Verso nuovi target terapeutici molecolari: l'eradicazione di HBV è un obiettivo possibile?

Massimo Levrero [1,2]

1. *DMISM - Dept of Internal Medicine, Sapienza University, Rome, Italy*
2. *IIT@Sapienza - Center for Life NanoSciences - Rome – Italy*

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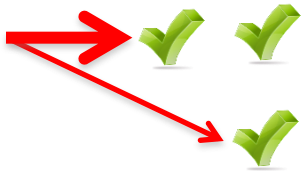
Disclosures

- BMS: Advisory Board and invited speaker; investigator
- Gilead: Advisory Board and invited speaker; investigator
- Janssen: Advisory Board and invited speaker, investigator
- MSD: Advisory Board and invited speaker; investigator
- Roche: Consultancy; invited speaker
- Tekmira: Advisory Board
- Medimmune: Advisory Board
- Galapagos: Advisory Board

HBV: concepts about « cure »

Long-term suppression of viral replication (DAA)



Viral Suppression

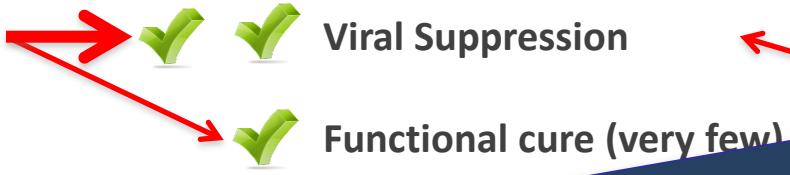
Functional cure (very few)

Eradication

Suppression of viral replication Immune control (IFNα)

HBV: concepts about « cure »

Long-term suppression of viral replication (DAA)



Viral Suppression

Functional cure (very few)

Suppression ation trol

long-term to life long therapies

risk of HCC persists

HBV: concepts about « cure »

Long-term suppression of viral replication (DAA)

Viral Suppression

Functional cure (very few)

Eradication

Suppression of viral replication Immune control (IFNα)

Strategies

Immune system

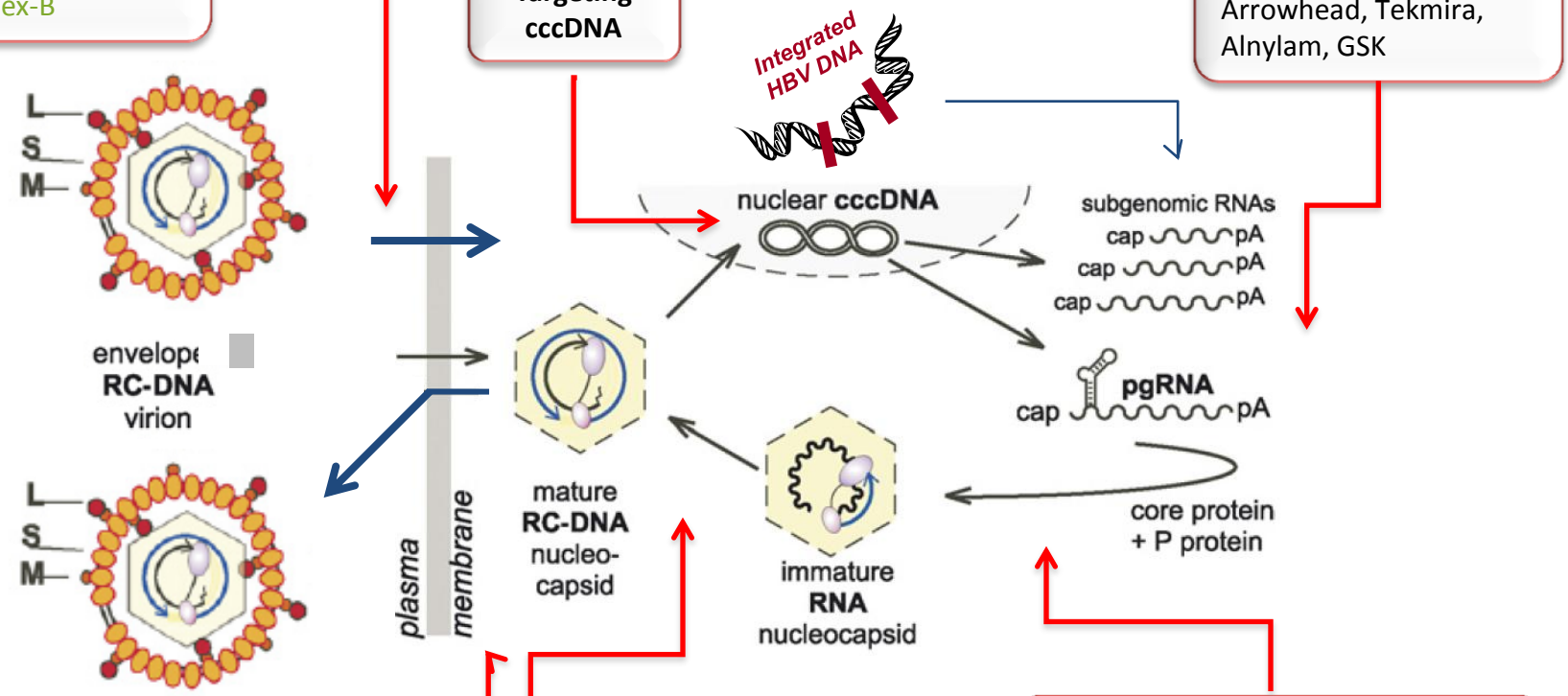
Viral targets

HBV cure landscape

Entry inhibitors
- Lipopeptides, e.g. Myrcludex-B

Targeting cccDNA

RNA interference, Arrowhead, Tekmira, Alnylam, GSK



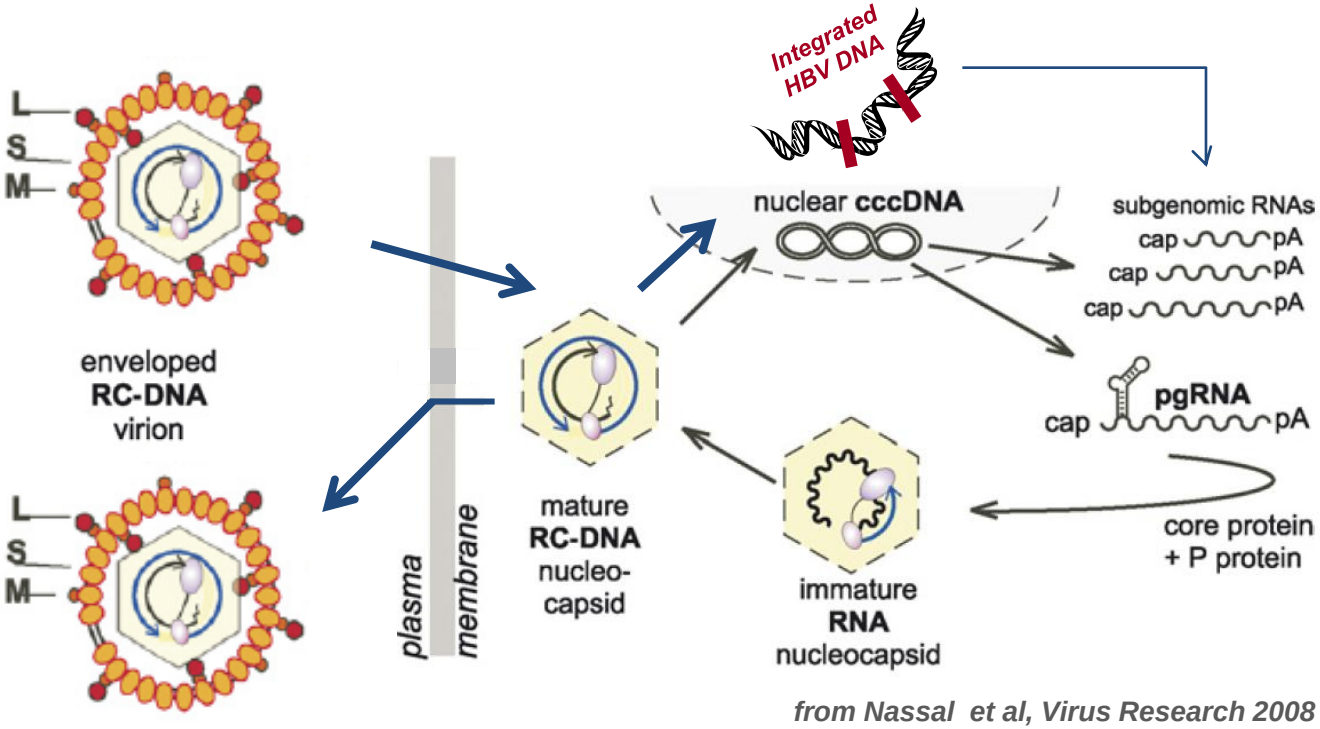
Inhibitors of HBsAg release, Replicor

Polymerase inhibitors
- Nucleoside analogues, e.g. Gilead, BMS
- Non-nucleoside, e.g. LB80380

Inhibition of nucleocapsid assembly Novira, AssemblyPHARMA, Gilead, Janssen

Immune modulation
- ***Toll-like receptors agonists***, Gilead, Roche
- ***Anti-PD-1 mAb***, BMS, Merck
- ***Vaccine therapy***: Transgene, Gilead, Roche Innovio, Medimmune, ITS

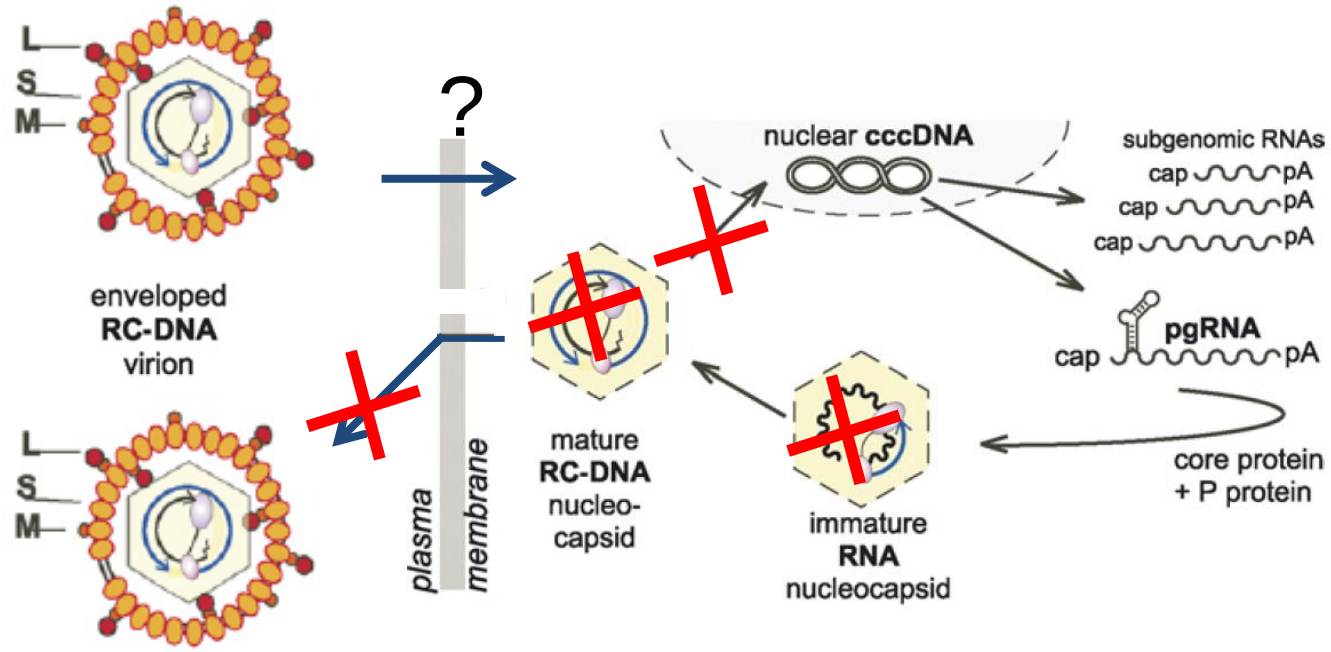
HBV cccDNA



from Nassal et al, Virus Research 2008

- template for all HBV mRNAs and the HBV pgRNA
- not directly targeted by NUCs
- may exist hepatic "latent" reservoire (non integrated functionally competent HBV genomes): OBI and inactive carriers

Antivirals do not directly target cccDNA

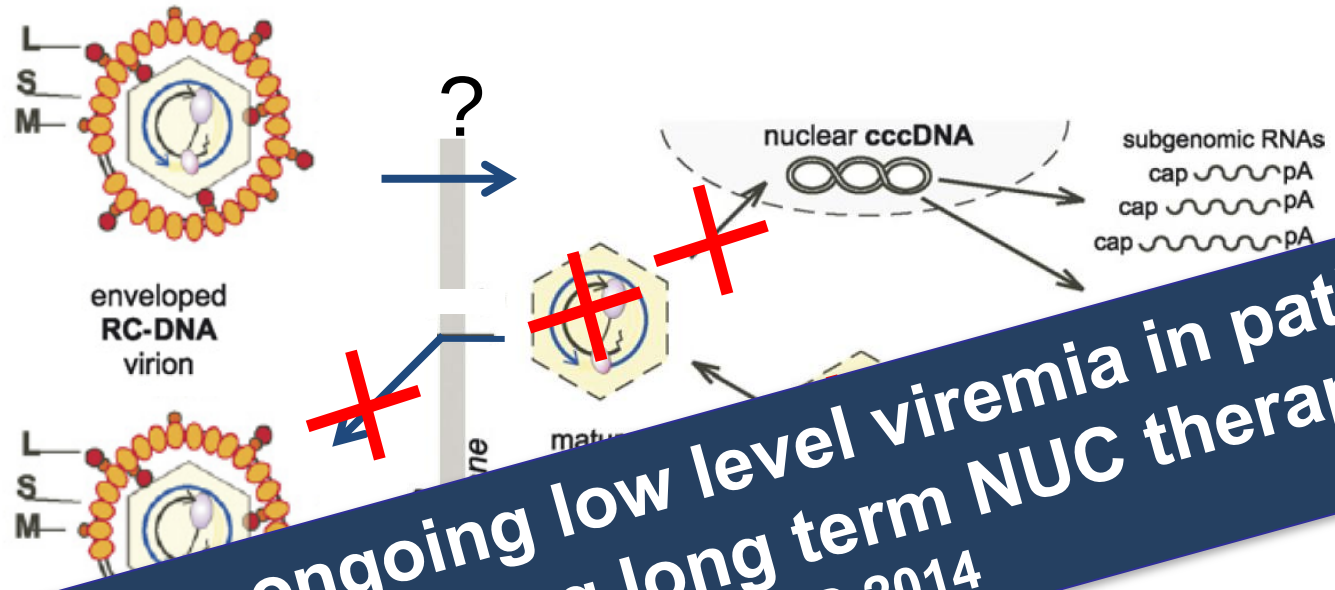


Modified from Nassal et al, Virus Research 2008

1 yr of monotherapy with nucleos(t)ide analogues (ADV, LAM, ETV) reduced median intrahepatic cccDNA amounts by 1 log

Zoulim,Petersen,Locarnini, Gastroenterology 2004,
Wong, Antivir Ther 2006, Sung, Gastroenterology 2005

Antivirals do not directly target cccDNA



Evidence for ongoing low level viremia in patients with CHB receiving long term NUC therapy

Marcellin , AASLD 2014

Modified from Nassal et al, Virus Research 2008

1 yr of monotherapy with nucleos(t)ide analogues (ADV, LAM, ETV) reduced median intrahepatic cccDNA amounts by 1 log

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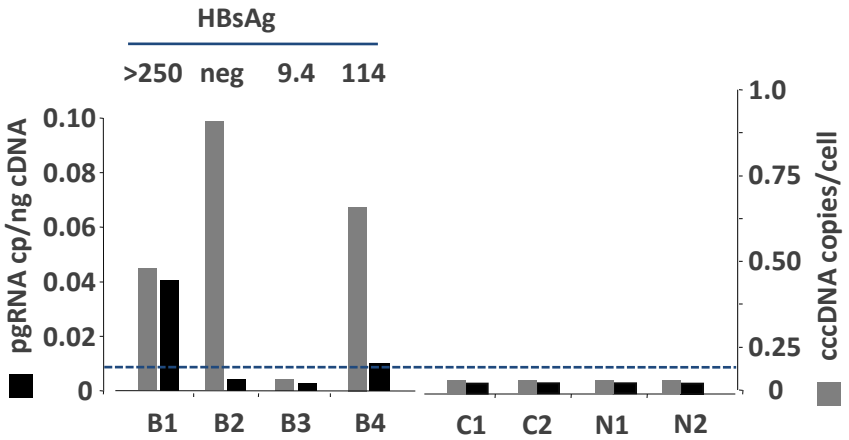
Persistence of cccDNA



Persistence of cccDNA in 3 out of 4 patients with long term HBV suppression under lamivudine
In 2 out 3 patients cccDNA is inactive (no pgRNA)

Belloni, Levrero, Gaeta HBV meeting 2010

- **Detected in the liver of NUCs long-term suppressed patients after HBsAg to anti-HBs seroconversion [Maynard, 2005; Belloni unpublished]**
- **Detected in the liver of HBsAg negative patients (occult HBV infection) [Werle-Lapostolle, 2004; Pollicino unpublished]**
- **Present in 30 /30 patients with occult HBV infection and HCC [Pollicino, 2004]**

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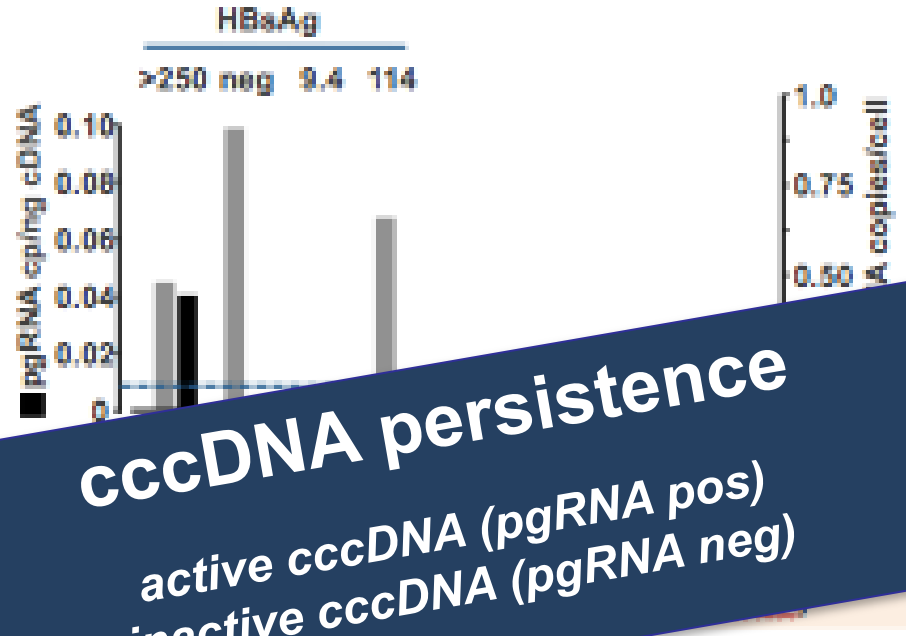
Persistence of cccDNA



cccDNA persistence

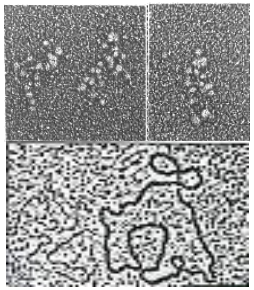
active cccDNA (pgRNA pos)
inactive cccDNA (pgRNA neg)

...irectly target cccDNA

persists in NUC long-term suppressed patients, occult HBV infection and subjects recovered from AVH

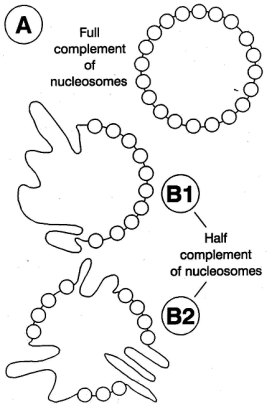
(Werle-Lapostolle 2004; Pollicino 2004; Maynard, 2005; Belloni, 2010)

The HBV cccDNA as a “minichromosome”



Bock, T. et al 1994.

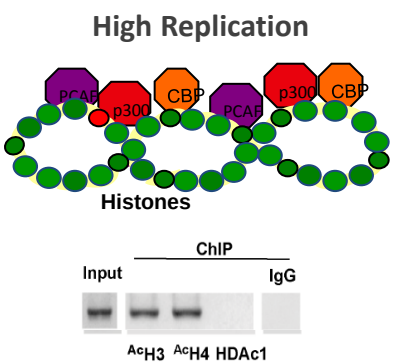
Bock, T. et al 2001

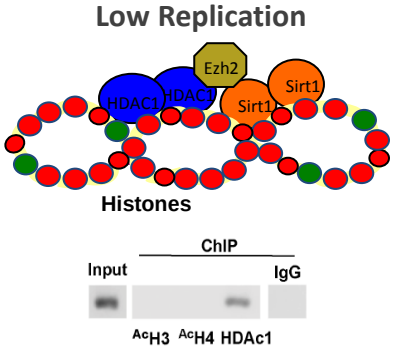


Newbold et al, 1995

Low Replication Phenotype
Quiescent or active
Medium to Low Viraemia

High Replication Phenotype
Transcriptionally Active
High Viraemia



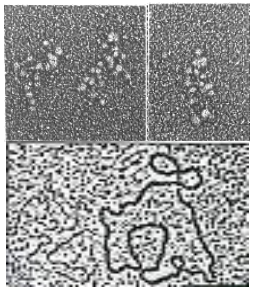


Pollicino, 2006; Belloni 2009)

- HBV cccDNA is organized as a minichromosome in the nucleus of infected cells by histone and non-histone proteins

  (Newbold 1995, Bock 2001, Pollicino 2006).

The HBV cccDNA as a “minichromosome”



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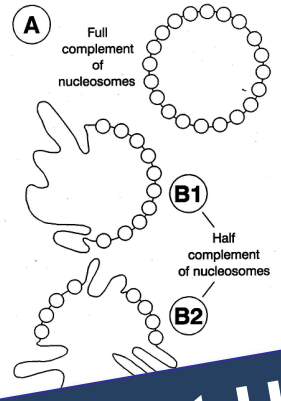

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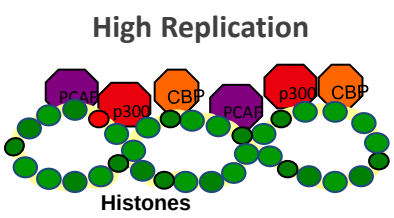
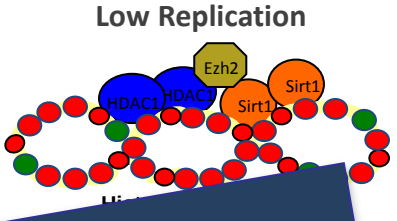
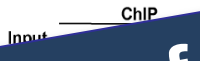






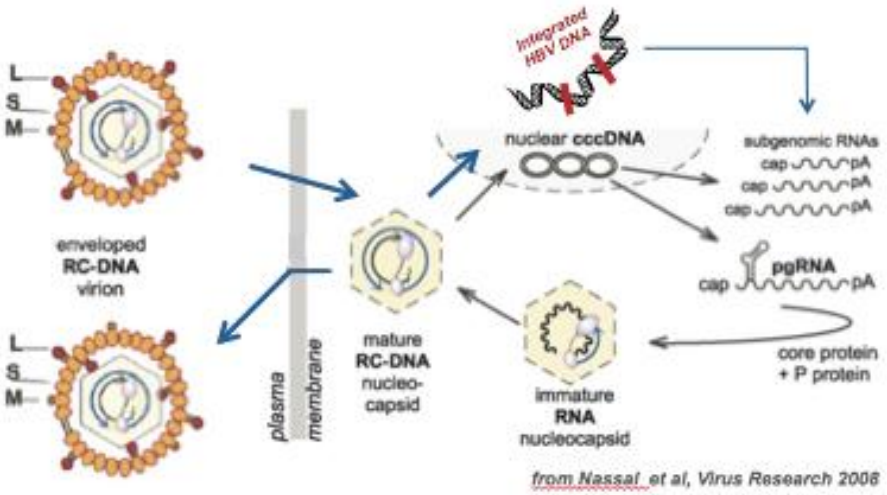
Pollicino, 2006; Belloni 2009)

Distinct HBV populations of cccDNA minichromosomes may exist

- [illegible] is organized as a minichromosome in the nucleus of infected cells by histone and non-histone proteins

  (Newbold 1995, Bock 2001, Pollicino 2006)

HBV cure – targeting cccDNA



- complete inbition of HBV replication
  - to avoid new hepatocytes infection and low level core particles recycling
- restoration of host innate and adaptive antiviral immunity against HBV
- direct targeting of cccDNA
  - inhibit cccDNA formation
  - cccDNA targeting endonucleases
  - induction of cccDNA cytosine deamination
  - destabilize cccDNA minichromosome

  - accelleration cccDNA loss during cell division
  - transcriptional silencing of cccDNA [FUNCTIONAL CURE]

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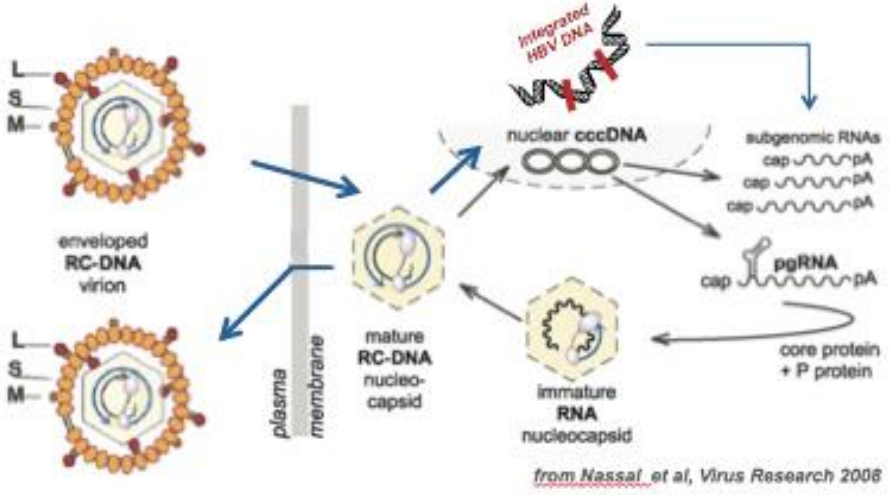
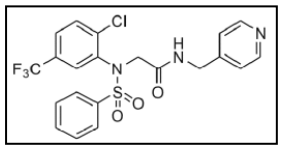
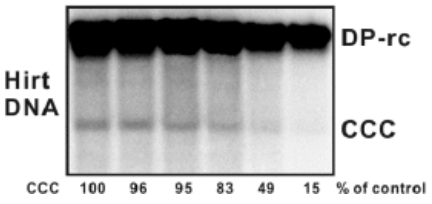
Identification of Disubstituted Sulfonamide Compounds as Specific Inhibitors of Hepatitis B Virus Covalently Closed Circular DNA Formation

cccDNA formation



CCC-0975





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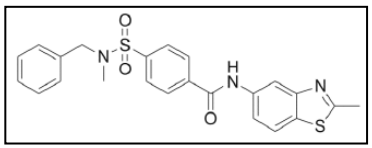

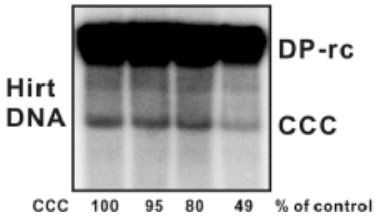

Cai et al., AAC 2012, 46, 4277.

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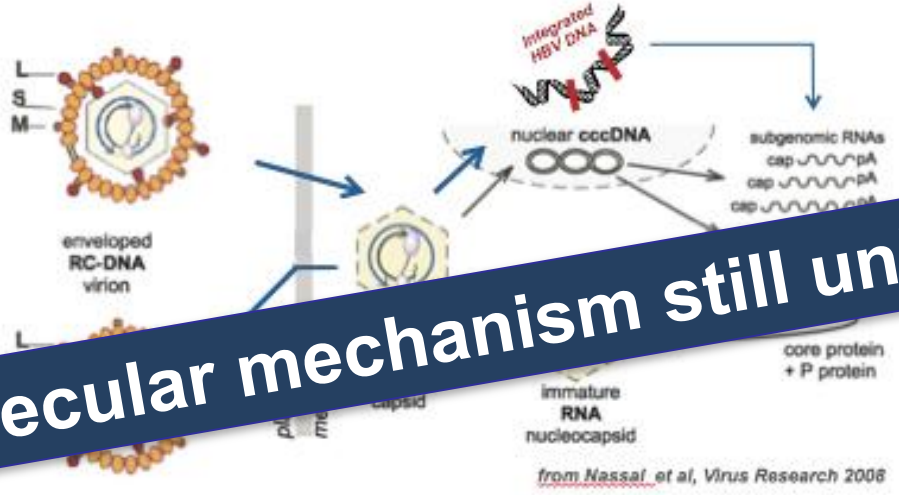
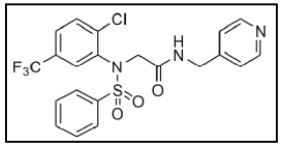
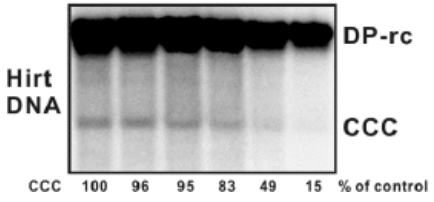
cccDNA formation



Molecular mechanism still unknown

CCC-0975





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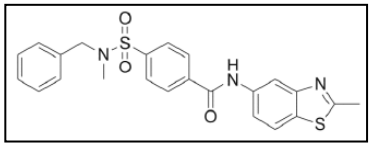

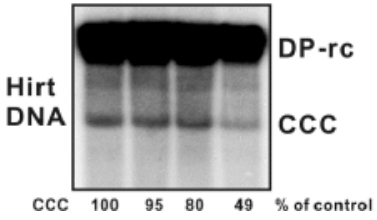

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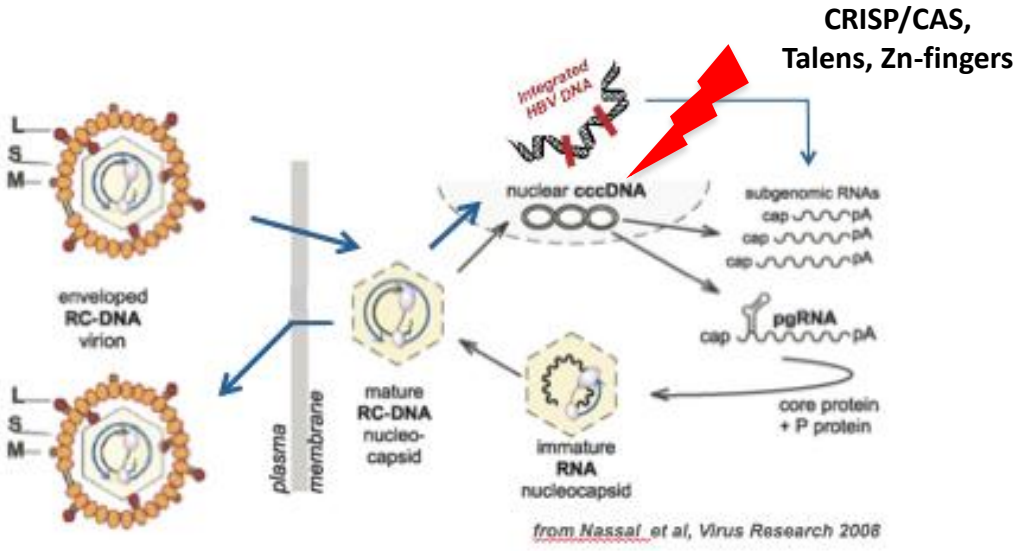
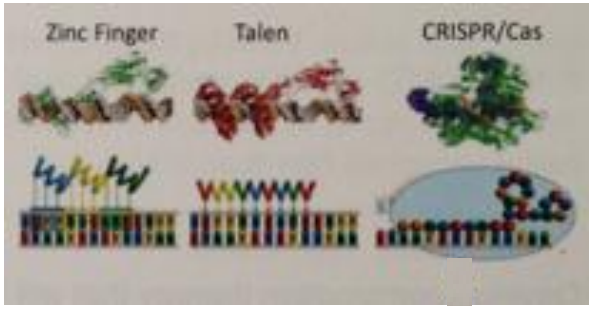
Cleavage of cccDNA by targeted gene disruption strategies



- **Zinc Finger** *[Weber, PlosOne, 2014]*
- **Talens** *[Chen, Mol Therapies, 2014]*
- **Bacterial CRISP / Cas RNA-guided DNA endonucleases**
  [Seeger, Mol Therapy Nucl Acids, 2014; [Lin, Mol Therapy Nucl Acids, 2014; [Kennedy, Virology, 2015]



Cleavage of cccDNA by targeted gene disruption strategies

Even if efficacy issues are solved, delivery may remain unpractical

- **Zinc Finger** *[Weber, PlosOne, 2014]*
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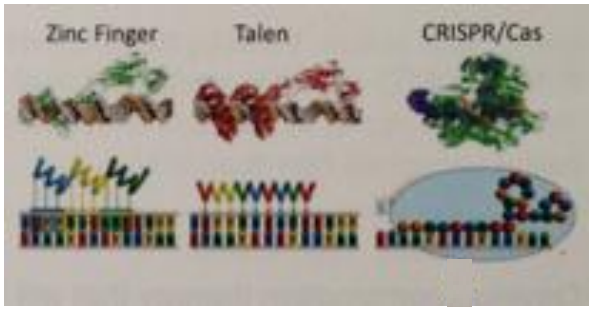
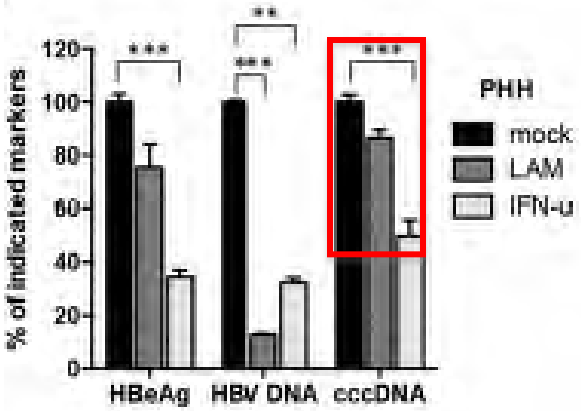
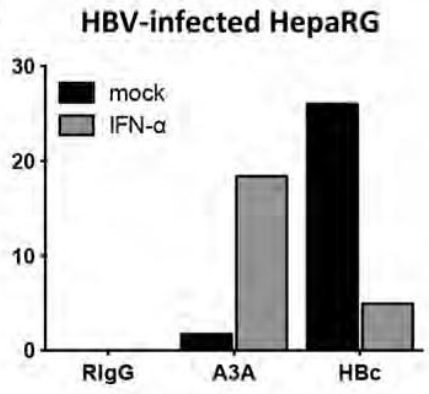
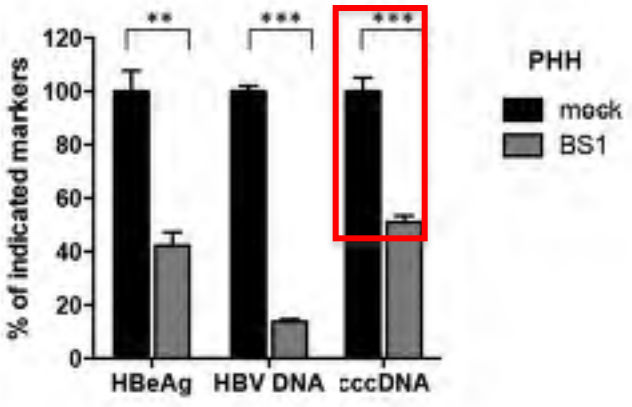
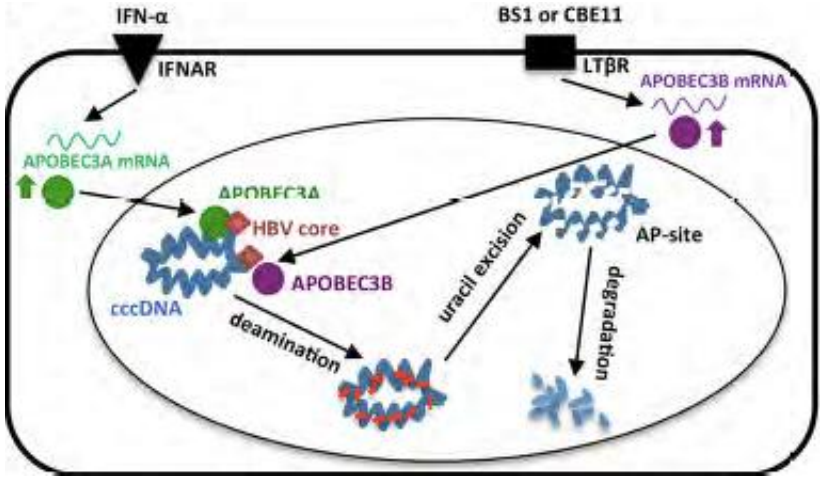


Specific and Nonhepatotoxic Degradation of Nuclear Hepatitis B Virus cccDNA





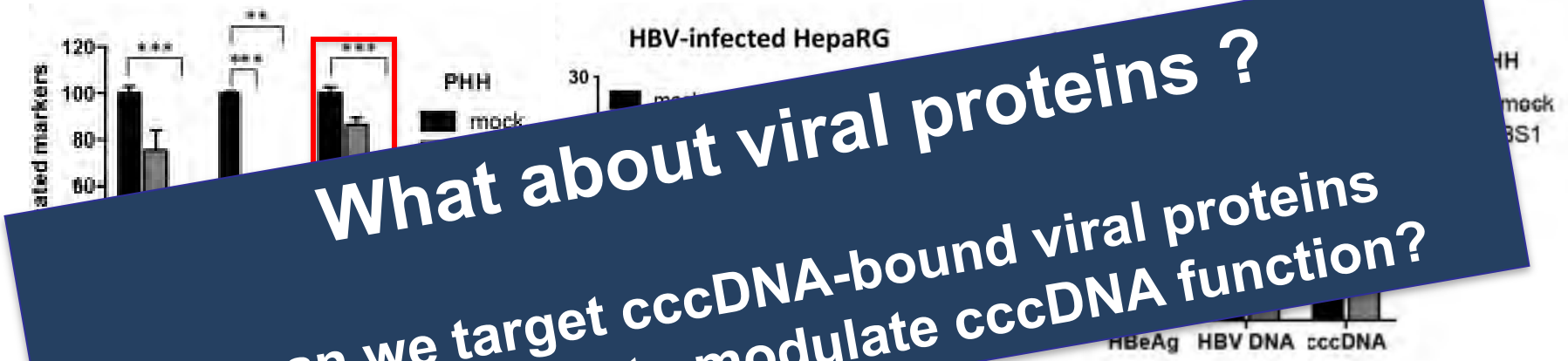




- Interferon-α and lymphotoxin-β-receptor activation up-regulated APOBEC3A and 3B cytidine-deaminases, respectively, in HBV-infected cells, primary hepatocytes and human liver-needle biopsies.
- HBV-core protein mediates the interaction with nuclear cccDNA resulting in cytidine-deamination, apurinic/apyrimidinic site formation and finally cccDNA degradation

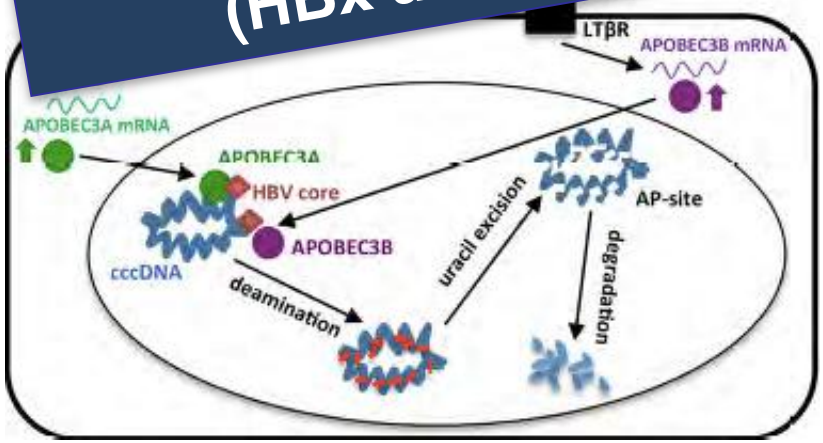
Lucifora et al. Science 343, 1221-8, 2014

Specific and Nonhepatotoxic Degradation of Nuclear Hepatitis B Virus cccDNA



What about viral proteins ?

can we target cccDNA-bound viral proteins (HBx and HBc) to modulate cccDNA function?



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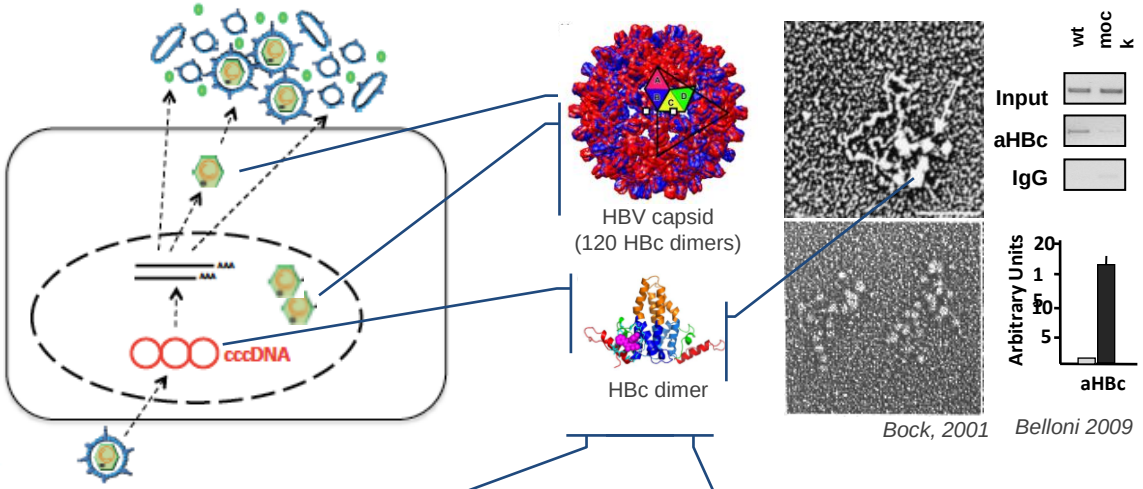
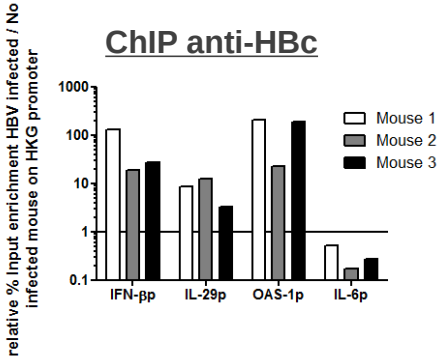
HBc protein / capsid

- HBc binds the cccDNA and modifies cccDNA nucleosome spacing



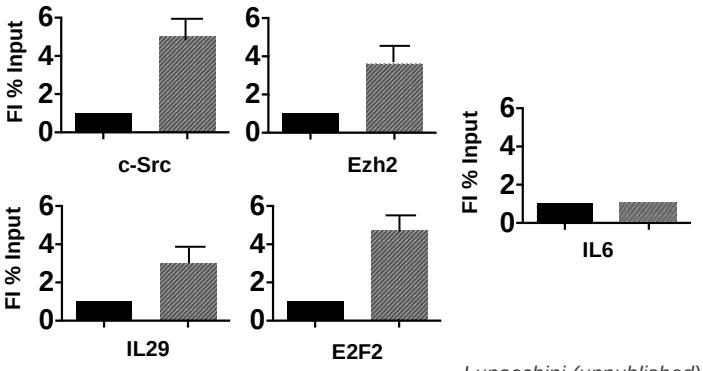
- HBc binds to (and represses) the IFN-b, IL-29 and OAS1 cellular promoters

(Durantel D, AASLD 2013)



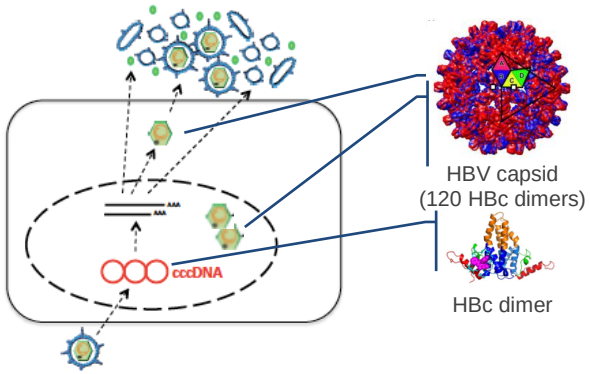
- HBc binds to cellular promoters and regulates gene expression

(Guo, BMC genomics, 2013)

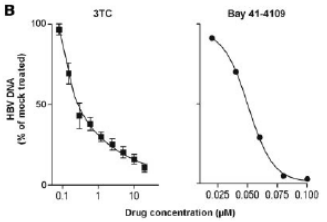


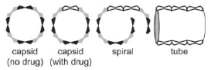
Lupacchini (unpublished)

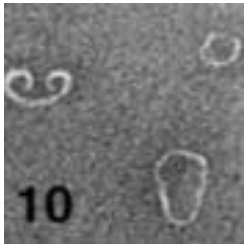
Assembly inhibitors
Core Protein Assembly Modulators (CpAMs)
Core inhibitors / Anti-capsid

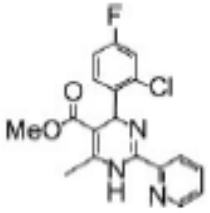


Hetero-aryl-dihydropyrimidines (HAPs) and the Phenyl-Propenamide derivatives, AT130 are a new class of antivirals that target the HBV capsid and inhibit HBV replication *in vitro* (Deres, Science 2003; Stray, PNAS 2005).

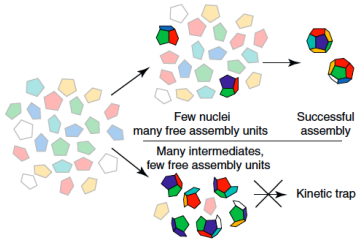


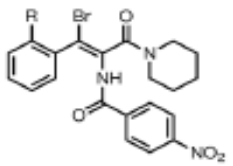




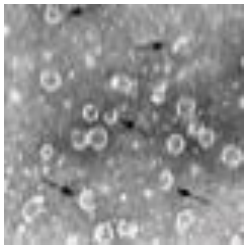


HAP12



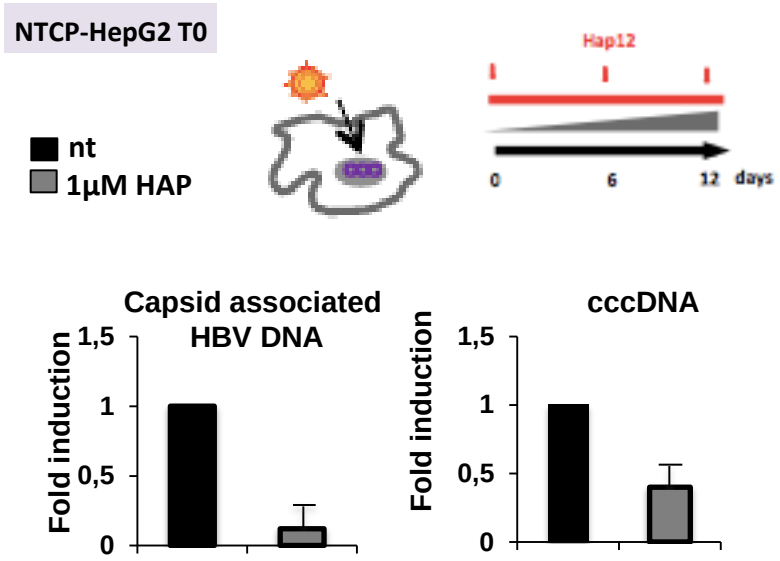


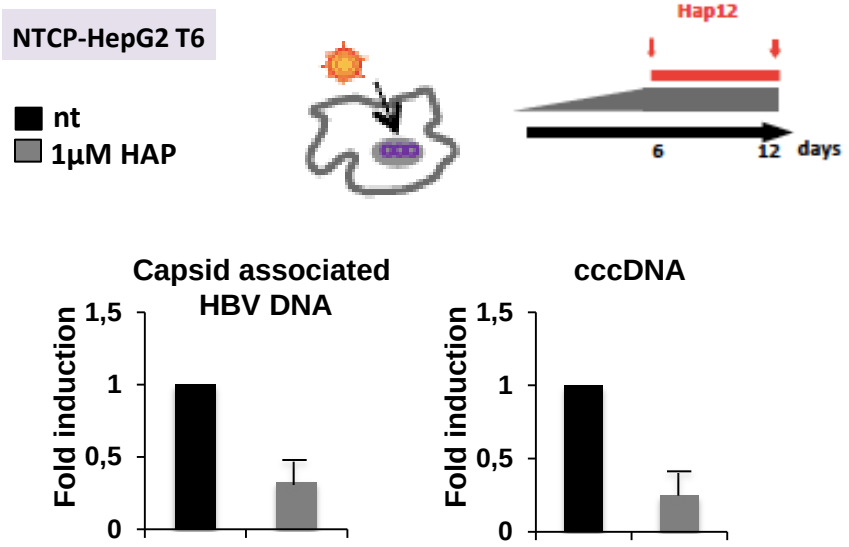
AT130



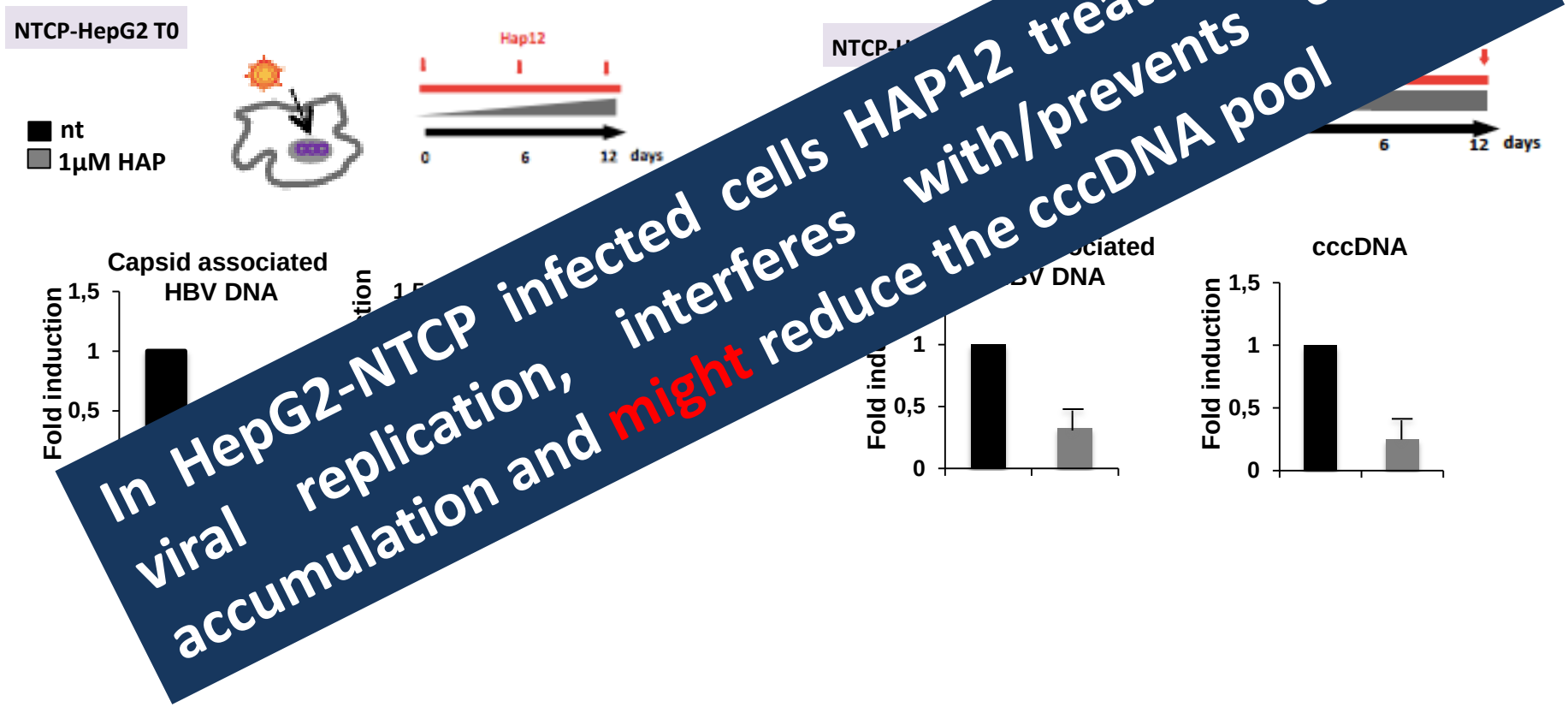
- HAP12 and AT130 misdirect HBV capsid assembly and block HBV replication.
- AT130 capsids are more "normalish" but inactive; HAP12 leads to aberrant structures

Core inhibitors affects the cccDNA formation/accumulation in HepG2-NTCP infected cells





Core inhibitors affects the cccDNA formation/accumulation in HepG2-NTCP infected cells



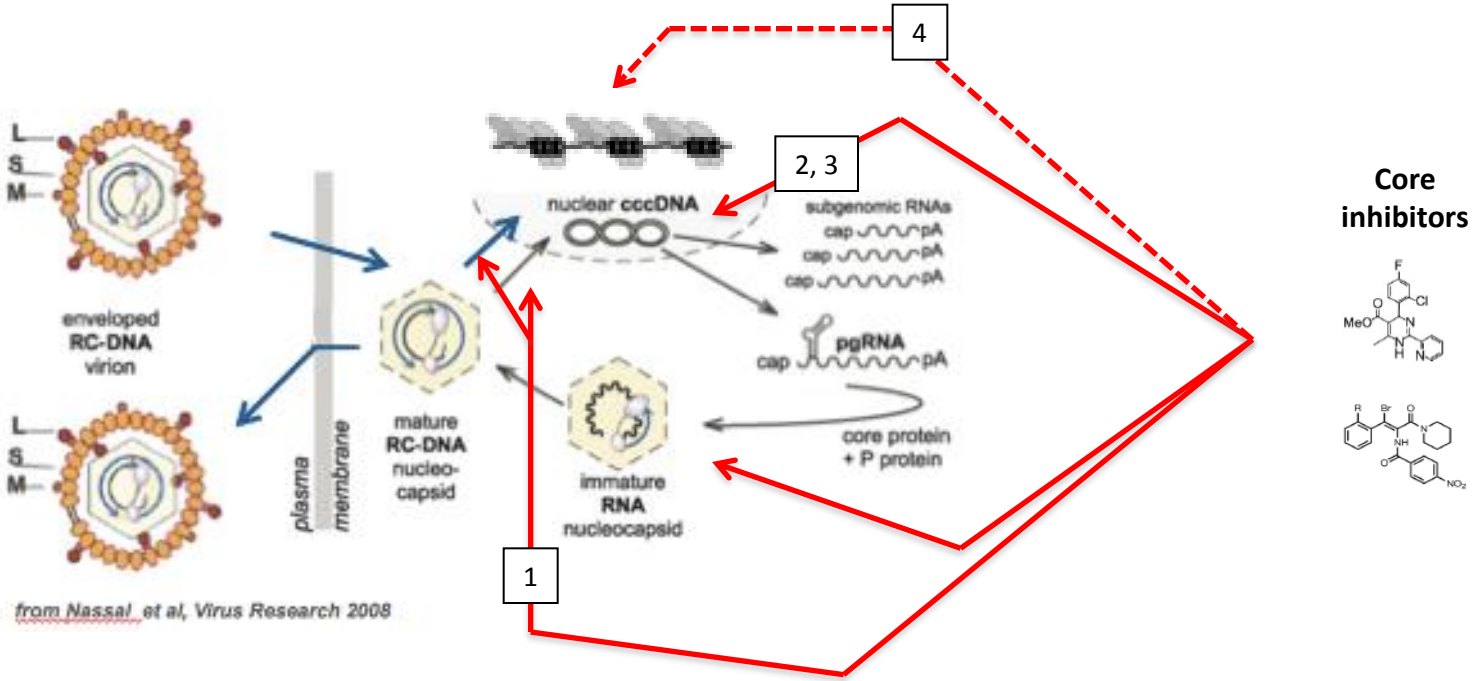
In HepG2-NTCP infected cells HAP12 treatment blocks viral replication, interferes with/prevents cccDNA accumulation and might reduce the cccDNA pool

Anti-capsid drugs

- **Core inhibitors (Hap12 and AT130) impact on Cp nuclear functions at multiple levels:**

  - **block new cccDNA accumulation (Rc-DNA delivery and/or core particles recycling)** [1]
  - **reduce the size of an *established* cccDNA pool** [2]
  - **inhibit HBc recruitment on the cccDNA** [3]

  The effects on HBc recruitment on cellular genes remains to be determined [4]

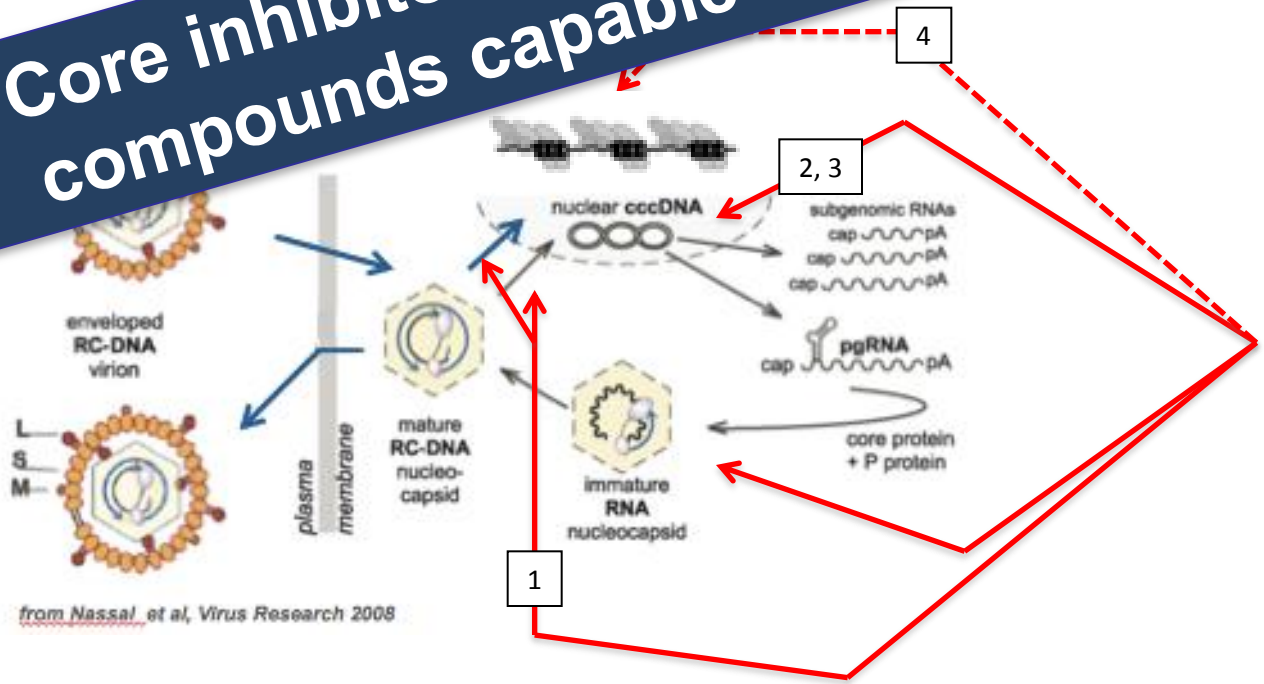


Anti-capsid drugs

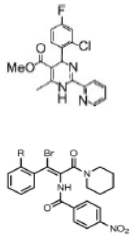
- **Core inhibitors (Hap12 and AT130) impact on Cp nuclear functions at multiple levels:**
  - **block new cccDNA accumulation (Rc-DNA delivery and/or core parti** 1
  - **reduce the size of an *established* cccDNA pool** 2
  - **inhibit HBc recruitment on the cccDNA** 3

The effects on HBc recruit… …e determined 4

Core inhibitors are the first "viral specific" compounds capable to target the cccDNA

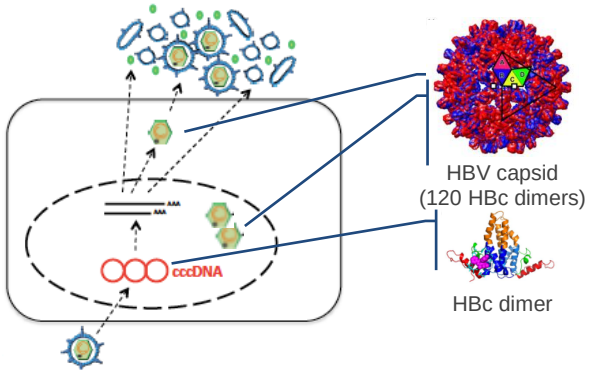


Core inhibitors



Core inhibitors / Anti-capsid

A growing family



Phenylpropenamide derivatives (AT61, AT130) [Gilead][Jansen]

Heteroaryldihydropyrimidines (HAP-1 and Bay 41-4109)

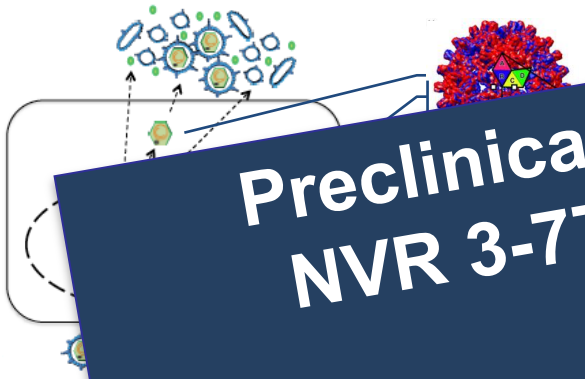
Sulfamoylbenzamide derivatives (DVR-23, DVR-56 and Novira Therapeutics NVR-1221) [Novira]

BCM-599 [2-amino-N-(2,6-dychloropyridin-3-yl) [acetamide family]

Isothiafludine (pg-RNA packaging)

Core inhibitors / Anti-capsid

A growing family



Phenylpropenamide derivatives (AT

Heter

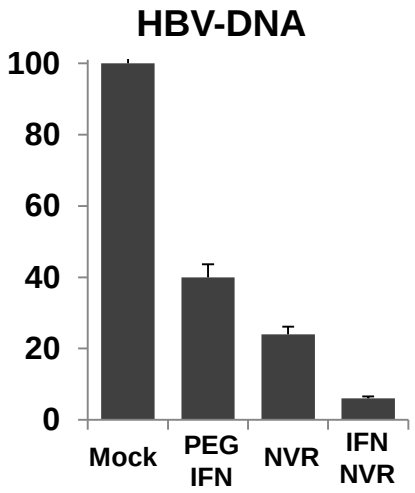
[acetamide family]

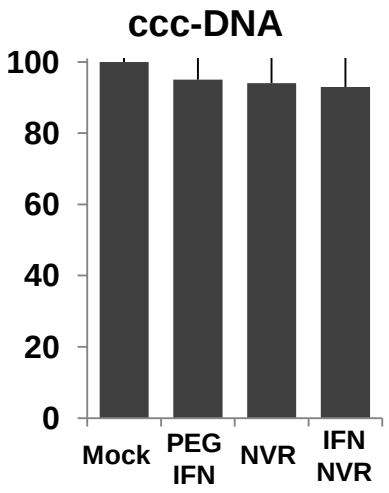
RNA packaging)

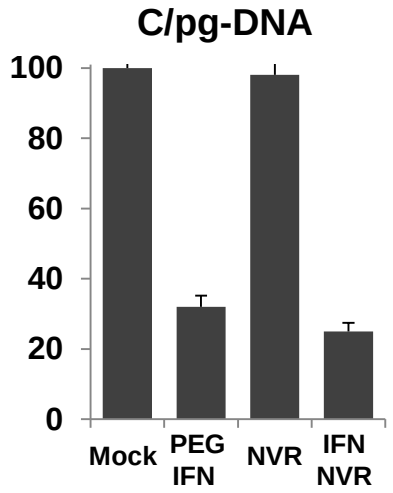
Preclinical and Early Clinical Profile of NVR 3-778, a Potential First-In-Class HBV Core Inhibitor

Gane, AASLD 2014

Preclinical characterization of the antiviral activity of NVR 3-778, a Potential First-In-Class HBV Core Inhibitor, *in vivo*







Klumpp et al. EASL 2015

Phase 1a Safety and Pharmacokinetics of NVR 3-778, a Potential First-In-Class HBV Core Inhibitor

LB-19

E.J. Gane[1], C. Schwabe[2], K. Walker[2], L. Flores[3], G.D. Hartman[3], K. Klumpp[3], S. Liaw[3] and N.A. Brown[3]

[1]New Zealand Liver Transplant Unit, Auckland City Hospital, Auckland, New Zealand; [2]Clinical Trials Unit, Auckland Clinical Studies, Auckland, New Zealand; [3]Novira Therapeutics, Inc., Doylestown, PA , U.S.A.

BACKGROUND

Chronic hepatitis B (CHB) is a persistent, potentially progressive necro-inflammatory liver disease associated with chronic infection with the Hepatitis B Virus (HBV). With endstage complications of cirrhosis-related liver failure and hepatocellular carcinoma, CHB accounts for an estimated 600,000 – 800,000 deaths annually worldwide, about twice as many as chronic HCV infection (Perz 2006, WHO 2014). The high prevalence of chronic HBV infection, low rates of post-treatment durable responses with current regulatory-approved therapies, and the high cost associated with life-long treatment indicate an urgent need for improved HBV antiviral therapies that can produce substantially higher rates of durable therapeutic responses with finite treatment duration.

NVR 3-778, a potent and selective orally bioavailable HBV core inhibitor discovered by Novira Therapeutics, inhibits HBV nucleocapsid assembly and potentially other core-mediated functions in the HBV lifecycle. NVR 3-778 may therefore offer multiple efficacy mechanisms for abrogating persistent HBV infection, including augmentation of host adaptive immune responses against HBV, inhibition of replication and cccDNA replenishment, prevention of reinfection, and eventual HBsAg loss and long-term cure. Completed nonclinical pharmacologic and toxicologic studies support clinical evaluation NVR 3-778 to improve durable treatment efficacy for HBV infected patients. We report the first trial of NVR 3-778 in human subjects, a Phase 1a dose-ranging trial of NVR 3-778 in healthy adult volunteers to evaluate safety and pharmacokinetics of orally administered NVR 3-778.

PRECLINICAL PROFILE OF NVR 3-778

Potent & selective antiviral activity in HBV-producing HepG2.2.15 cells

- 50% inhibitory concentration (EC_{50}) = 0.24 μM
- 90% inhibitory concentration (EC_{90}) = 0.62 μM
- Active *in vitro* across all tested HBV genotypes (A, B, C, D)

Pre-clinical pharmacology & toxicology studies supportive of clinical development for the treatment of HBV infection

- No adverse effects in safety pharmacology studies in animals (respiratory, cardiac, CNS)
- Negative in genotoxicity assessments
- The safety profile from 7- and 28-day toxicology studies was satisfactory and supports clinical studies at dose levels that may deliver antiviral efficacy in HBV patients

PHASE 1A STUDY OBJECTIVES

Primary Objectives

1. Assess dose-related safety and tolerability of NVR 3-778 after oral doses of 50 mg and up to 1200 mg/day, in healthy adult volunteers
2. Assess pharmacokinetics (PK) of NVR 3-778, with single & multiple oral doses of 50 mg and up to 1200 mg/day, in healthy volunteers

Secondary Objectives

1. Assess potential differences in systemic PK profile of NVR 3-778 in healthy volunteers when administered with and without food, i.e., with fed and fasted dosing

KEY INCLUSION CRITERIA

1. Male or female, between 18 and 65 yrs of age. Females must not be of childbearing potential
2. Body Mass Index (BMI) 18-32 kg/m^2
3. In good health, with:
   - No history of chronic or recurrent medical conditions requiring frequent medical intervention or pharmacologic management
   - No symptoms of ongoing illness, at the time of screening
   - No clinically significant abnormalities in vital signs, and no significant abnormalities on physical examination at screen
4. No significant abnormalities in 12-lead electrocardiograms (ECGs) at screen
5. Ability and willingness to give informed consent

STUDY DESIGN

4 single-dose cohorts (A, B, C, D)

- Sequential escalating doses

8 subjects/cohort

- Randomized 6:2 (active study drug:placebo)
- Single doses of 50, 150, 400, then 800 mg
- Single doses administered after overnight fast
  - 7 days' observation, safety and PK
- Preliminary food-effect assessment
  - Cohort A subjects first receive 50 mg fasted dose
  - After washout, cohort A subjects receive second 50 mg dose, with standardized high-fat breakfast

Fifth cohort (E): 14-day multiple-dose cohort, fasted dosing

- 200 mg QD dosing selected based on PK data for cohorts A-D

Interim safety reviews to advance to successive cohorts

POPULATION & DEMOGRAPHICS

DEMOGRAPHICS

- 40 eligible subjects enrolled (8/cohort x 5 cohorts)
- 38 males, 2 females
- 35 Caucasians, 5 other (2 Asian Indian, 2 Hawaiian/Pacific Islander, 1 New Caledonian)

DISPOSITION

- All subjects completed study
- No premature discontinuations
- No treatment modifications

RESULTS: DOSE-RELATED SAFETY AND PHARMACOKINETICS

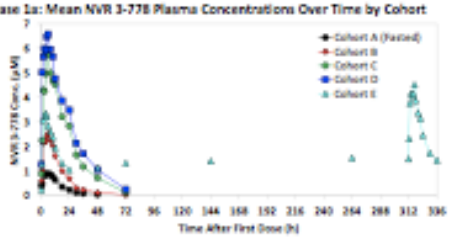
All Clinical Adverse Events (AEs), Regardless of Relatedness to Study Drug

AE Term	Overall #patients with AE	Cohort A N= 12 2 Doses	Cohort B N=6 1 Dose	Cohort C N=6 1 Dose	Cohort D N=6 1 Dose	Cohort E N=6 14 Doses	Placebo N= 10
Headache	5	1	1	1	2	0	0
Pre-syncope	3	1	1	0	0	0	1
Nasal Congestion	2	0	0	1	1	0	0
Tooth Fracture	2	1	0	0	0	0	0
Constipation	2	0	1	0	0	0	1
Phlebitis (intravenous)	2	1	0	0	0	0	2

AEs with single occurrences: Dizziness, hypoesthesia, paresthesia, cough, oropharyngeal pain, thermal burn (heating pad), ligament sprain, back pain, myalgia, neck pain, pain in jaw, diarrhea, pain (unspecified), fatigue, insomnia, upper respiratory tract infection, decreased appetite, tooth extraction

- No pattern of treatment-related (active vs. placebo Rx) or dose-related AEs
- All AEs are of common types, seen in any population of trial subjects
- Most AEs were not attributed to study treatment (next table, right)
- AEs mild (grade 1), except 2 grade 2 AEs not attributed to treatment (sprain, tooth pain)

Phase 1a: Mean NVR 3-778 Plasma Concentrations Over Time by Cohort



- Cohort E (200 mg QD): peak levels (C_{max}) = 3.5 μM, 24-hr trough levels > 1 μM
- Exposures exceed HBV inhibitory conc. in HepG2 cells: EC_{50} = 0.24 μM; EC_{90} = 0.62 μM
- Doses > 200 mg may afford continuous HBV inhibition with QD dosing schedule
- Possible steady-state PK within 5-5 days

Cohorts A-E: Calculated PK Parameters

Cohort Dose	C_{max} (μM)	T_{max}** (h)	$t_{1/2}$ (h)	AUC_{inf} (μM*h)	AUC_{tau} (μM*h)	CL/F (L/h)	Vz/F (L)
A 50mg (fasted)	1.18 (12.7)	3.7 (72.5)	7.8 (53.8)	13.8 (18.8)	16.6 (23.2)	7.6 (30.2)	80.9 (15.4)
A 50mg (fed)	1.42 (32.9)	7.8 (71.5)	7.8 (21.2)	15.1 (15.4)	17.9 (21.8)	6.8 (28.5)	73.3 (9.2)
B 150mg	3.09 (10.2)	4.0 (1.0-8.0)	10.1 (39.5)	36.7 (23.8)	47.7 (31.0)	7.9 (32.6)	107.2 (31.0)
C 400mg	6.60 (51.3)	6.0 (1.0-8.0)	10.9 (40.6)	95.2 (39.5)	178 (36.1)	5.9 (36.7)	158.6 (31.5)
D 800mg	7.46 (30.5)	3.8 (2.0-8.0)	15.2 (8.0)	114 (21.0)	187 (11.7)	10.0 (12.5)	217.0 (7.1)
E 200mg (Day 1)	3.50 (25.4)	3.3 (2.0-5.0)	10.3 (36.5)	47.0 (28.2)	64.4 (37.6)	8.5 (54.0)	109.2 (14.6)

*All table entries are mean with (%CV) except T_{max} as noted.
**T_{max} reported as mean and (range)

- C_{max} > 1 μM with 50 mg lowest dose; T_{max} = 4-7 hr;
- $T_{1/2}$ = 8-10 hr with lower doses, 15-19 hr with higher doses
- Low covariance for key PK parameters predicts consistent effects in patients

Clinical AEs Considered Possibly Related to Study Drug

AE Term	Overall #patients with AE	Cohort A N= 12 2 Doses	Cohort B N=6 1 Dose	Cohort C N=6 1 Dose	Cohort D N=6 1 Dose	Cohort E N=6 14 Doses	Placebo N= 10
Headache	3	0	0	1	1	0	0
Fatigue	1	0	0	0	0	1	0
Decreased Appetite	1	0	0	0	0	1	0

- Very few treatment-attributed AEs
- 3 headaches (mild), seen only at higher doses – but not seen with prolonged dosing
  - Headaches and GI complaints are most common AEs in trials of normal subjects, with placebo or active treatment

All Graded Laboratory Abnormalities

Cohort	Randomization #	Lab Analyte	Visit [1]	Lab Value	ULN/LLN
B	R1010	Creatinine	Day 4	106	ULN = 105
B	R1015[2]	ALT	Day 8	74	ULN = 45
D	R1031	Total Bilirubin	Day 8	36	ULN = 24
E	R1036	Creatinine	Day 4	108	ULN = 105
E	R1036[3]	Neutrophils	Day 14	1.3	LLN = 1.9
E	R1036[3]	Neutrophils	Day 15	1.44	LLN = 1.9

[1]Placebo recipient; [2]subject had low neutrophil count at Baseline

- Very few lab abnormalities among the 40 study subjects, scattered types
- No recurrent pattern by organ system
- All lab abnormalities were transient, mild (grade 1)

CONCLUSIONS

- NVR 3-778 has sub-micromolar activity against HBV in HepG2.2.15 cells
  - Broad HBV genotype activity (A,B,C,D tested)
- Accelerates capsid mis-assembly *in vitro*
- Potential to inhibit HBV assembly and other core-mediated roles *in vivo*
- Satisfactory preclinical pharmacology/toxicology
  - Consistent oral bioavailability in animals
  - Good safety at exposure levels likely to afford anti-HBV efficacy
- Phase 1a Dose-Ranging Results in Adult Healthy Volunteers:
  - Satisfactory safety for single and 14-day QD dosing
    - No pattern of treatment-related clinical AEs or lab abnormalities
  - Encouraging PK results in human volunteers support likelihood of satisfactory PK in HBV patients, with QD dosing
  - Oral doses ≥ 200 mg QD in volunteers result in peak plasma levels of 3-7 μM and 24 hr trough levels > 1.5 μM, multifold above HBV inhibitory concentrations *in vitro*
- Phase 1a results indicate feasibility of achieving potentially effective concentrations of NVR 3-778 in HBV patients, at well-tolerated dose levels
- Phase 1a data support ongoing Phase 1b dose-ranging study in HBV patients, in which NVR 3-778 will be evaluated alone and in combination with other HBV agents (interferons and HBV nucleosides/nucleotides)

REFERENCES

Perz JF et al. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. J. Hepatology 2006; 45:529–538

WHO Hepatitis B Fact Sheet; WHO.org July 2014

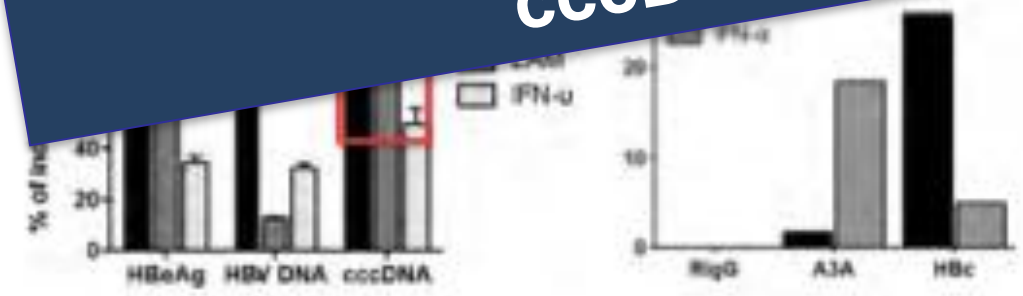
DISCLOSURES

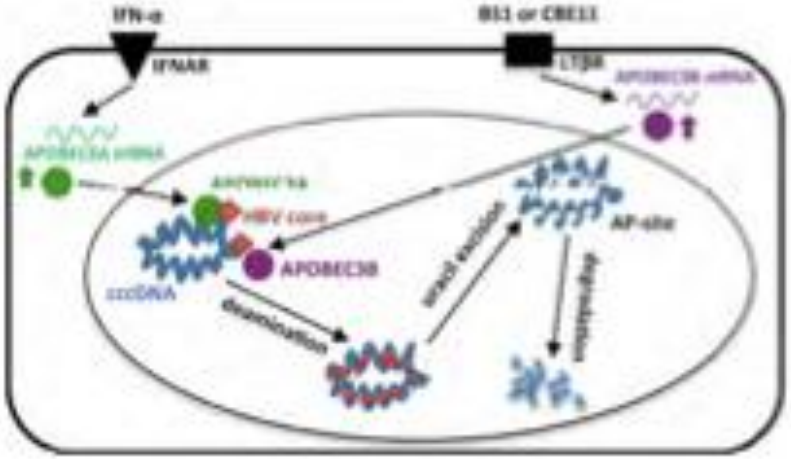
Messrs Flores, Hartman, Klumpp, Brown & Ms Liaw are employees of Novira Therapeutics, Inc.

Specific and Nonhepatotoxic
Degradation of N

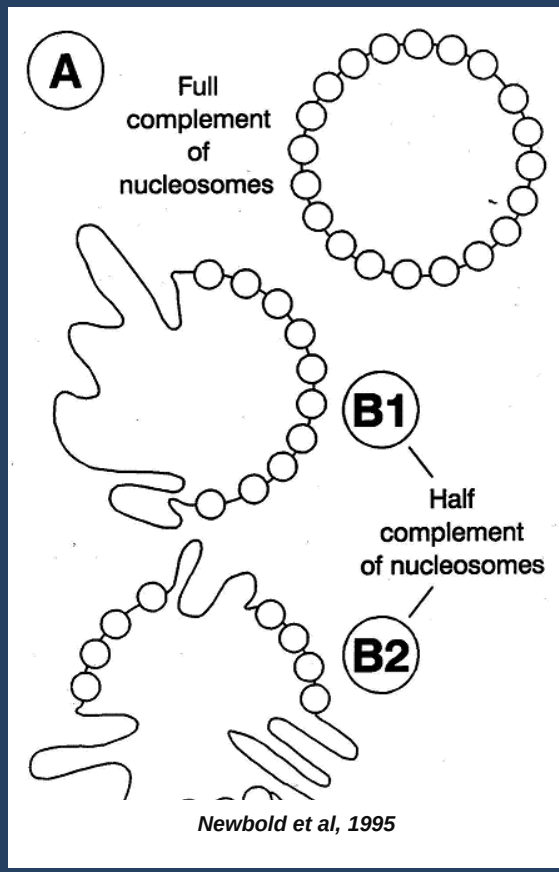
IFNα, LTβ and potentially Hap12 all exert a strong but partial effect on the cccDNA pool

Are they targeting only a subset of cccDNA molecules?



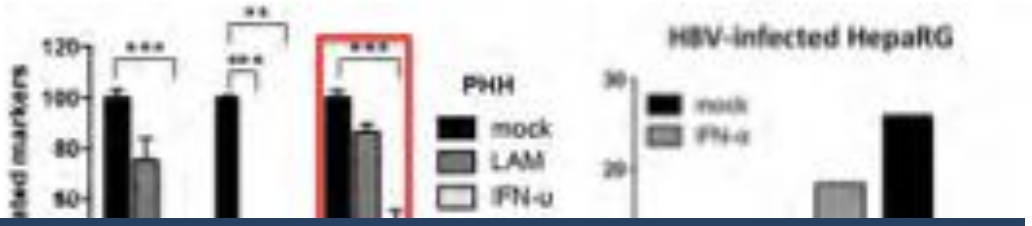


- Interferon
activation
cytidine-de
infected
human live
- HBV-core
with nucle
deaminat
formation



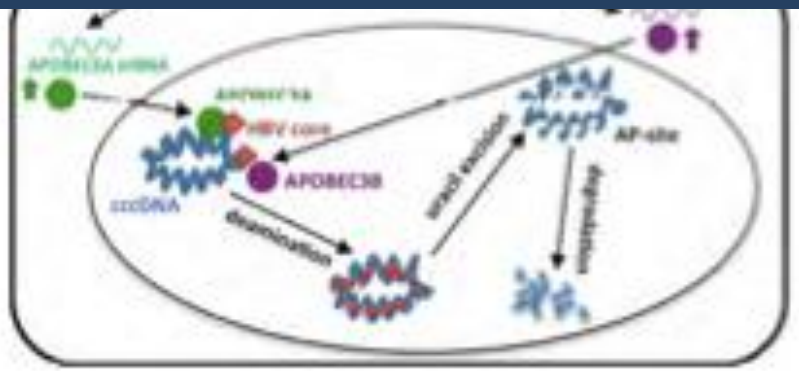
Newbold et al, 1995

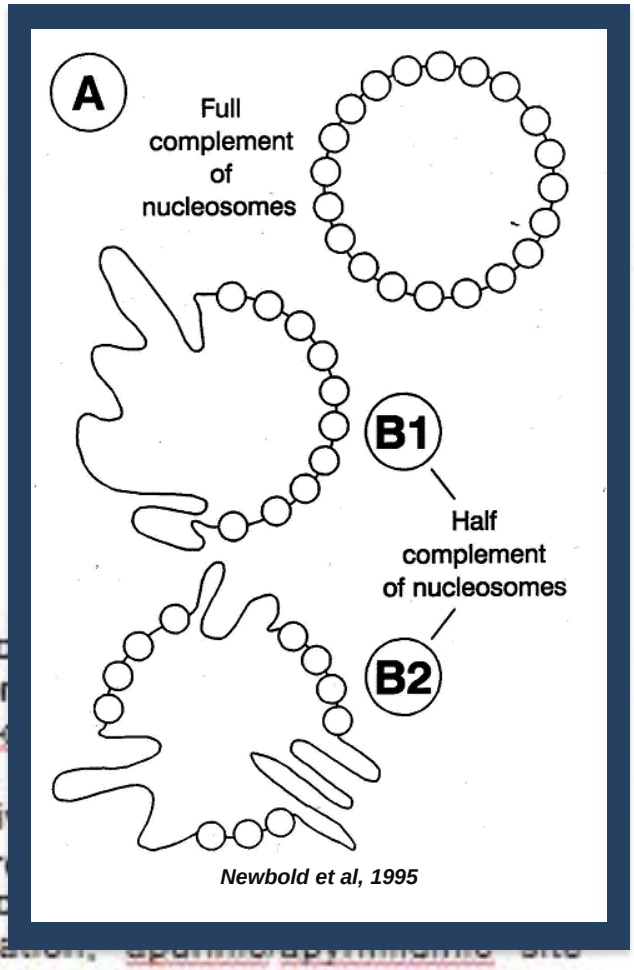
Specific and Nonhepatotoxic Degradation of Nuclear Hepatitis B Virus cccDNA



Should we "open / activate the cccDNA to better target the cccDNA pool

Similar to strategiues currently explored in HIV

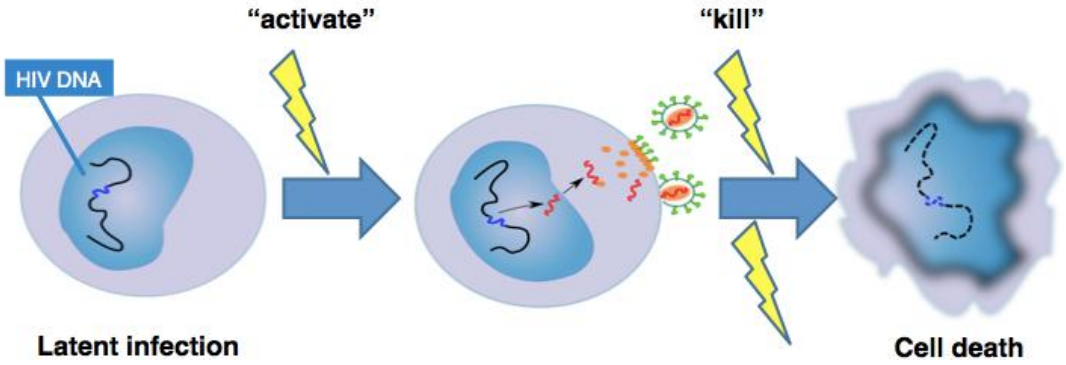




- HBV-cor
with nuc
deamina
formation and finally cccDNA degradation

Lucifora et al. Science 343, 1221-8, 2014

HBV "*Kick and Kill*" strategy

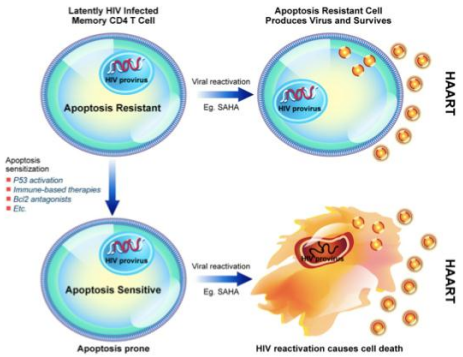


Increase immune clearance

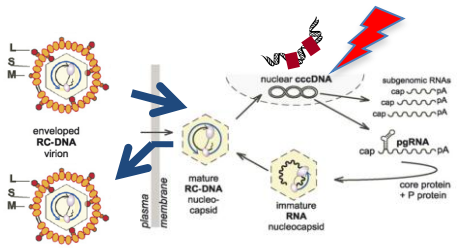
therapeutic vaccine

anti-PD1 or anti-PDL1

Increase apoptosis susceptibility



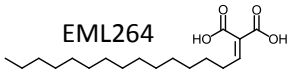
Target cccDNA



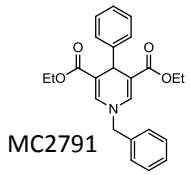
Modified from S. Lewin, IAS 2013

Targeting cccDNA-bound HATs and HDACs by “epigenetic” compounds

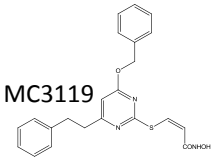
PCAF/p300 inhibitor

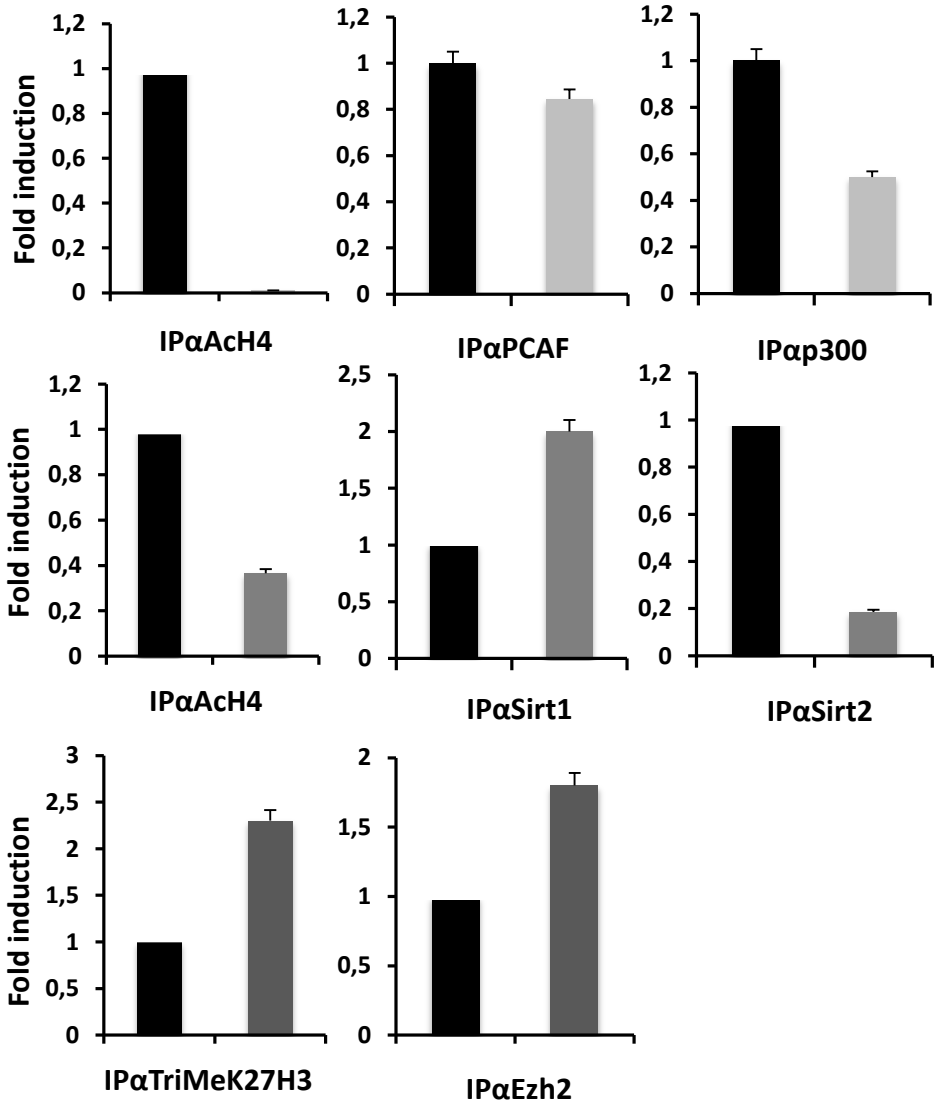


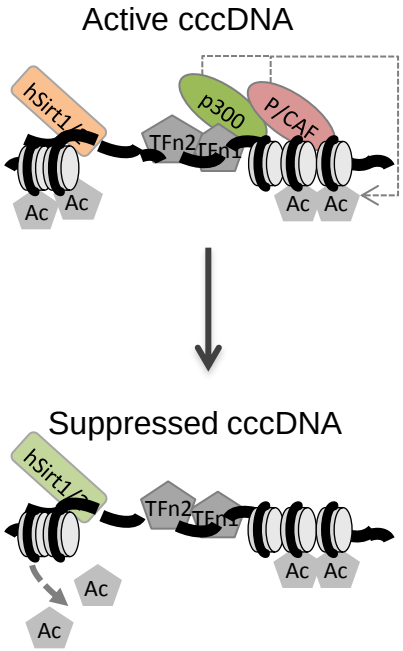
Sirt1/2 stimulator



JMD3 inhibitor





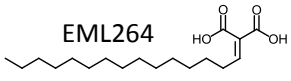


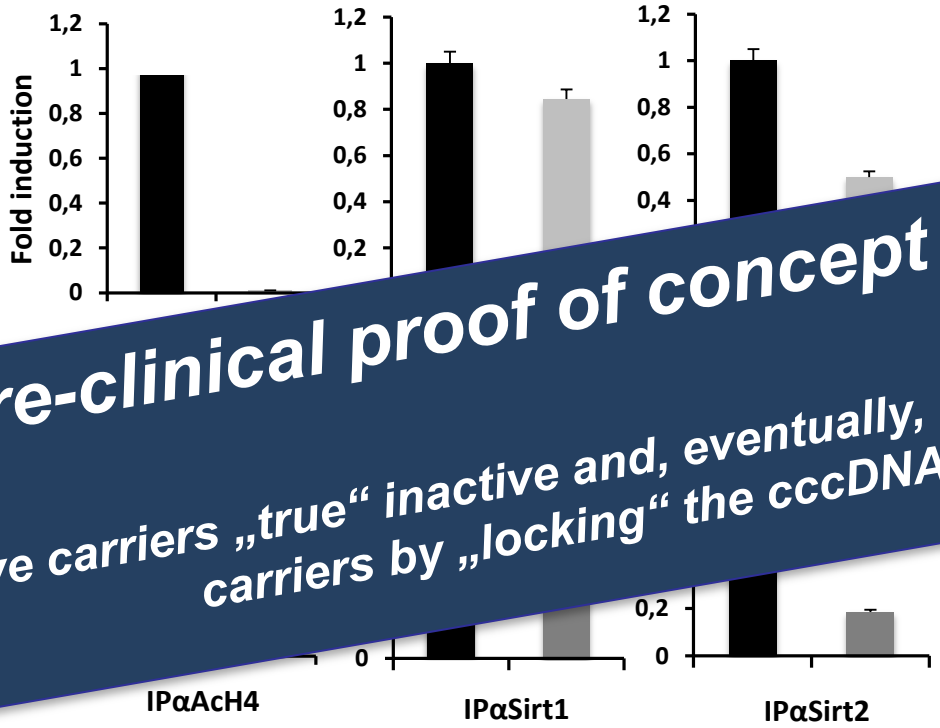
EMs modify chromatin remodelling enzymes recruitment onto HBV minichromosome

Targeting cccDNA-bound HATs and HDACs by “epigenetic” compounds

PCAF/p300 inhibitor

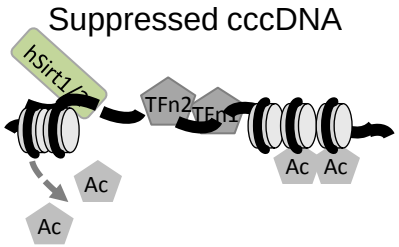
EML264





Pre-clinical proof of concept stage

Make active carriers „true“ inactive and, eventually, over time „occult“ carriers by „locking“ the cccDNA



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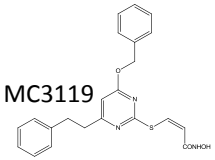
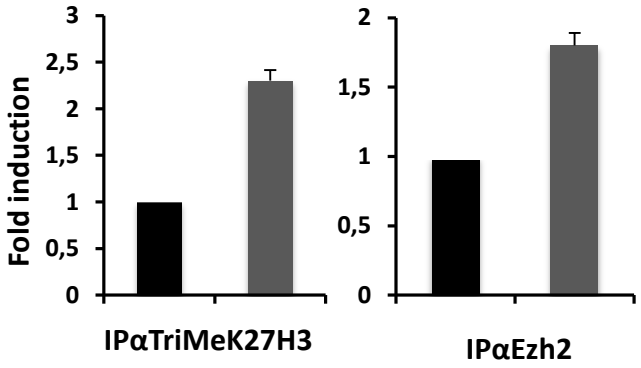
MC3119





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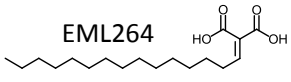
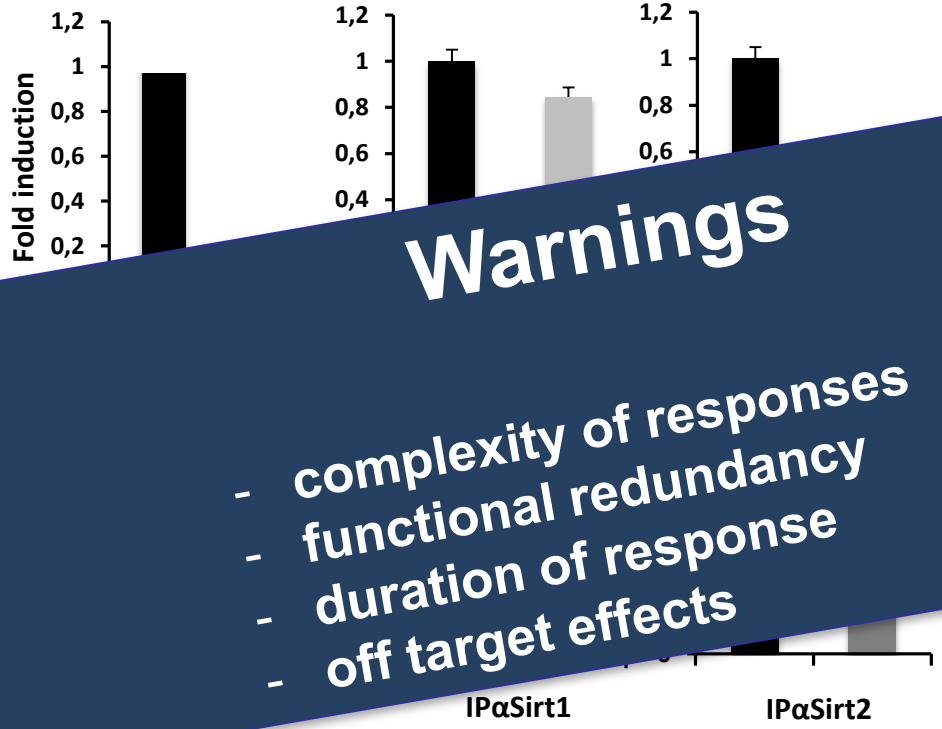


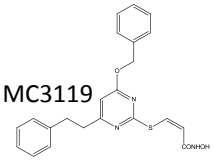


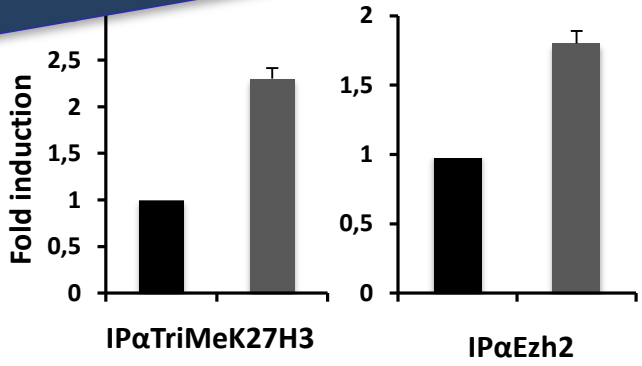
Warnings

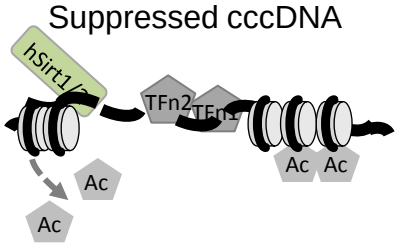
- complexity of responses
- functional redundancy
- duration of response
- off target effects

JMD3 inhibitor

MC3119







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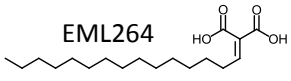

Sirt1/2 sti

JMD3 inhibitor

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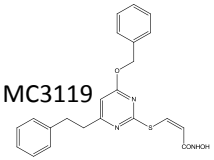
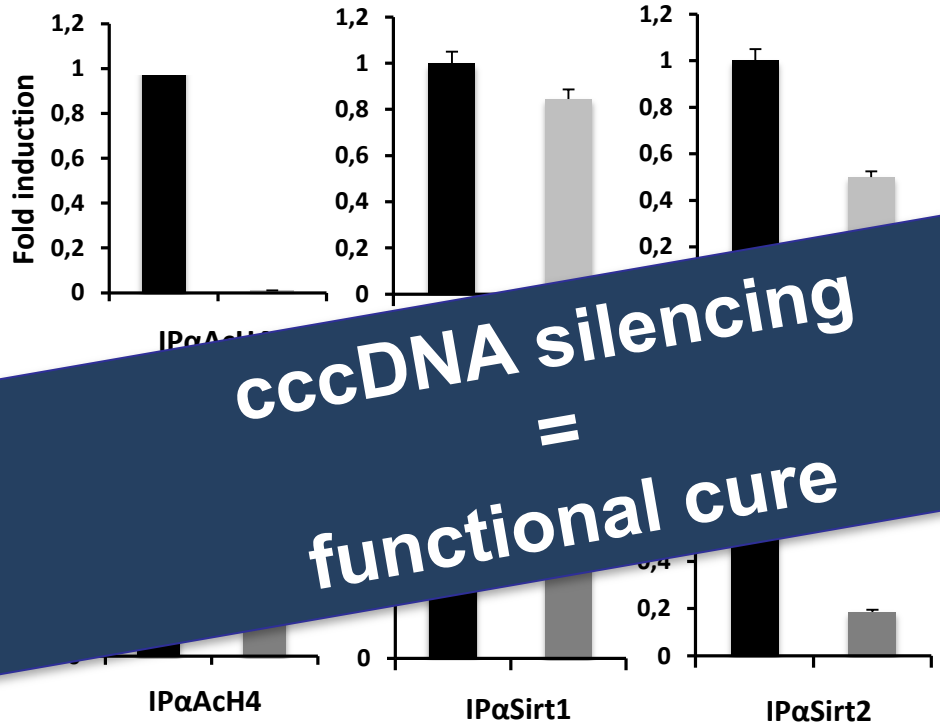
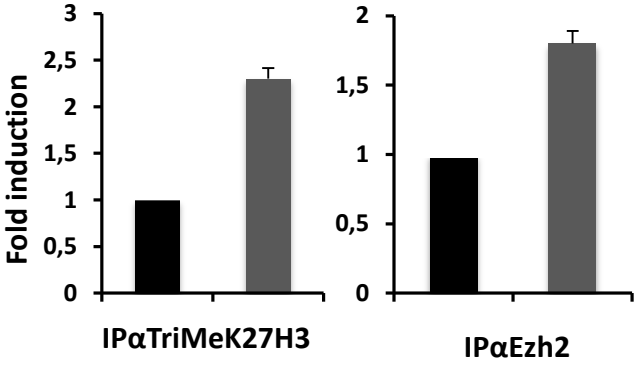
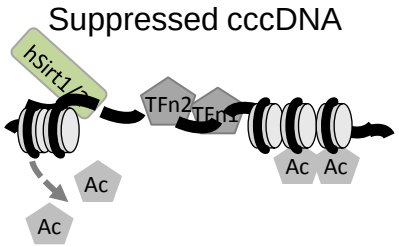




cccDNA silencing = functional cure





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HBV cure landscape

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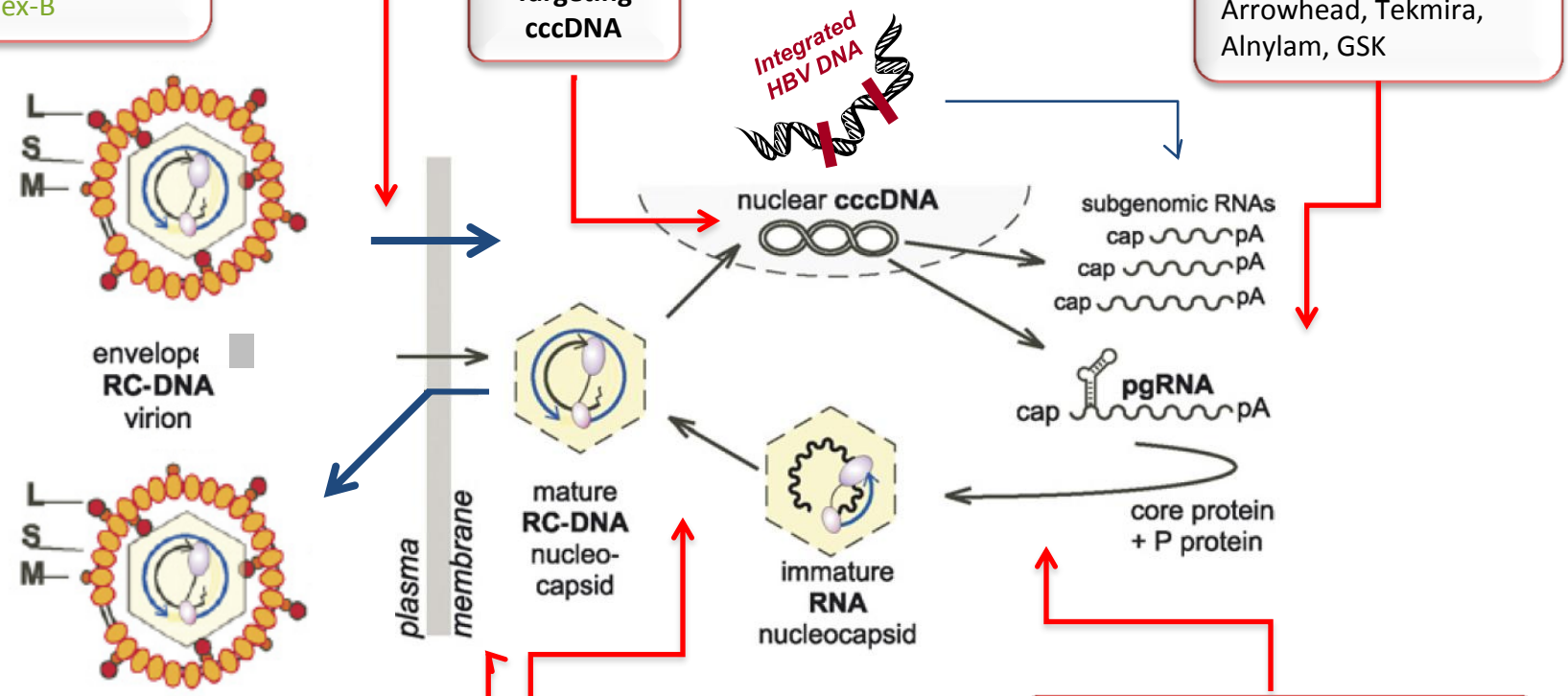
Targeting cccDNA

RNA interference, Arrowhead, Tekmira, Alnylam, GSK



Inhibitors of HBsAg release, Replicor

Polymerase inhibitors
- Nucleoside analogues, e.g. Gilead, BMS
- Non-nucleoside, e.g. LB80380

Inhibition of nucleocapsid assembly Novira, AssemblyPHARMA, Gilead, Janssen

Immune modulation
- ***Toll-like receptors agonists***, Gilead, Roche
- ***Anti-PD-1 mAb***, BMS, Merck
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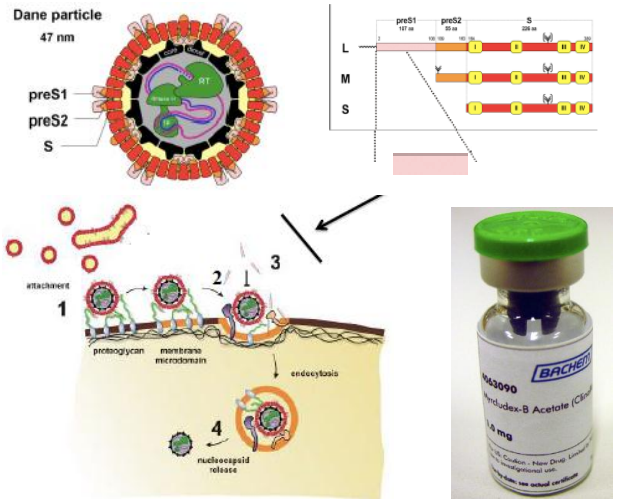
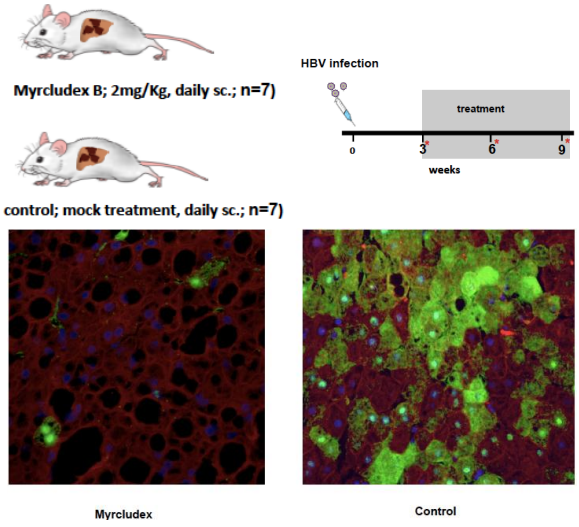
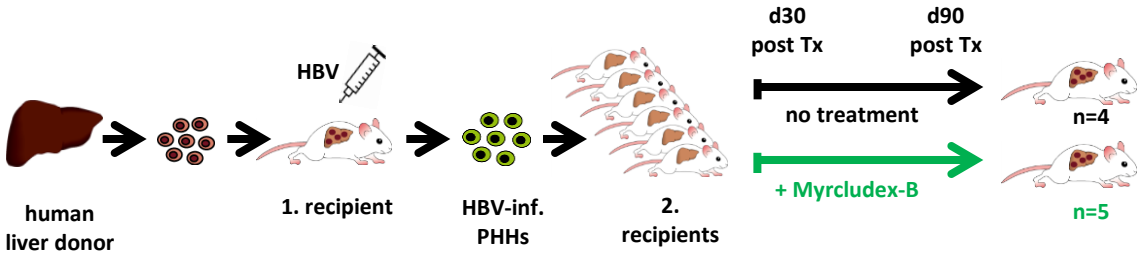
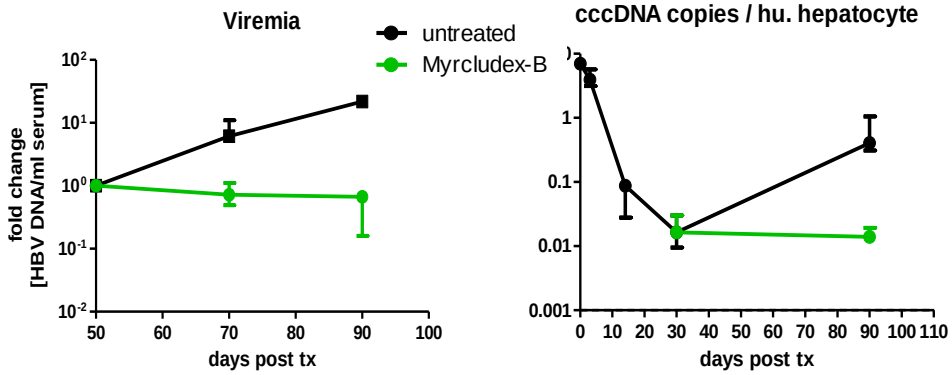
Myrcludex B: Targeting Entry of HBV into Hepatocytes





Entry inhitor plus cell proliferation support loss of cccDNA and HBsAg

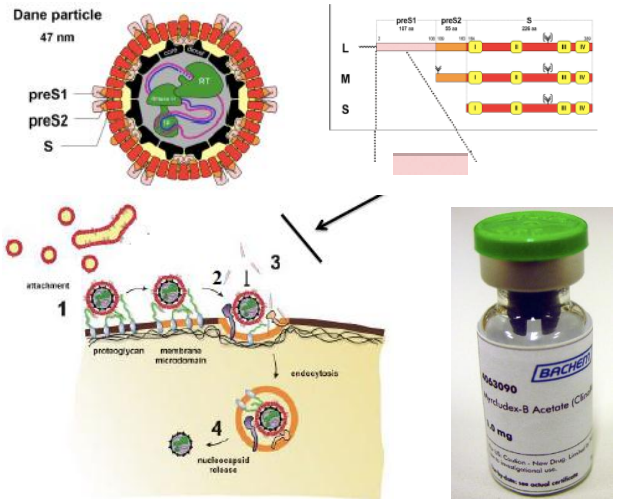


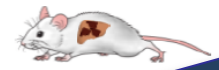


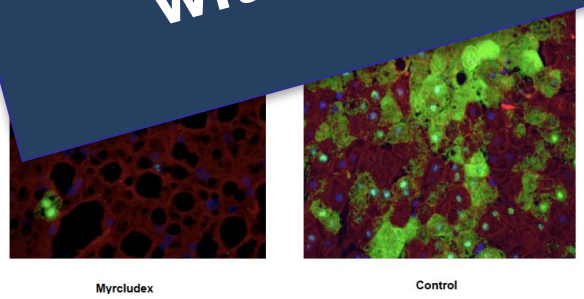
Cell proliferation combined with antiviral treatment to block re-infection (Myrcludex B) promoted cccDNA clearance in the majority of the human hepatocytes.

Allweiss, Petersen, Dandri et al, EASL 2014, O101

Myrcludex B: Targeting Entry of HBV into Hepatocytes







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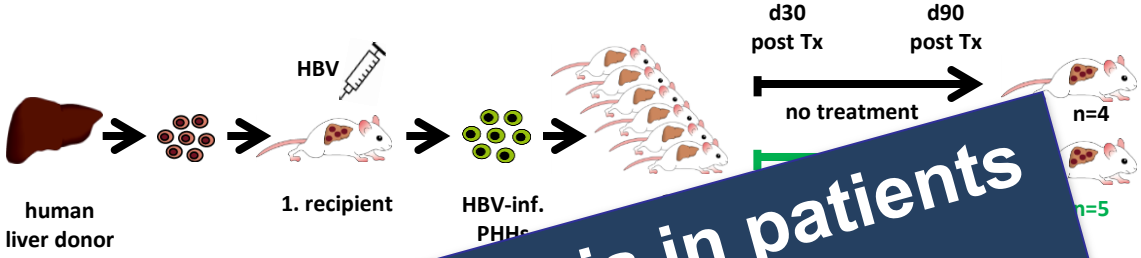

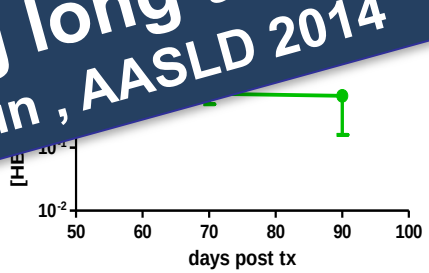

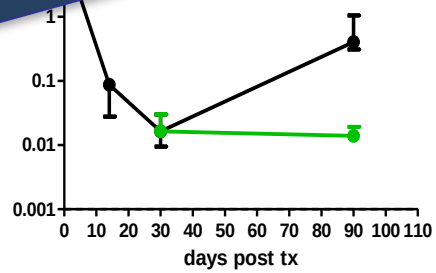

Evidence for ongoing low level viremia in patients with CHB receiving long term NUC therapy
Marcellin , AASLD 2014

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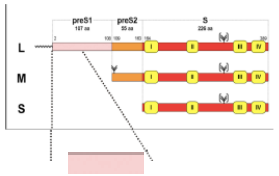
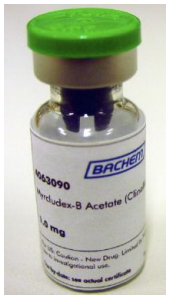
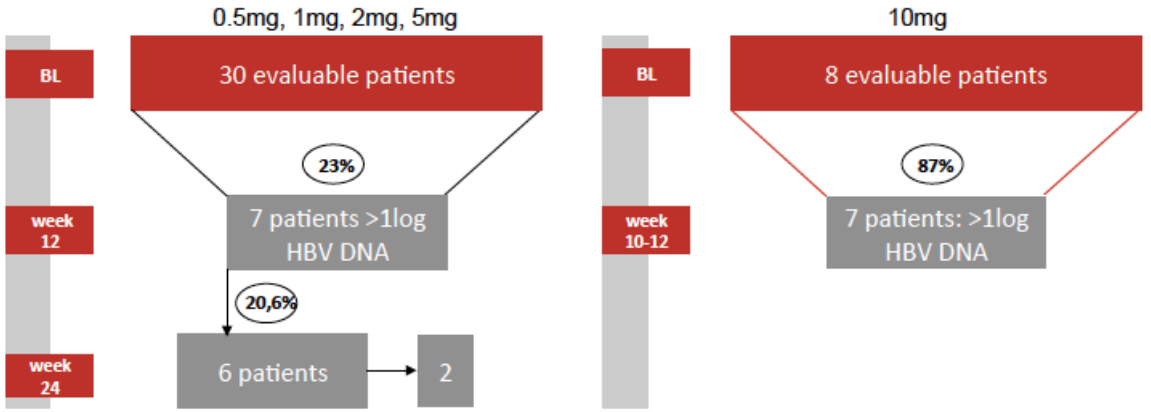




HBV Phase 2a Results



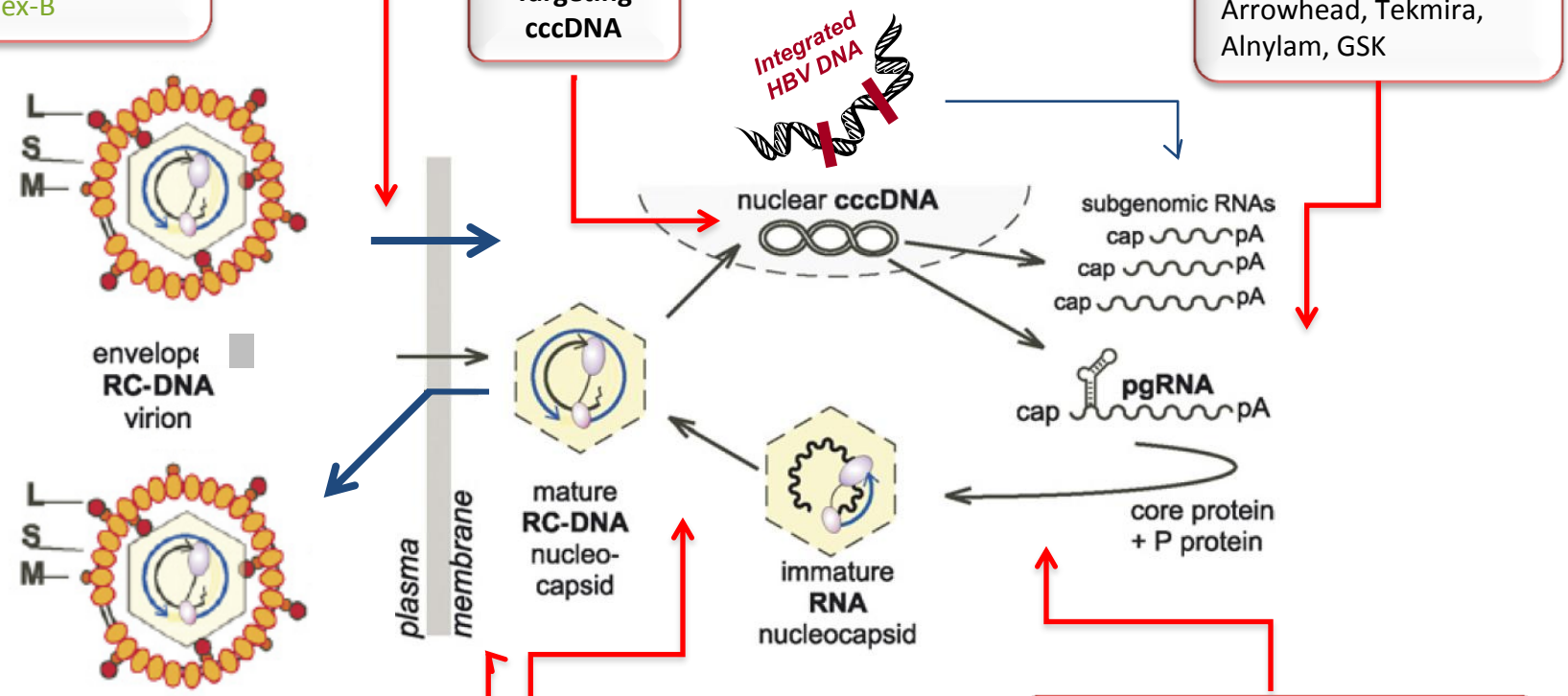
HDV Pilot Study

HBV cure landscape

Entry inhibitors
- Lipopeptides, e.g. Myrcludex-B

Targeting cccDNA

RNA interference, Arrowhead, Tekmira, Alnylam, GSK



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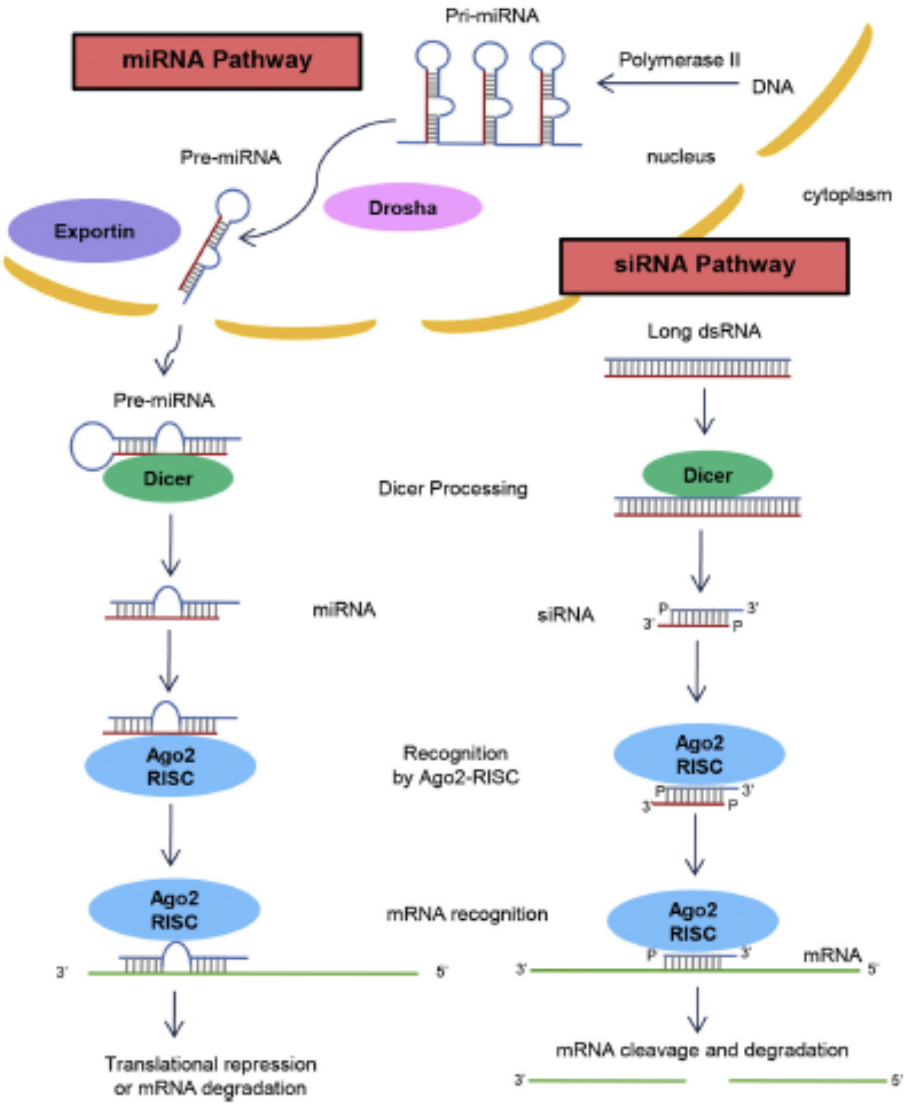
Immune modulation
- ***Toll-like receptors agonists***, Gilead, Roche
- ***Anti-PD-1 mAb***, BMS, Merck
- ***Vaccine therapy***: Transgene, Gilead, Roche Innovio, Medimmune, ITS

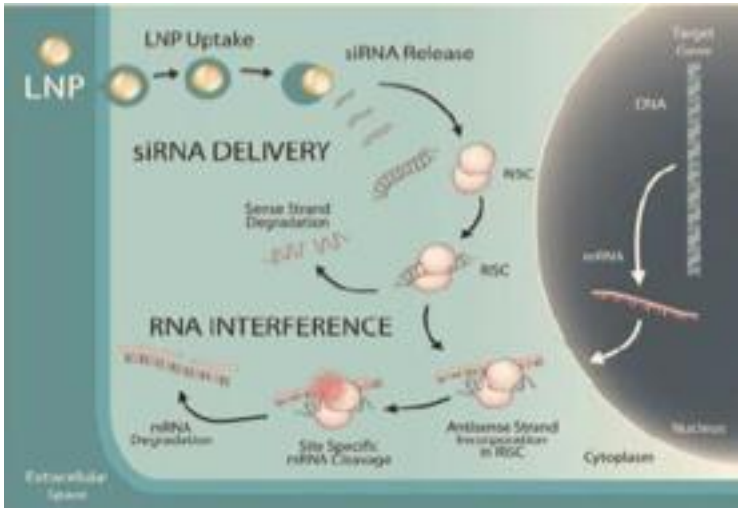
RNA-interference (RNAi) mechanism:

knockdown of disease causing genes



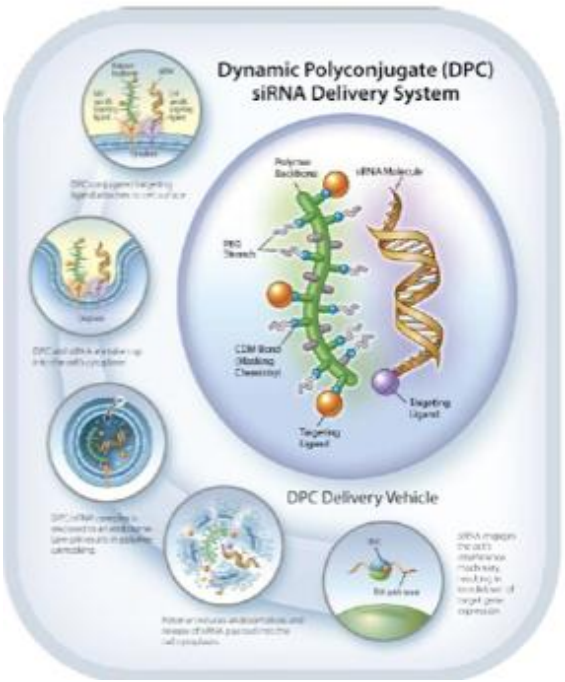
RNA-interference (RNAi) mechanism:
role of delivery

Tekmira OncoCore Biopharma, Inc.



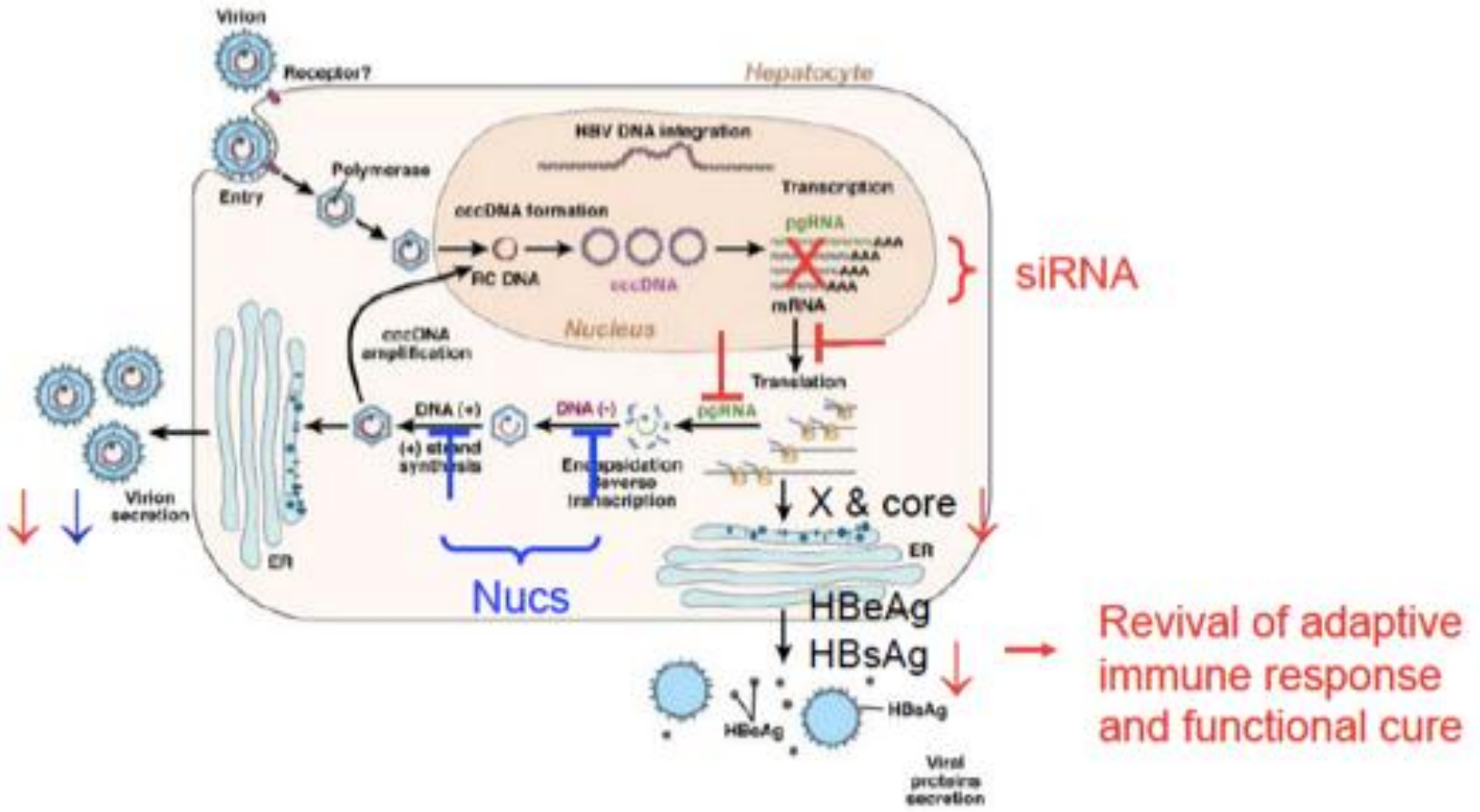
1. Shield siRNA from serum nuclease to avoid degradation
2. Induce cellular uptake by target organ/cells
3. Promote endosomal escape
4. Potent delivery of payload to RISC machinery

Arrowhead Research Corporation



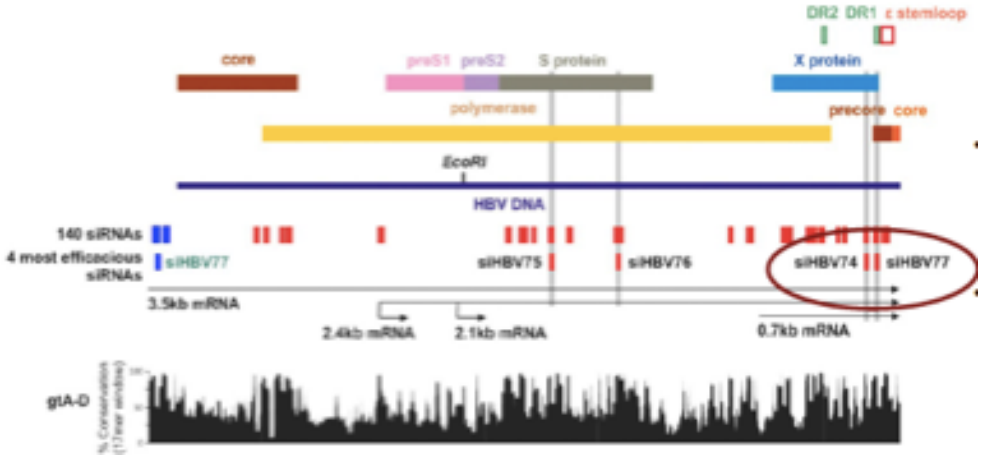
- **DPC polymer composition and physical characteristics**
  - Amphipathic peptide
  - peptide amines reversibly "masked" with CDM
  - Slightly negatively charged
- **Cellular uptake of peptide is ligand-driven (N-acetyl galactosamine (NAG)) for hepatocytes)**
- **siRNA is made liver tropic by attachment of lipophilic ligand (e.g. cholesterol)**
- **↓ pH in endosomes drives peptide unmasking**
- **Unmasked peptide disrupts endosomal membrane**

RNAi treatment for chronic hepatitis B

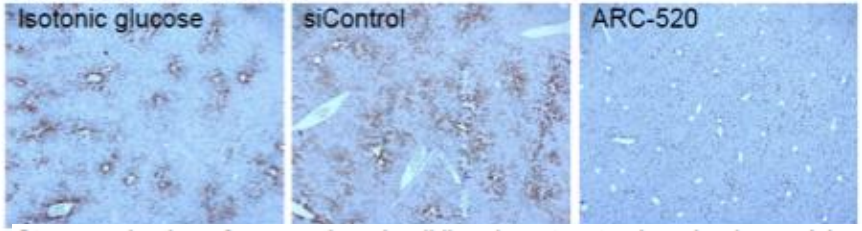


RNAi treatment for chronic hepatitis B:

siRNA design and preclinical program

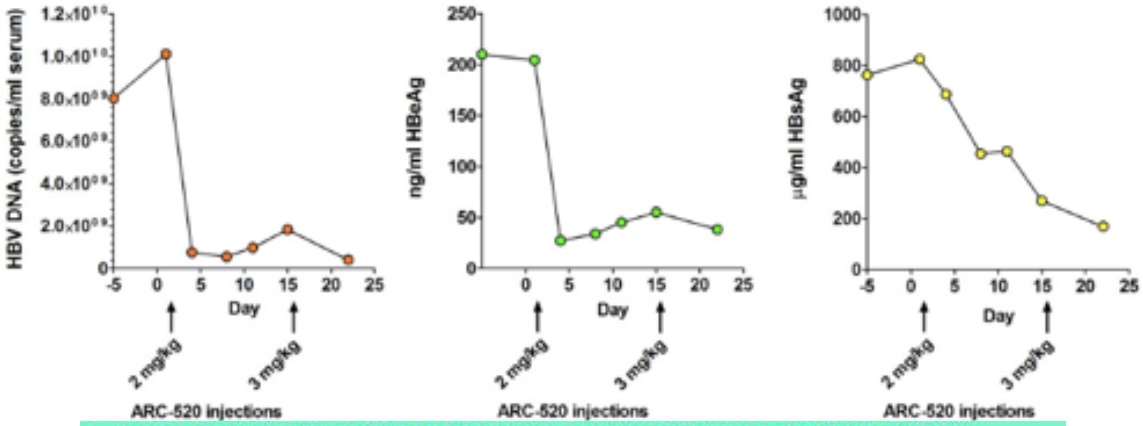


Anti-HBcAg immunostain



Strong reduction of core antigen in all liver hepatocytes in animals receiving ARC-520

Wooddell et al, Mol Ther 2013 May; 21(5) 973-85

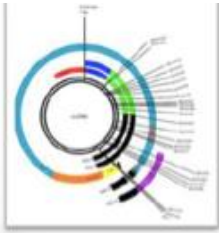


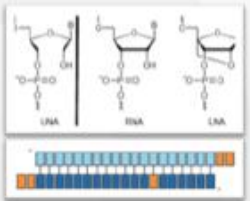
- Log_{10} reduction in HBV DNA (95%), HBeAg (90%) and HBsAg (90%)
- First demonstration of RNAi efficacy in the chimp HBV model
- KD comparable to that achieved in mouse HBV models at similar dose level
- Further reduction after a subsequent dose

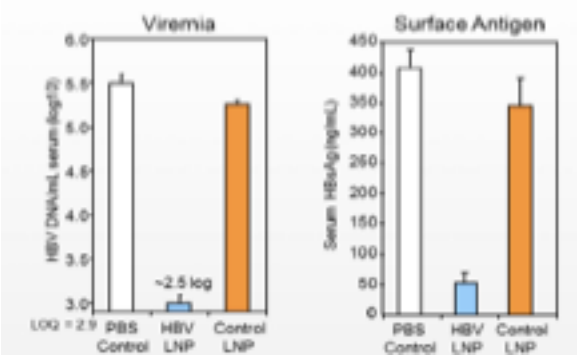
RNAi treatment for chronic hepatitis B:
siRNA design and preclinical program

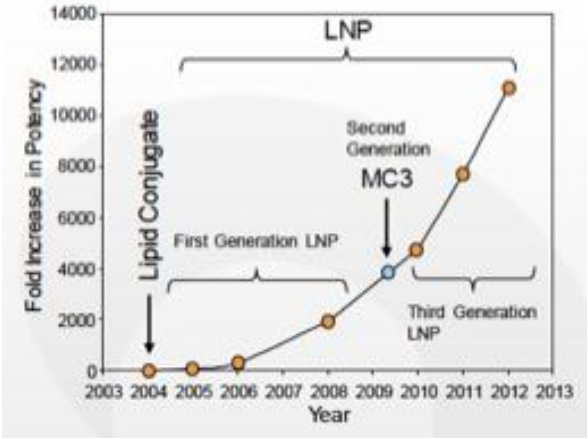
Tekmira ONCORE BIOPHARMA, INC.

- 1000's of HBV genomic sequences analyzed and 150 conserved sites screened
  - Multiple genotypes included
  - All mRNA encoding surface antigen (HBsAg)
  - > 90% conservation across all genotypes
- Lead triggers assessed with chemical modification
  - Abrogating immune-stimulation with proprietary know-how
  - **Unlocked Nucleobase Analogs ("UNA")** reducing miRNA-like off-target effects
- Payload Cocktail – multiple triggers
  - Increased efficacy across genotypes
  - Decreased risk of viral resistance





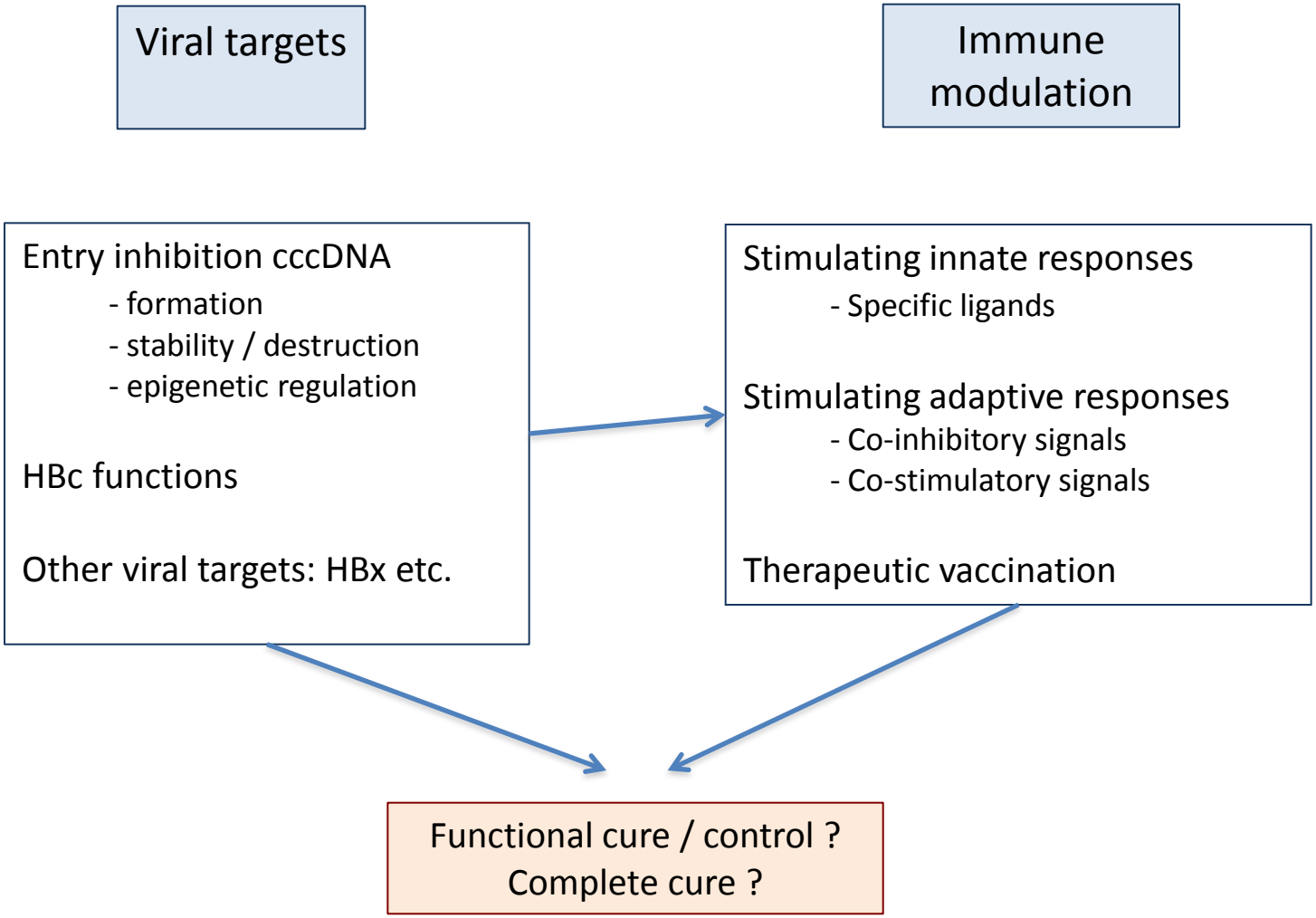




Expanded TI / reduced toxicity / eliminates need for pre-medication

What Might HBV Cure Will Look Like?

let's keep an open mind



Modified from F Zoulim 6.2014

What Might HBV Cure Will Look Like?

let's keep an open mind

Potent NA → to prevent viral spread and cccDNA re-amplification
[new anti-Pol; anti-RNAseH][anti-capsid]

⊕

cccDNA inhibitor → inhibit cccDNA formation [CCC-0975, entry inhibitors …]
deplete cccDNA [LTb, anti-capsid …]
silence cccDNA pool [epigenetic drugs, IFNa …][anti-HBx]

⊕

Entry inhibitors → Entry inhibitors [Mircludex and others]

⊕

Immune Activator → activate or restore antiviral immunity
[target HBs: siRNA, HBs secretion blockers]
[innate immunity stimulators, anti caspsid]
[anti-PD1/anti-PDL1

Modified from S. Locarnini 6.2014

Thanks to

Laboratory of Gene Expression

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Gianna Aurora Palumbo
Leonardo Lupacchini
Francesca Guerrieri
Natalia Pediconi
Ludovica Calvo
Debora Salerno
Silvia di Cocco

Collaborations:

Adam Zlotnick
Lichun Li
Department of Molecular & Cellular Biochemistry,
Indiana University, Bloomington, USA

Uri Lopatin
Adam Zlotnick
Assembly Pharmaceuticals

Fabien Zoulim
Barbara Testoni
David Durantel
INSERM U761 -Lyon

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