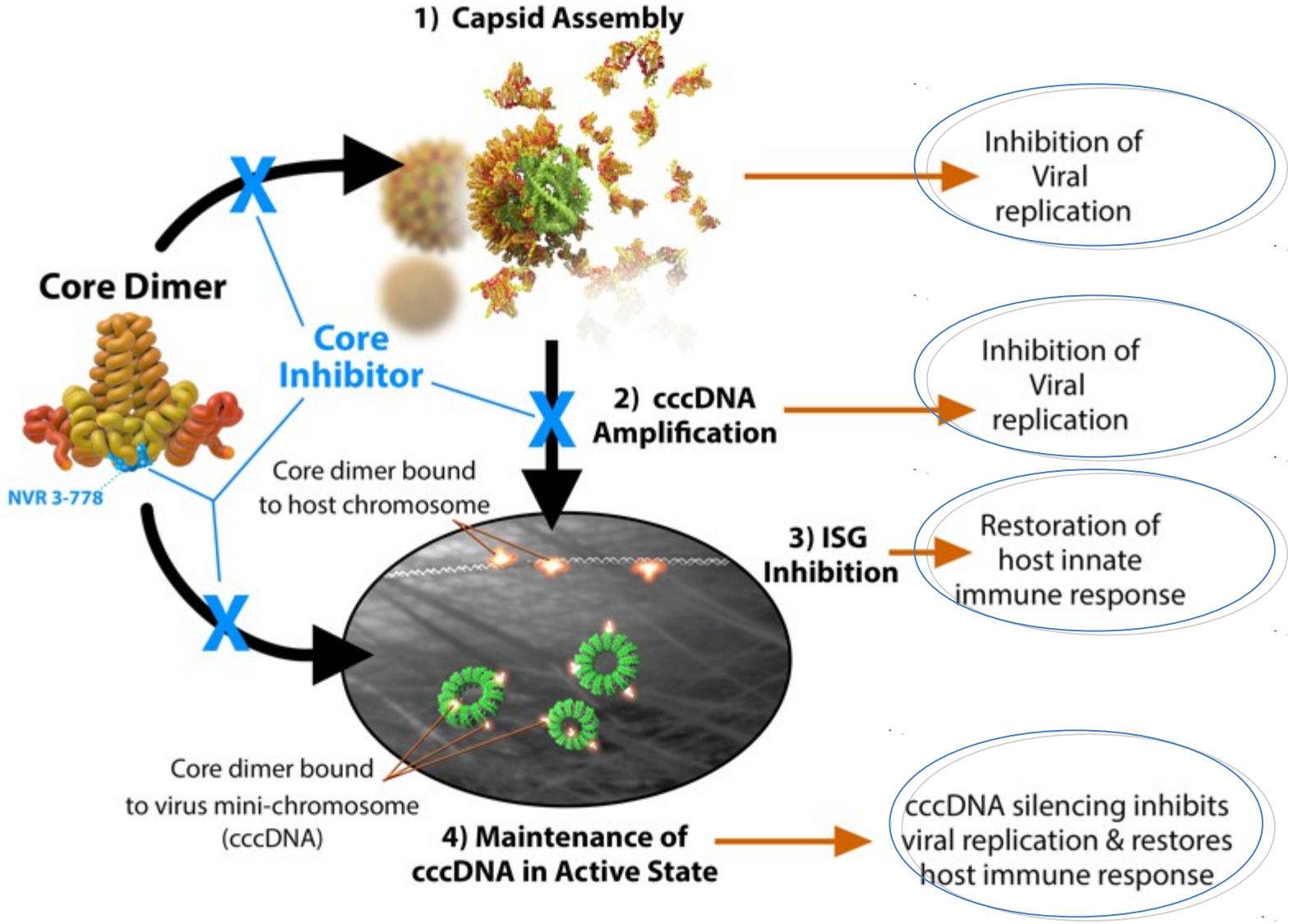




- The effect is rapid
- Blocked by proteasome inhibitors
- Blocked by E3 ligase inhibitors
- No changes in Smc5/6 mRNA levels

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

Targeting the HBV Nucleocapsid



Targeting HBV Nucleocapsids

Heteroaryldihydropyrimidines

Destabilization of nucleocapsids

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

Phenylpropenamide derivatives

Prevent pgRNA encapsidation

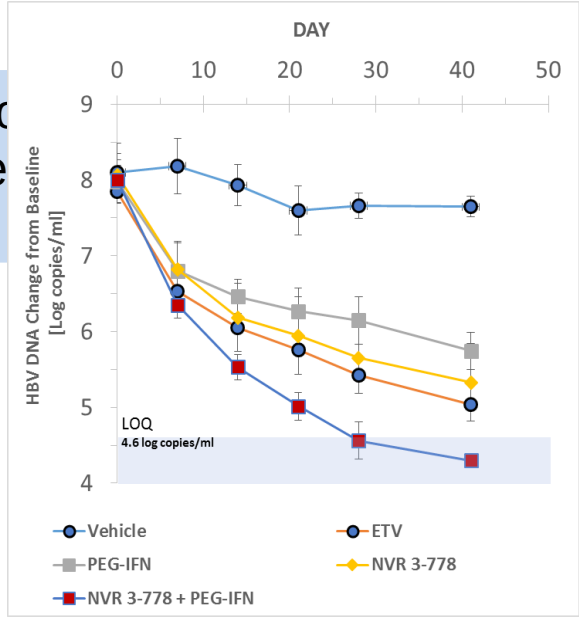
Feld, J et al 2007. AVR; 76:168-177

Antimicrob Agents Chemother. 2002.

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

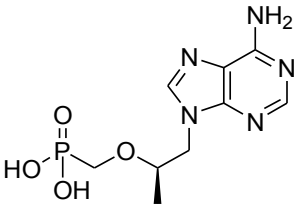
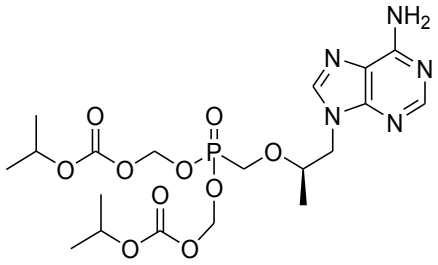
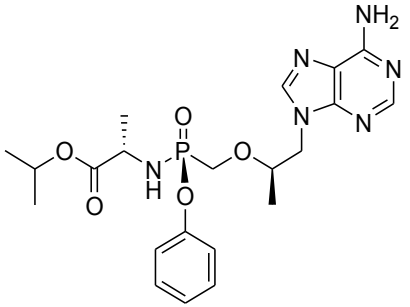
Lam A, et al. AASLD 2015, San Francisco. #33

[sulphonamide/sulfamoyl benzimide derivatives]

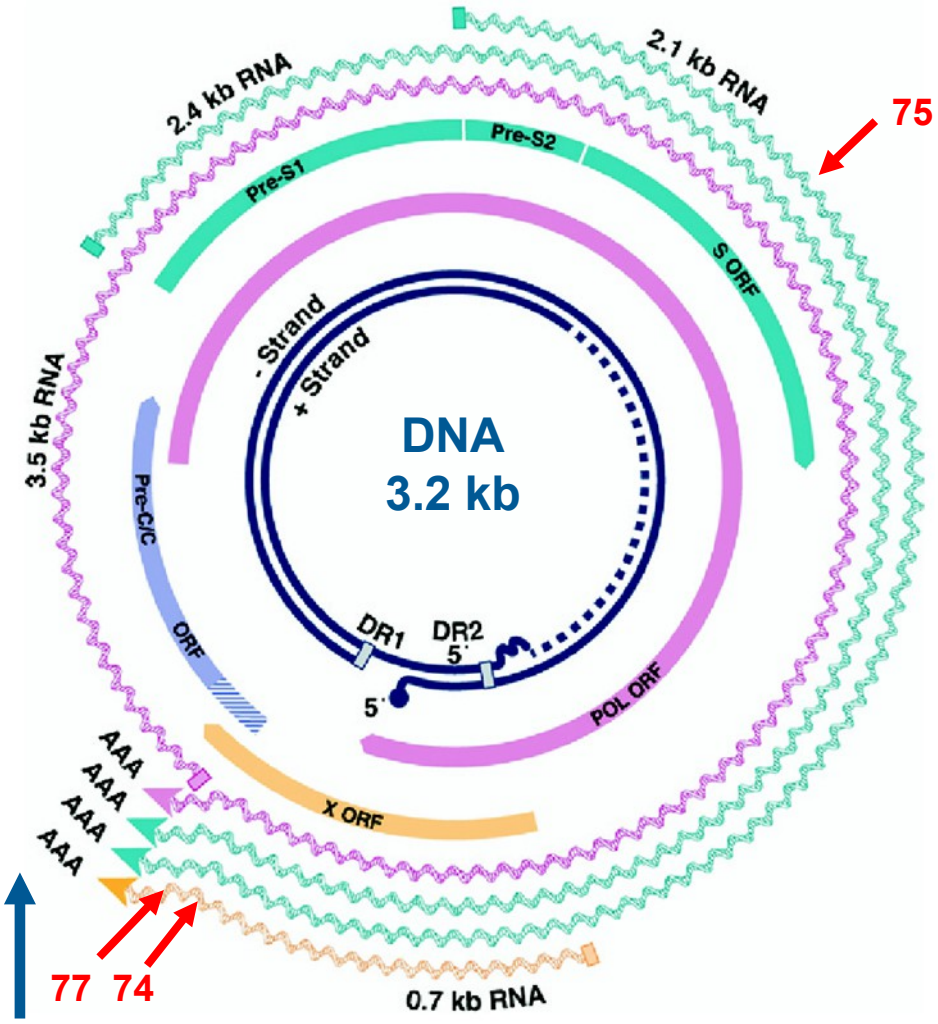


Reverse Transcription: Improved Potency of NA Tenofovir Alafenamide (TAF)

- 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

HBV genome and siRNA target sites

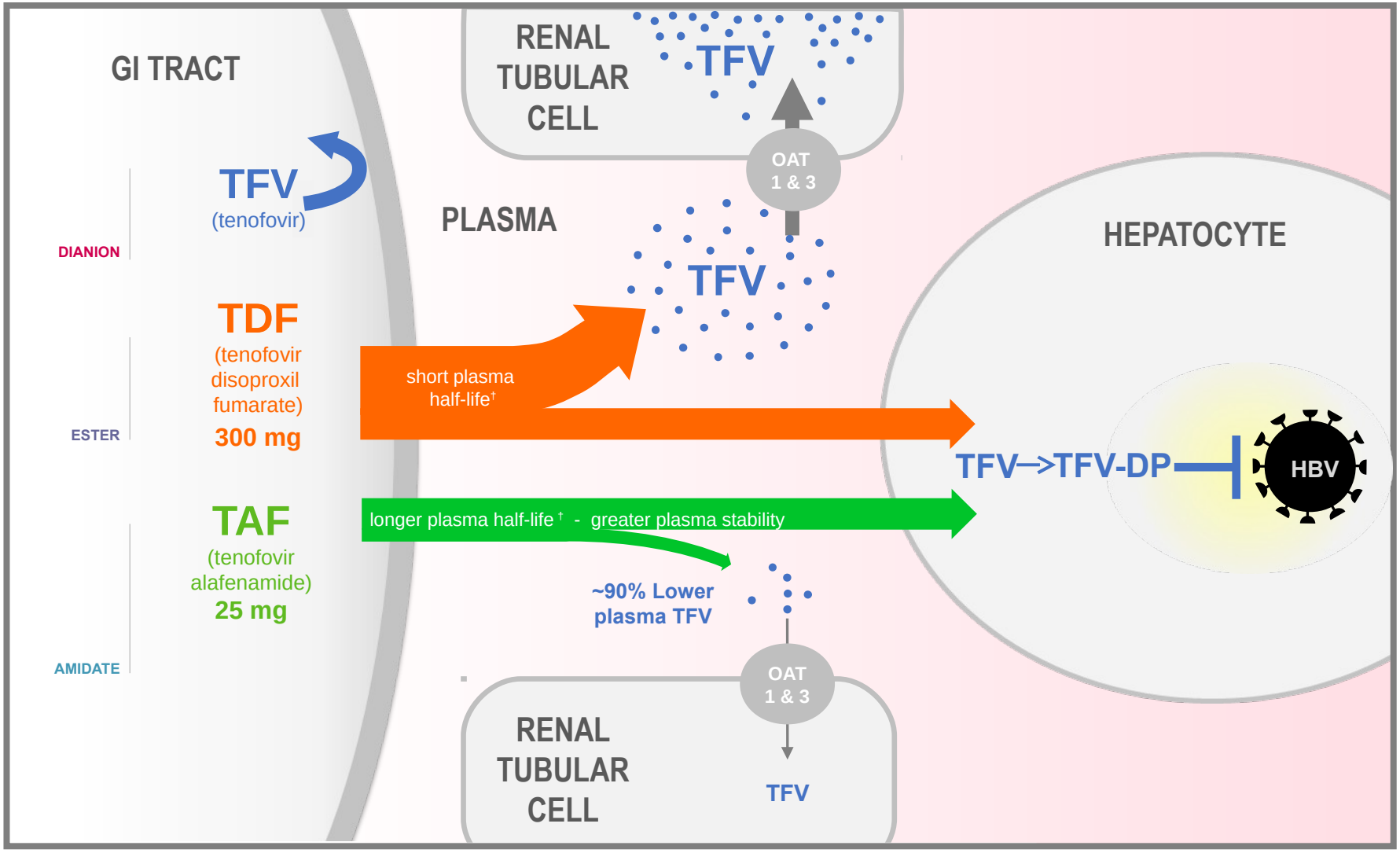


Same polyadenylation signal for all mRNAs

- **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

Tenofovir alafenamide (TAF) – A Novel Prodrug of Tenofovir

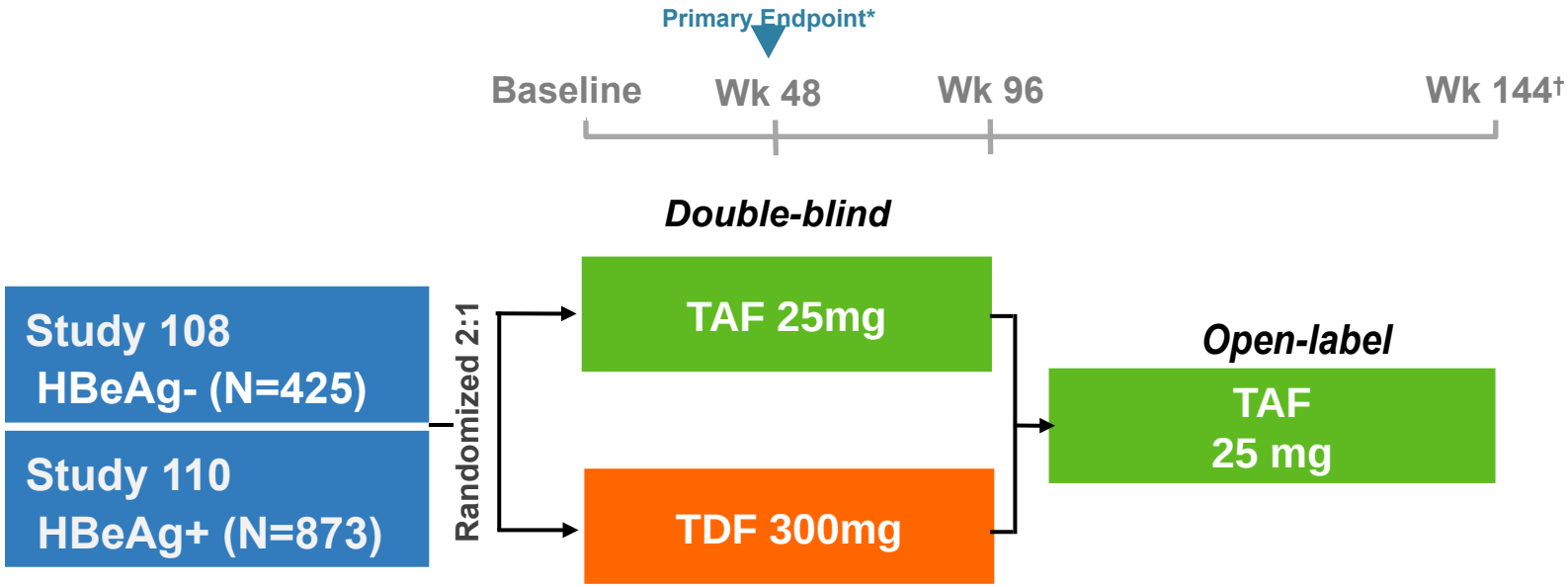


† $T_{1/2}$ based on *in vitro* plasma data - TDF = 0.4 minutes, TAF = 90 minutes.

Lee W et. *Antimicr Agents Chemo* 2005;49(5):1898-1906. Birkus G et al. *Antimicr Agents Chemo* 2007;51(2):543-550. Babusis D, et al. *Mol Pharm* 2013;10(2):459-66. Ruane P, et al. *J Acquir Immune Defic Syndr* 2013; 63:449-5. Sax P, et al. *JAIDS* 2014. 2014 Sep 1;67(1):52-8. Sax P, et al. *Lancet* 2015. Jun 27;385(9987):2606-15. Agarwal K et al. *J Hepatology* 2015; 62: 533-540; Buti EASL 2016, Oral GS06; Chan, EASL 2016, Oral GS12

Study 108 and 110: Phase 3 CHB Studies: TAF vs TDF

TAF HBV Phase 3 Program



- Two phase 3, randomised, double-blind studies
- Inclusion criteria
  - HBV DNA ≥20,000 IU/mL; ALT >60 U/L (males), >38 U/L (females)
- Primary endpoint (non inferiority margin of 10%):
  - HBV DNA <29 IU/mL at Week 48
- Key secondary safety endpoints
  - Bone mineral density and renal parameters at Week 48

†Amendment to extend double-blind to Week 144 and open-label phase to Week 384 (Year 8) is currently underway
*Non-inferiority margin of 10%
Buti EASL 2016, Oral GS06; Chan, EASL 2016, Oral GS12

HBV DNA Response at 48 Weeks

Study 108 (HBeAg-subjects)

HBV DNA <29 IU/mL (%)

Log_{10} HBV DNA Change

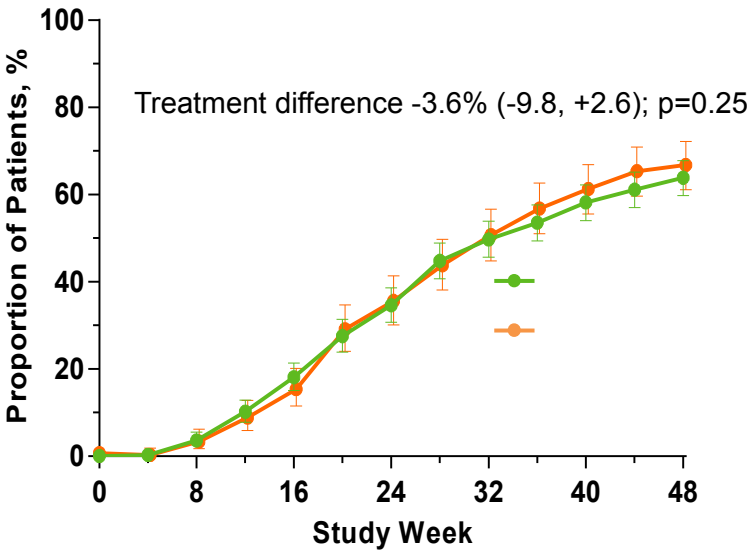
- Similar and non-inferior rates of virologic suppression with TAF and TDF at Week 48
- No resistance detected in either treatment group

Buti, EASL 2016, Oral GS06

HBV DNA Response at 48 Weeks

Study 110 (HBeAg+ subjects)

HBV DNA <29 IU/mL (%)



Log_{10} HBV DNA Change

- Similar and non-inferior rates of virologic suppression with TAF and TDF at Week 48
- No resistance detected in either treatment group

Chan, EASL 2016, Oral GS12

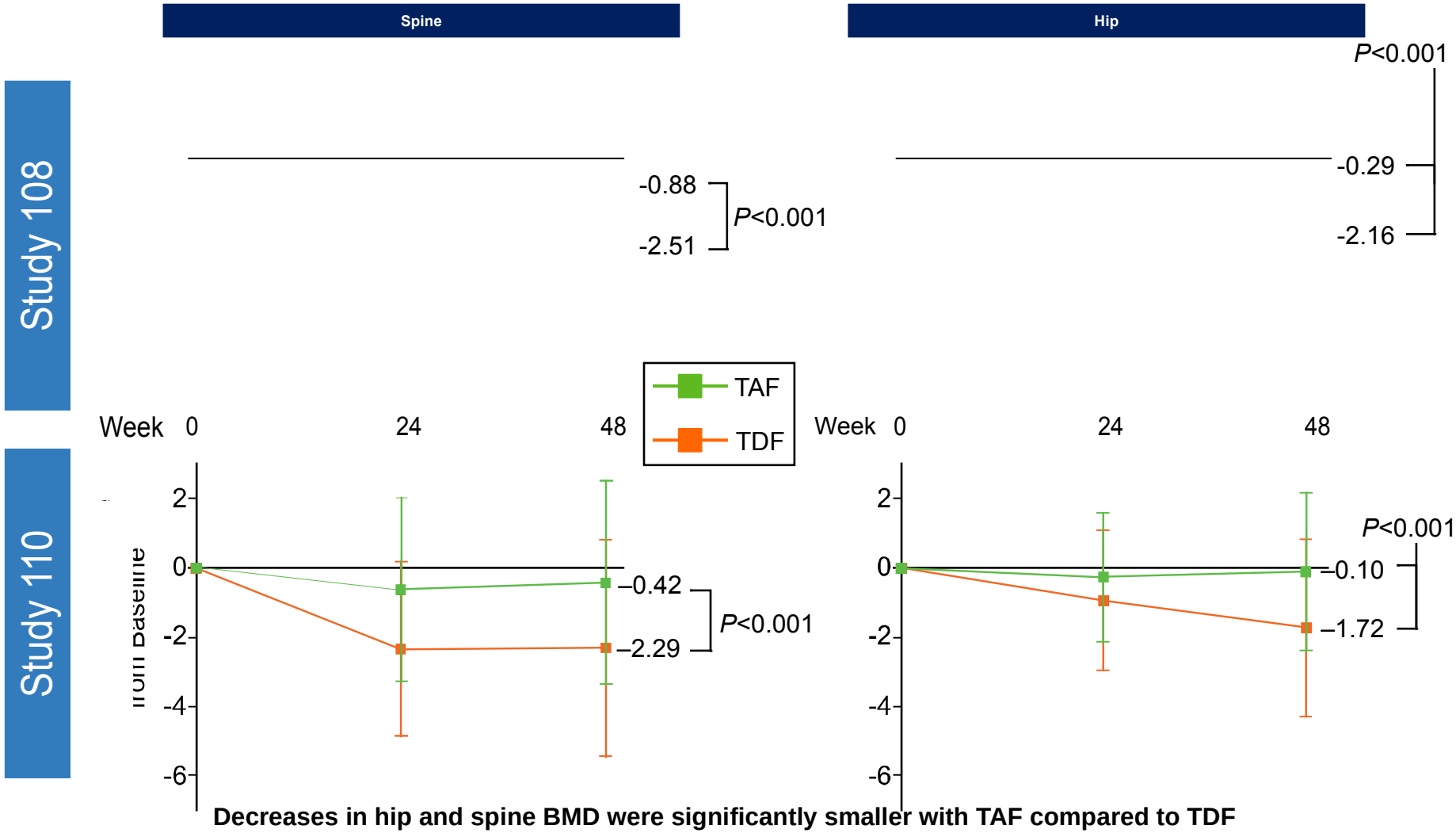
‡

Results: Renal Safety

	TAF n=866	TDF n=432	P-value
Change in sCr, mg/dL	0.010 (0.11)	0.024 (0.10)	0.012

Continuous data are expressed as mean (SD)
sCr, serum creatinine; eGFR$_{CG}$, creatinine clearance by Cockcroft-Gault
Buti EASL 2016, Oral GS06
Chan, EASL 2016, Oral GS12
Gilead Sciences, Data on File

Changes in Spine and Hip BMD Through Week 48



Decreases in hip and spine BMD were significantly smaller with TAF compared to TDF

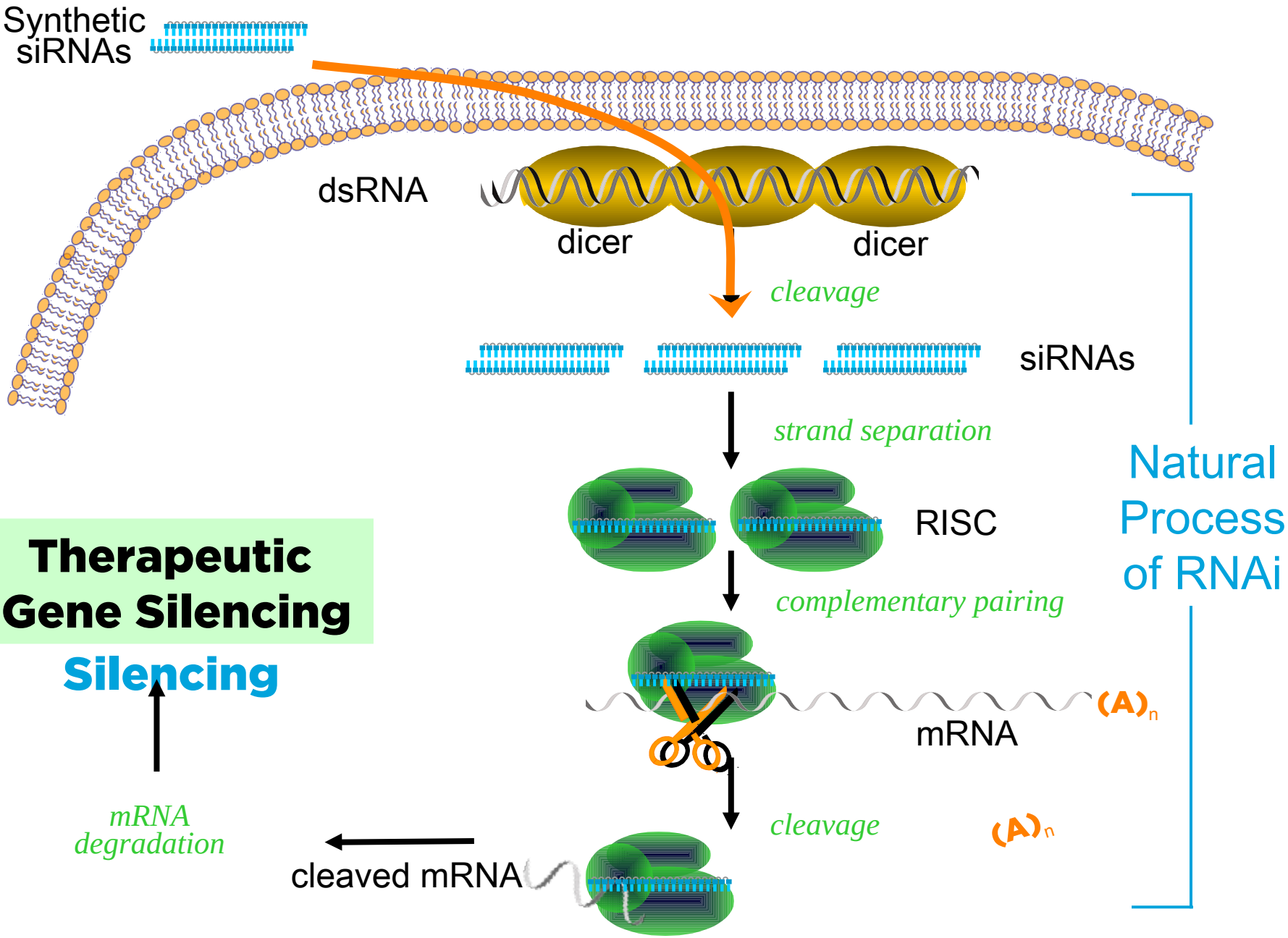
Buti, EASL 2016, Oral GS06
Chan, EASL 2016, Oral GS12

Authors' Conclusions

- Treatment with TAF for 48 weeks demonstrated:
  - Non-inferior efficacy to TDF for the proportion with HBV DNA <29 IU/mL
  - Improved rates of ALT normalization
  - No resistance development in either treatment group
  - Rates of HBeAg loss and seroconversion similar to TDF in Study 110
- TAF was well tolerated in HBeAg-negative and -positive patients
  - Treatment-emergent AEs similar to TDF
  - Significantly smaller increases in SCr (integrated safety analysis) and decreases in $eGFR_{CG}$ compared to TDF, with improved markers of renal tubular function
  - Significantly less declines in hip and spine BMD compared to TDF, with improved bone biomarkers

Buti, EASL 2016, Oral GS06
Chan, EASL 2016, Oral GS12

Mechanism of RNA Interference (RNAi)



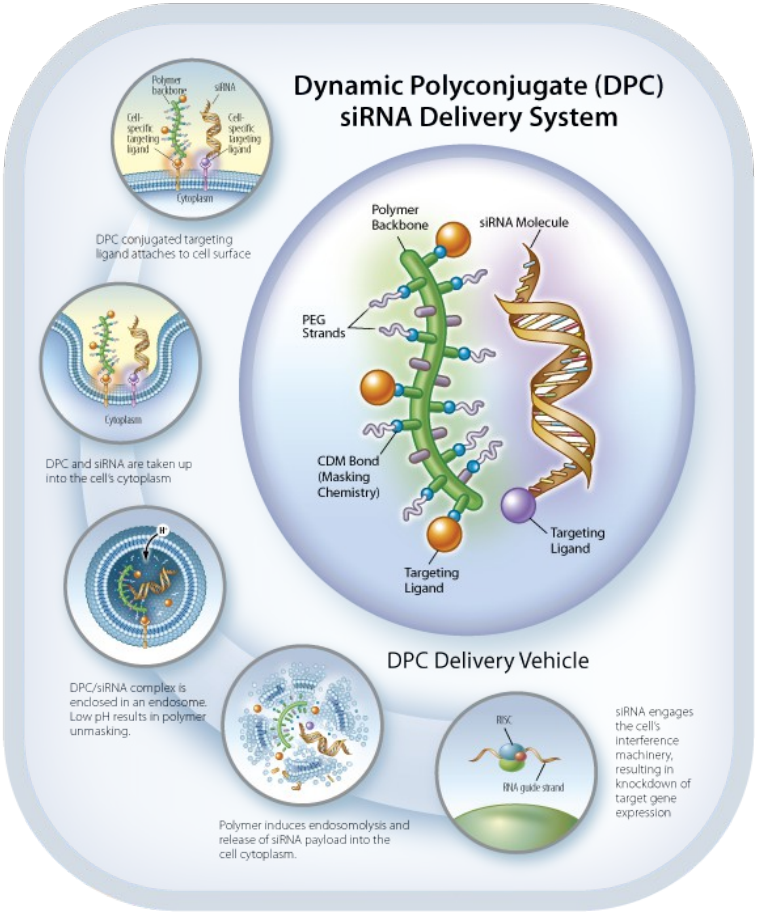
Groups Involved in RNAi Therapy and HBV

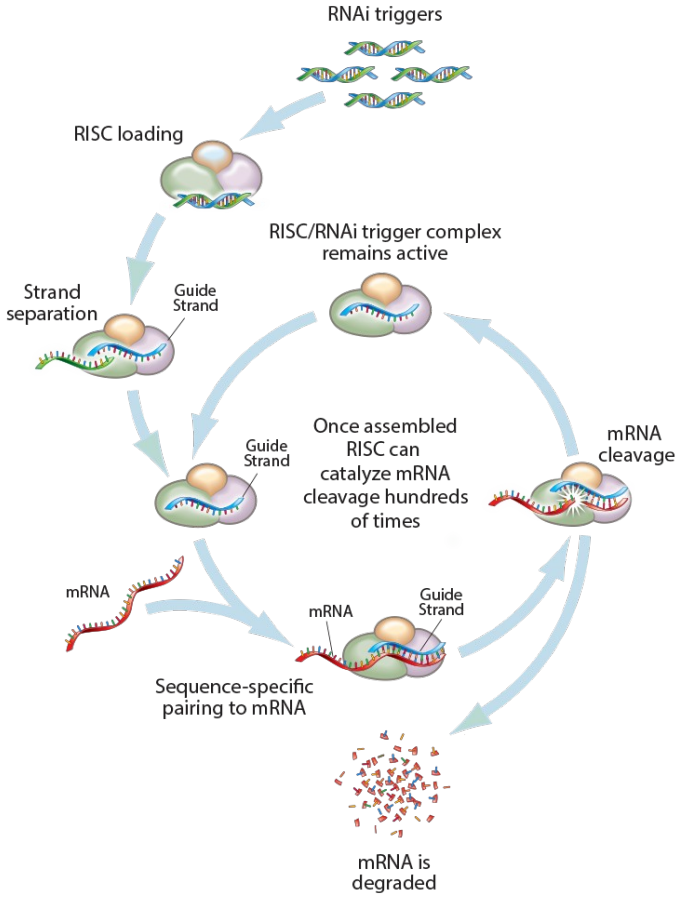
- Arrowhead Pharmaceuticals
  - ARC-520 (phase 2)
  - ARC-521 (phase 1/2)
- Arbutus Biopharma
  - ARB-1467 (phase 1/2)
- Alnylam Pharmaceuticals
  - ALN-HBV (phase 1)

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

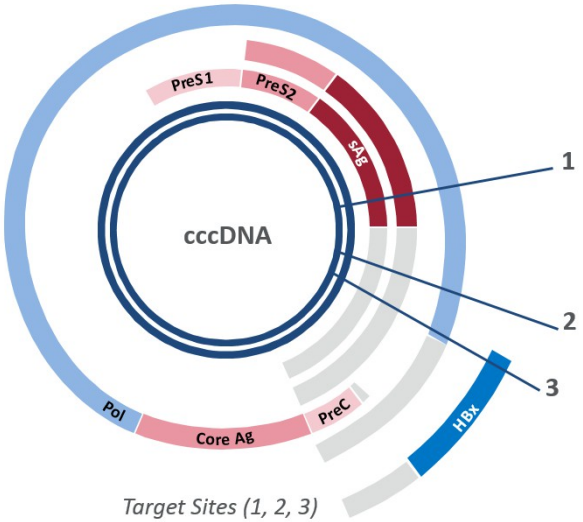




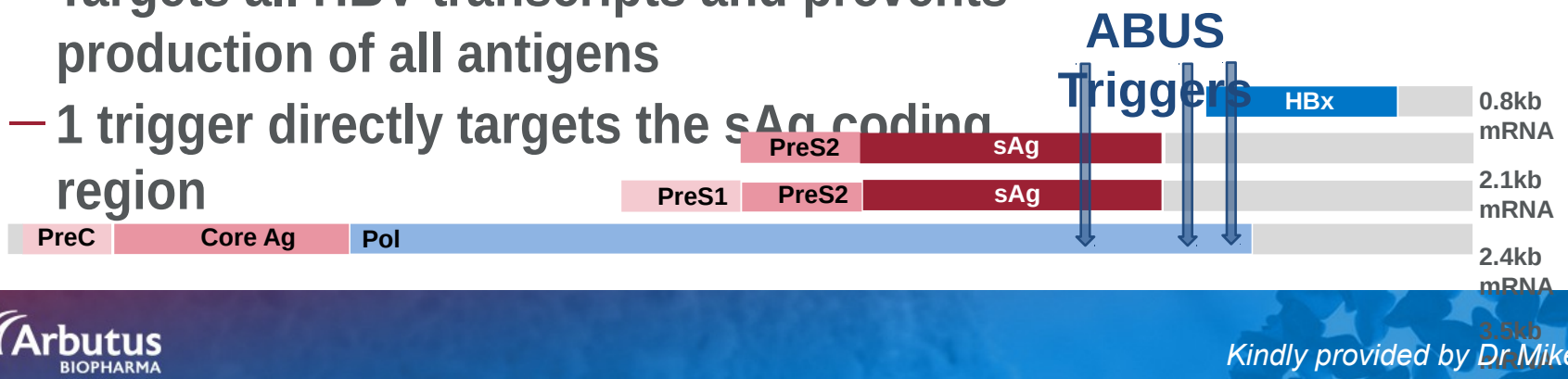
From: ArrowheadPharma.com/science

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



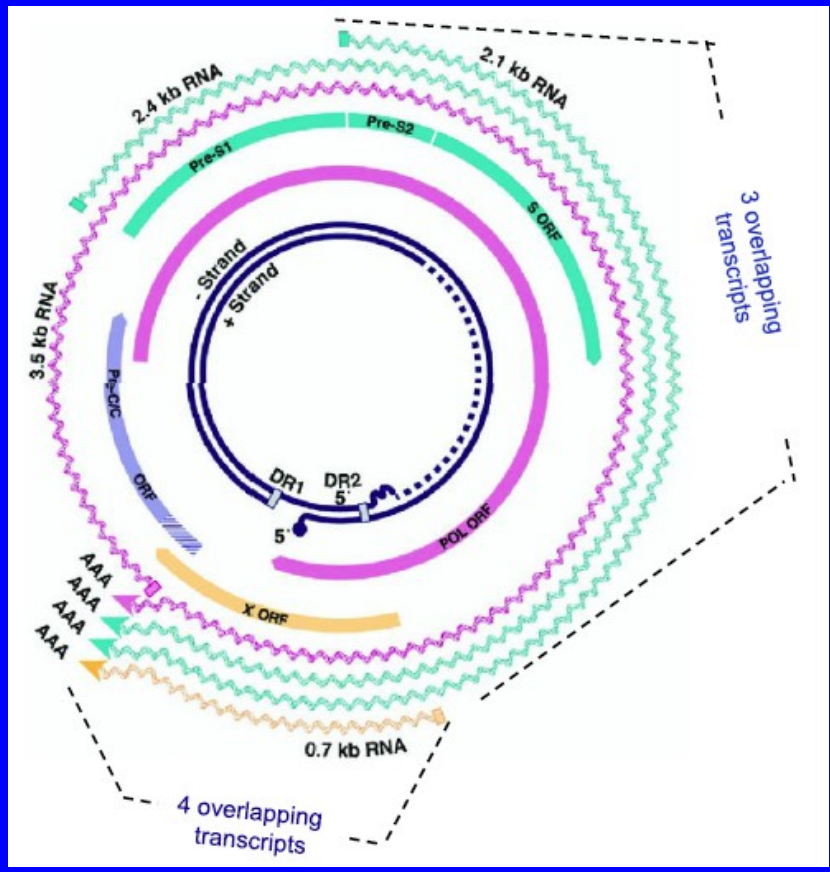
Target Sites (1, 2, 3)



Kindly provided by Dr Mike Sofia

Alnylam RNAi (Preclinical)

- Delivery: **Multi-component lipid nanoparticles** for delivery to the liver via LDL receptor
- Triantennary GalNac 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)

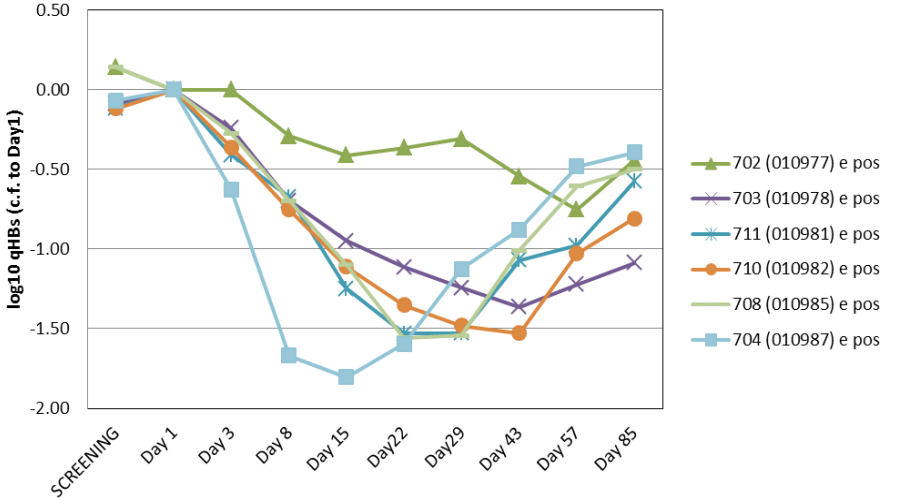
Direct antiviral effect lasted up to 57 days after a single dose of ARC-520, delayed response duration >85 days

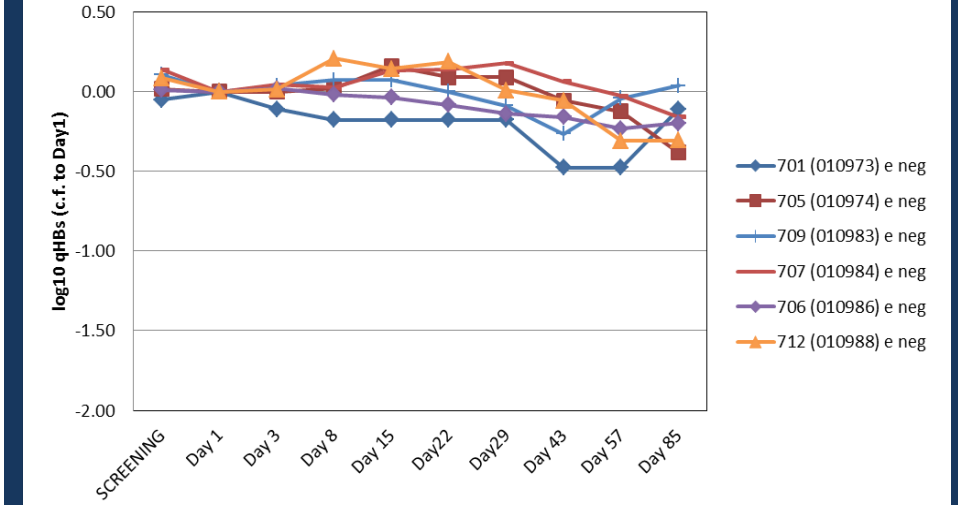
- Small dose-related reduction in HBsAg
- Maximum effective dose not reached
- HBV DNA results pending in ETV naïve patients

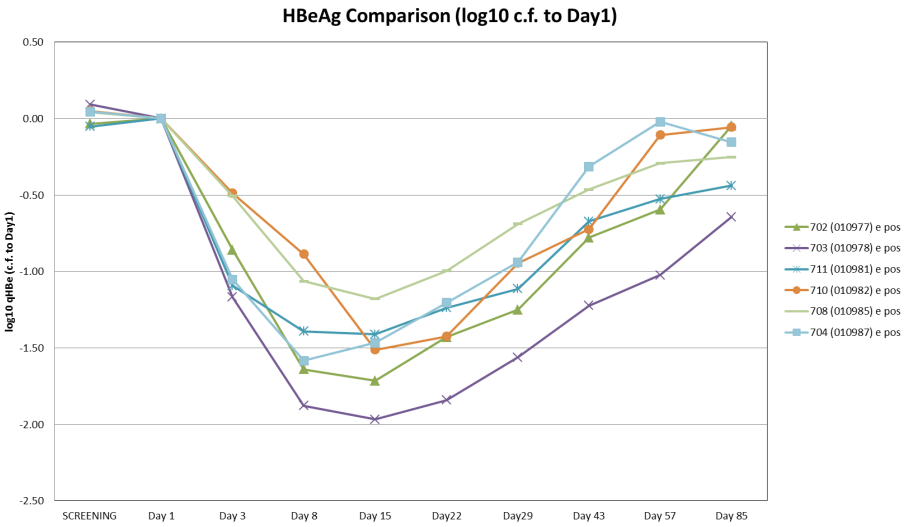
Yuen M-F, et al. AASLD 2015, San Francisco. #LB-9

ARC-520 RNAi: clinical responses

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







HBsAg:

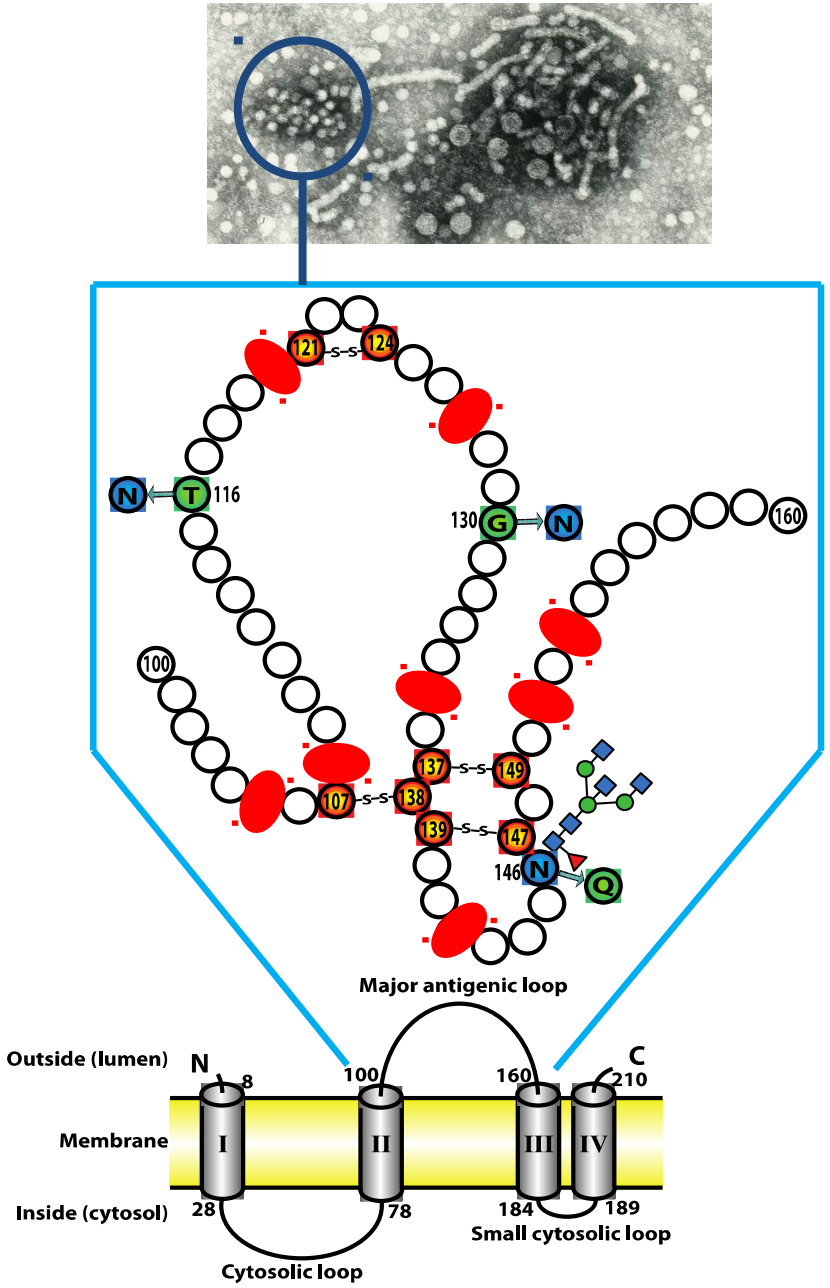
•> 1log drop in HBsAg achieved by all HBe pos subjects

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

HBeAg:

•> 1log decline in HBeAg achieved

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**

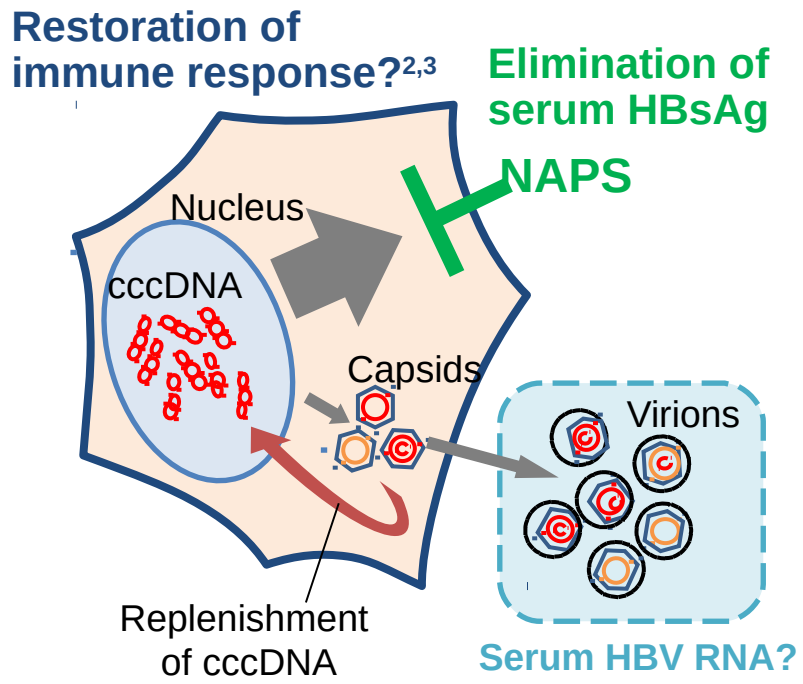
Fattovich G, et al. Am J Gastro 1998; 93:896-900. Werle-Lapostolle B, et al. Gastroenterology 2004; 126(7):1750-1758. Perrillo R. Hepatology 2009; 49:1063-1065

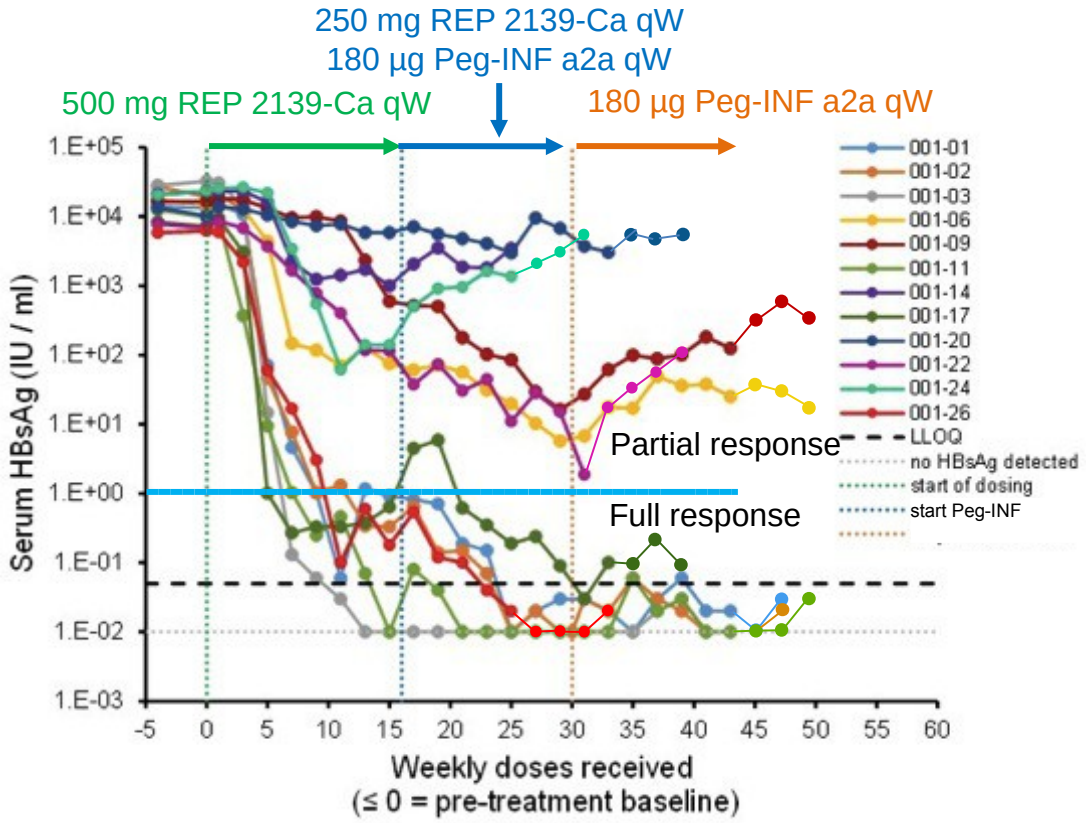
Nucleic Acid Polymers (NAP): Virion Assembly and HBsAg Egress

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]



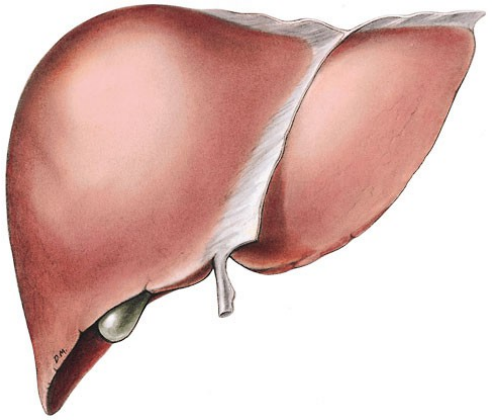


Bazinet M, et al. AASLD 2015, San Francisco. #31.

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

Hepatitis B Virus Immune Evasion/Exhaustion

- **Hepatotropic virus**
  -
    -
    -
    -



- **High Antigen burden**
  -
    -
    -

- **Immune regulation at the innate and adaptive level**

Rationale for Immunotherapy in HBV

- **Spontaneous sustained immune control in adults following acute infection**

- **Bone marrow transplant > Immune reconstitution > clearance of chronic HBV**

- **Patients that spontaneously clear chronic HBV display robust T cell responses**

Potential Immunological Targets for HBV Therapy

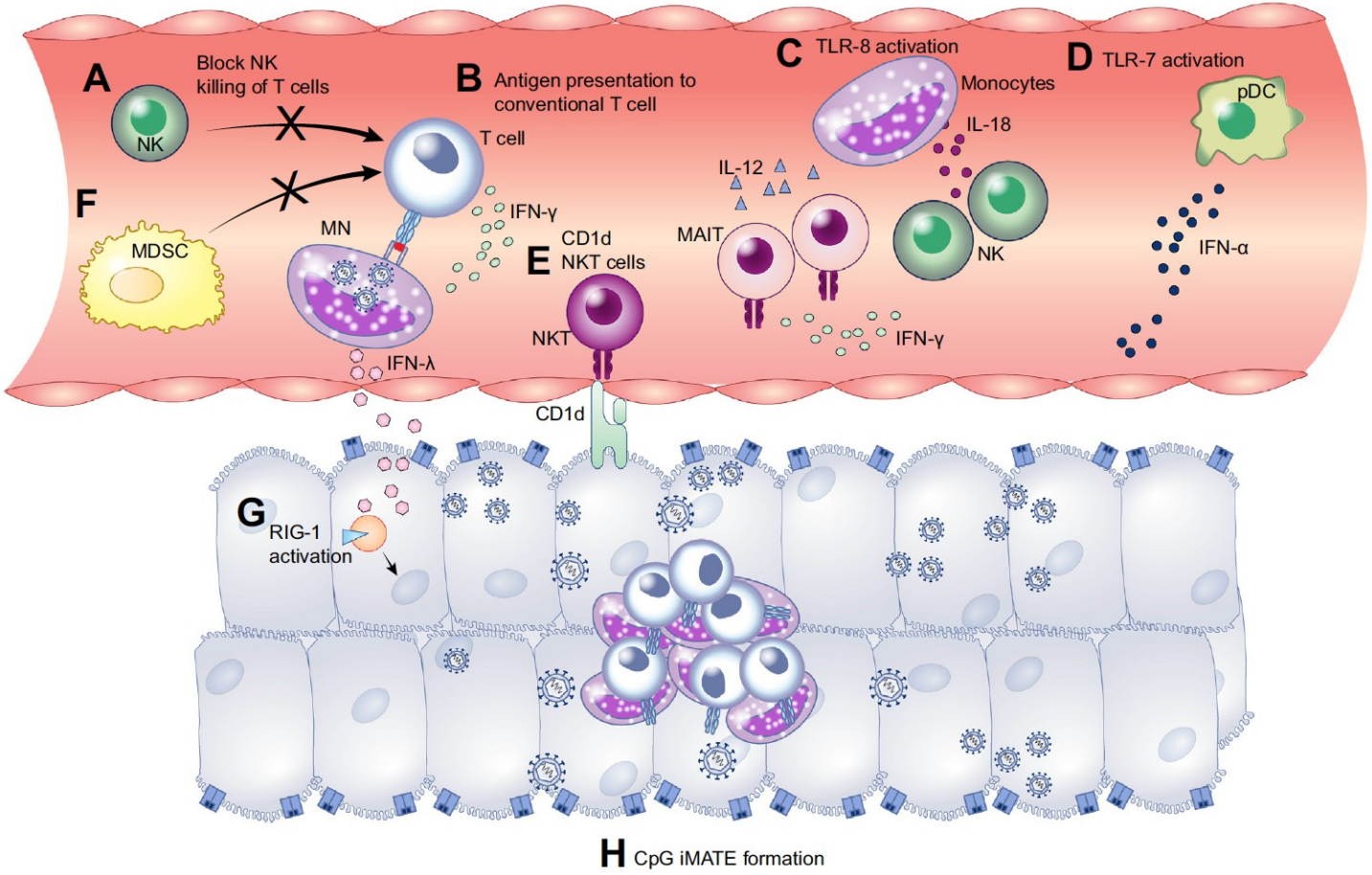
Innate

-
-

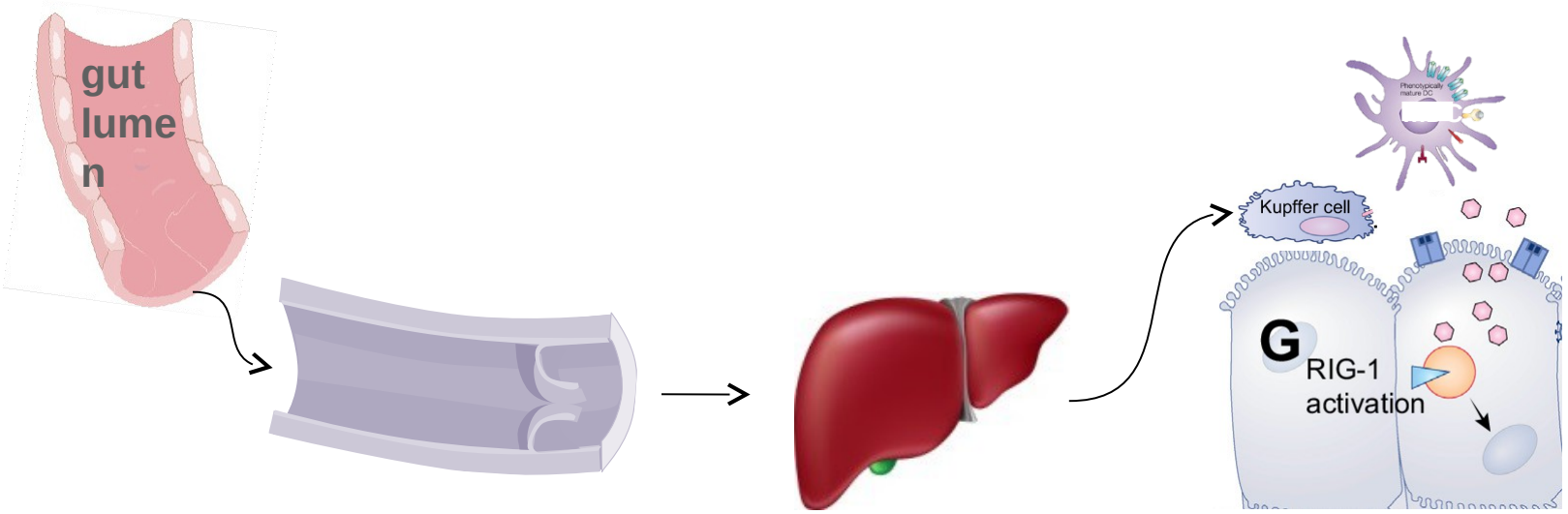
Adaptive

-
-

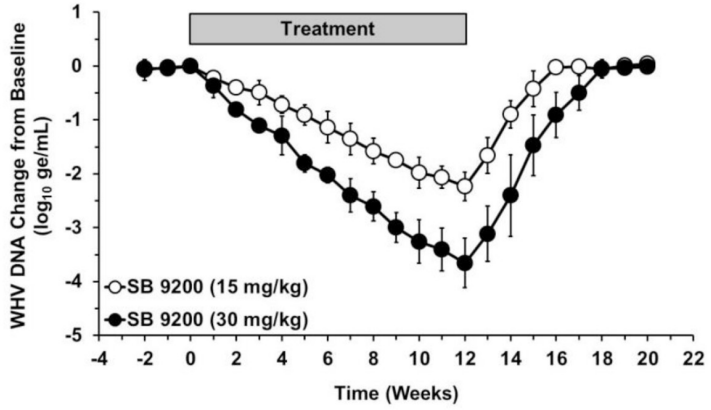
Opportunities for Innate-targeted Immunotherapy - Stimulating antiviral cytokine production --

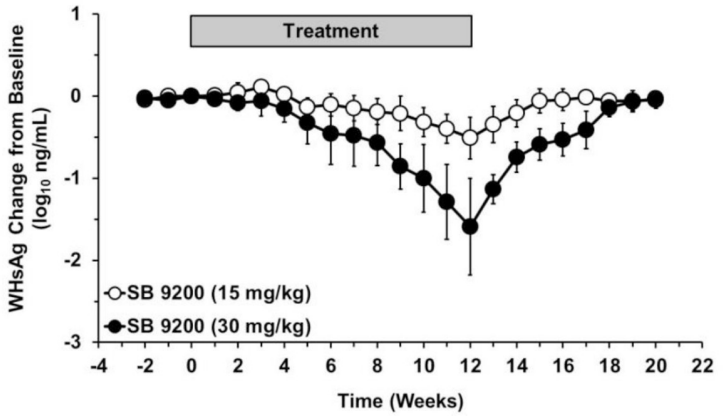


Direct triggering of RIG-I in infected hepatocytes

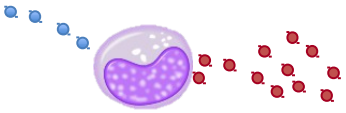


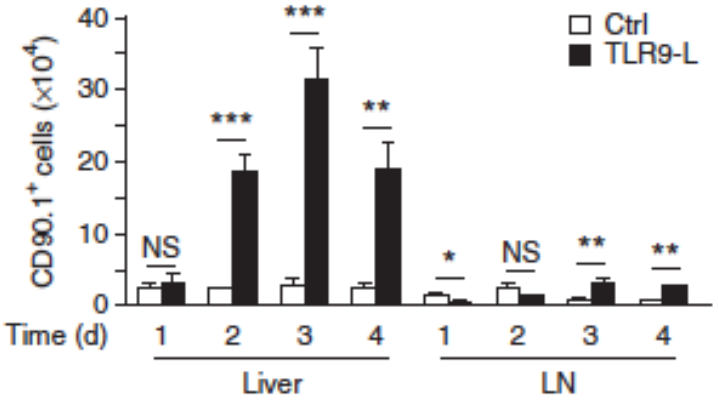
Chronic Woodchuck Hepatitis Virus Infection model

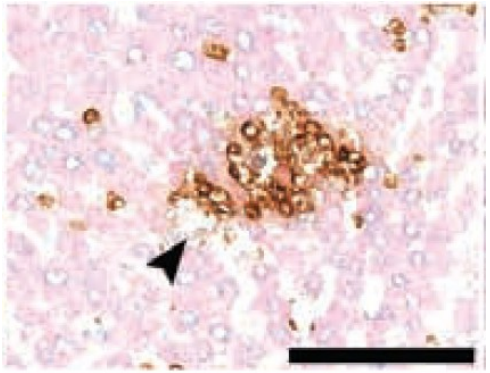


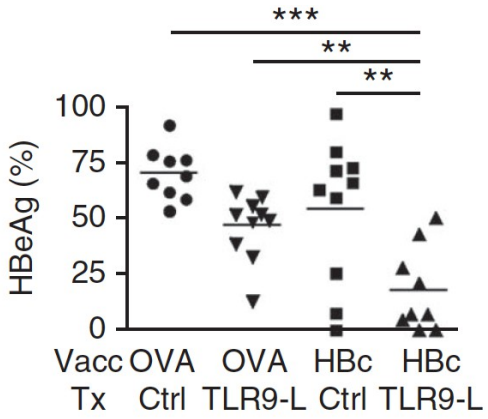


CpG induction of iMATEs









Potential Immunological Targets for HBV Therapy

Innate

-
-

Adaptive

-
-

Therapeutic Vaccination in Chronic HBV Infection

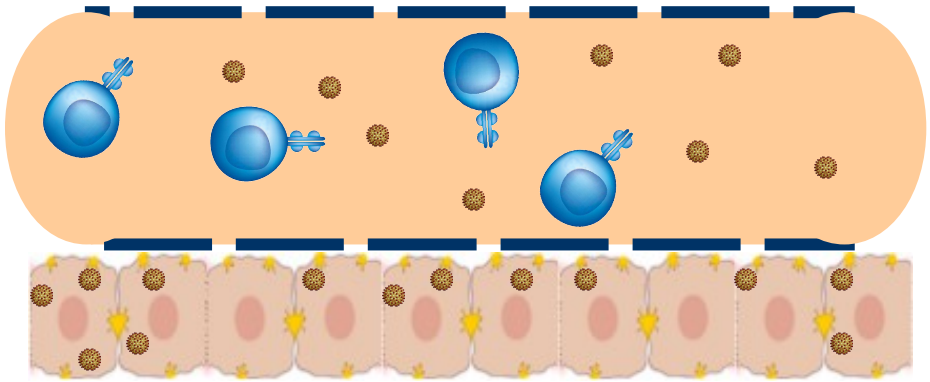
Inhibition of HBV-specific T cell Function

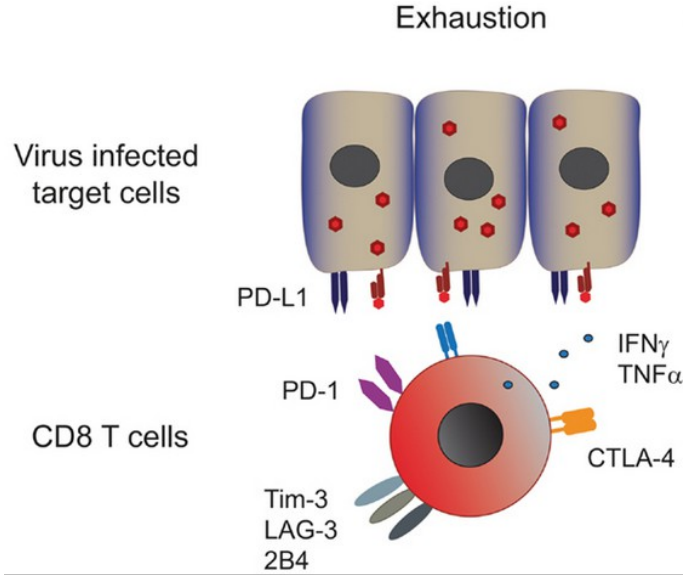
Chronic

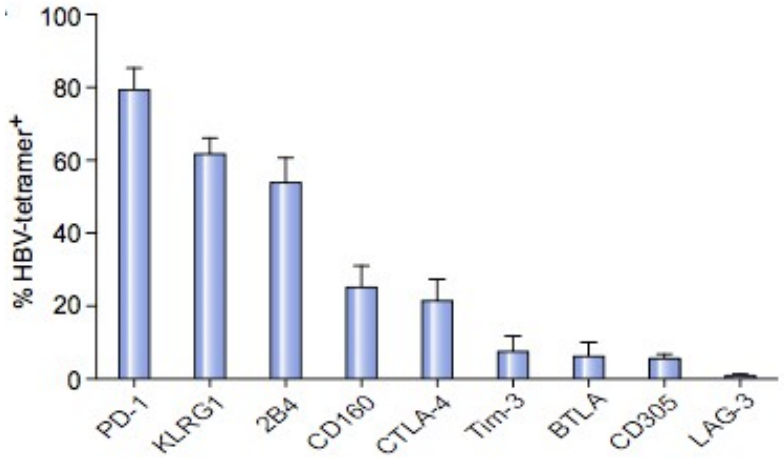
Weak T cell response + no effective antibodies

1.
   a.

1.
   a.
   b.
   c.

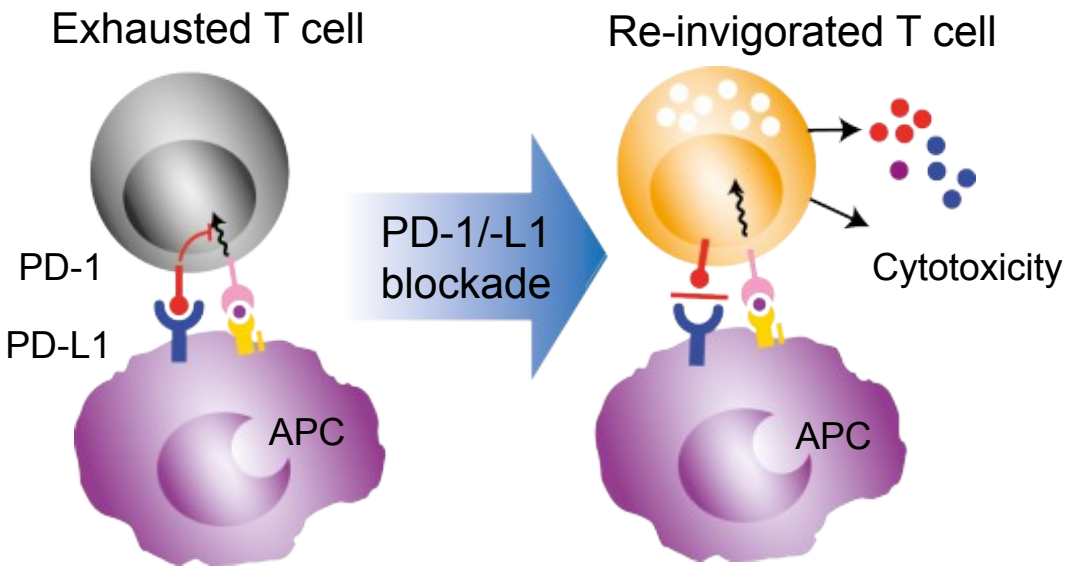




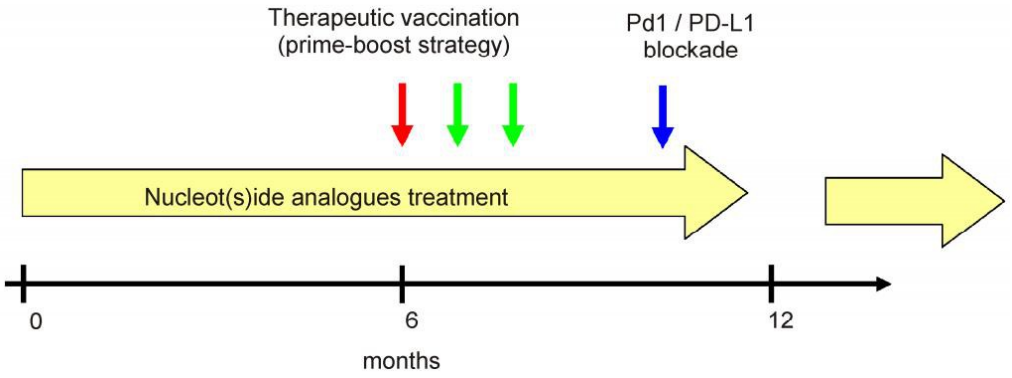


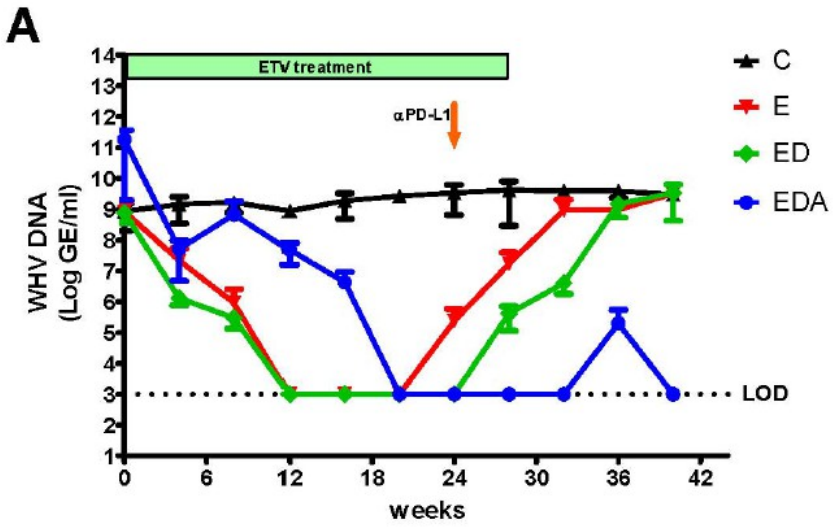
Therapeutic vaccination with checkpoint modulation

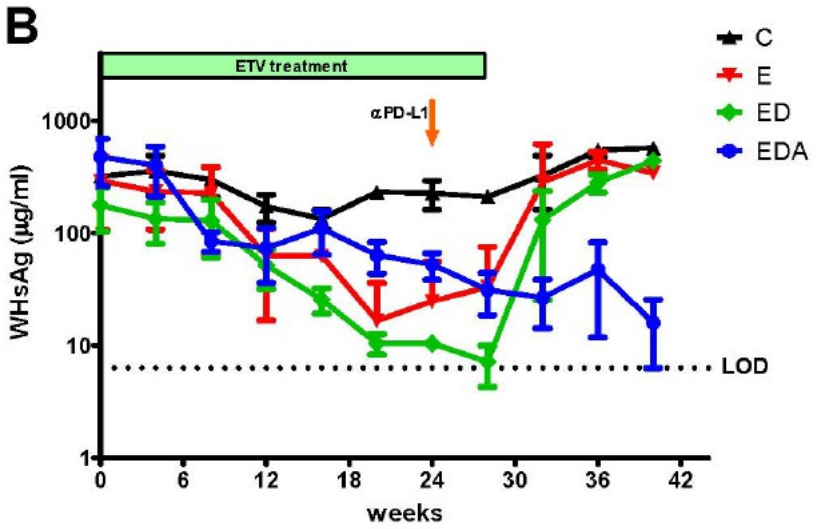
The NEW ENGLAND JOURNAL of MEDICINE
Safety and Activity of Anti–PD-L1 Antibody in Patients with Advanced Cancer
N ENGL J MED 366;26 NEJM.ORG JUNE 28, 2012



Therapeutic vaccination with checkpoint modulation







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)

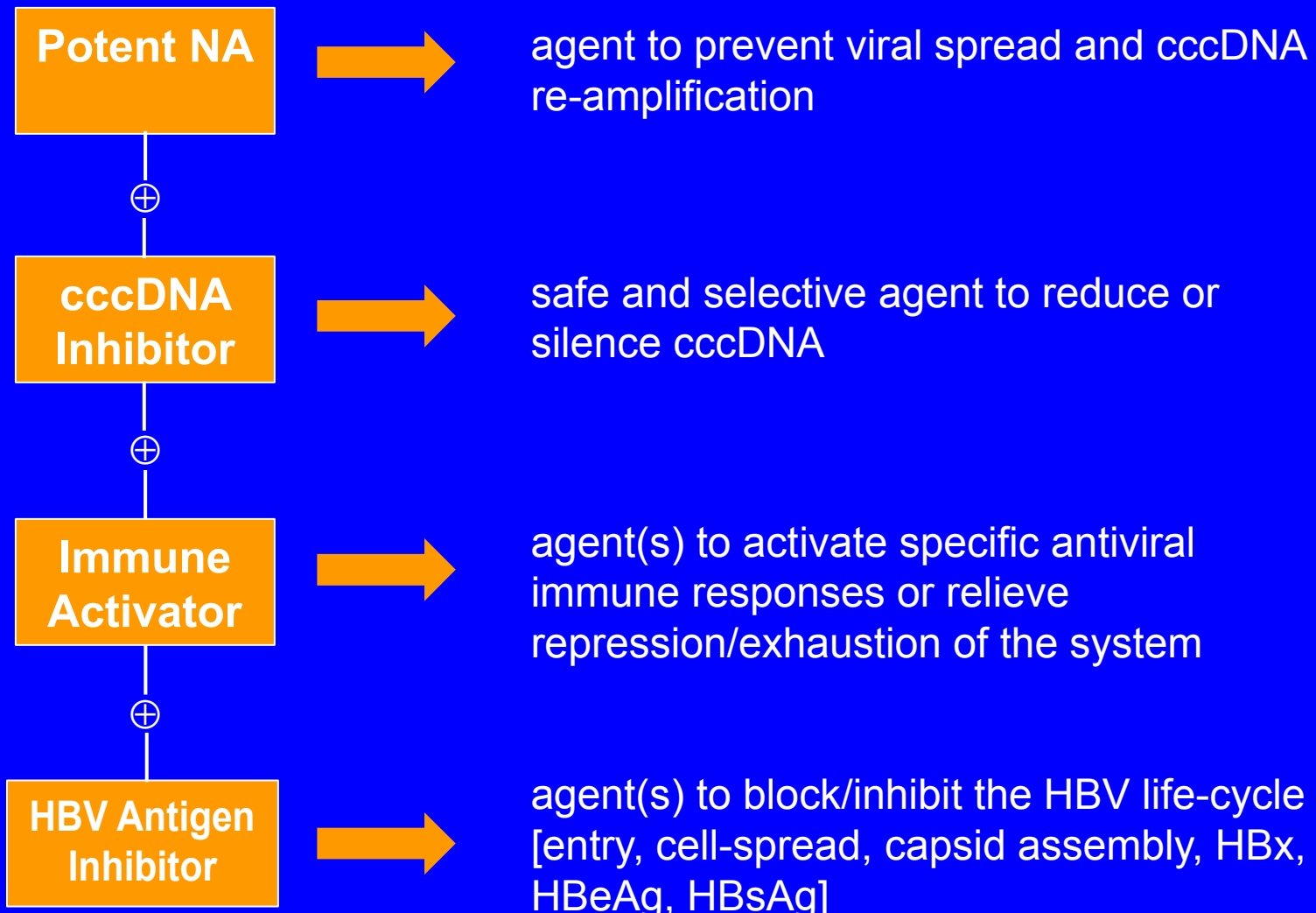
Hadziyannis, S et al 2012. Gastro;143:629.
Liang, Y et al 2011. Aliment Pharacol Ther;34:344.
Patwardham, N et al 2014. Aliment Pharmacol Ther;40:804.
He, D et al 2013. BMC Infec Dis;13:458.
Jeng, W-J et al 2013. Hepatol;58:1888.

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