Hepatitis B: current challenges and perspective for cure

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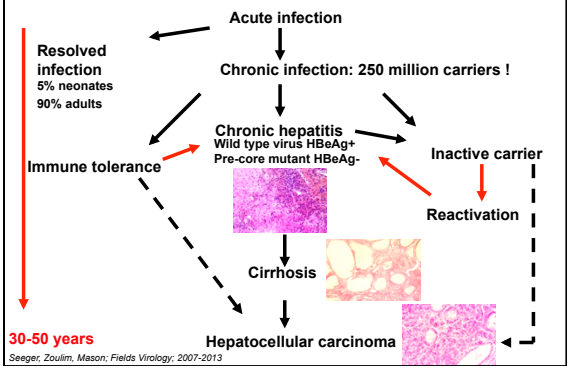
The burden of chronic HBV infections



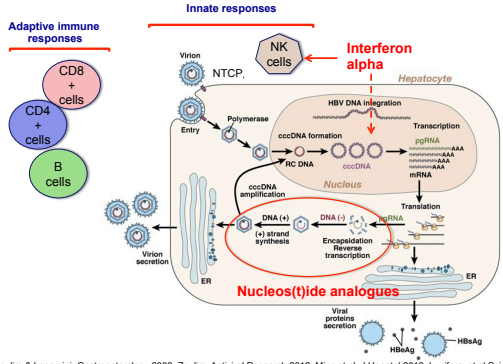
Seeger, Zoulim, Mason; Fields Virology; 2007-2013

Results of current antivirals

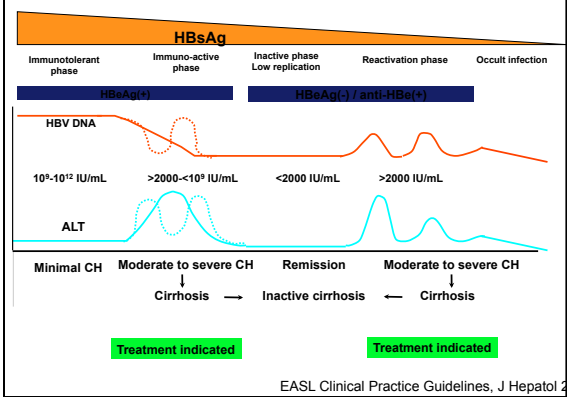
Antivirals approved for the treament of chronic hepatitis B

Drug Type	Approved
Nucleoside analogs	• Lamivudine • Entecavir • Telbivudine
Nucleotide analogs	• Adefovir dipivoxil • Tenofovir disoproxil fumarate
Cytokines	• Interferon alfa • Pegylated Interferon alfa-2a

Mode of action of antivirals for CHB



Zoulim & Locarnini, Gastroenterology 2009; Zoulim Antiviral Research 2012; Mico et al J Hepatol 2013; Lucifora et al Science 2014



EASL Clinical Practice Guidelines, J Hepatol 2

Treatment indications

Patients in the immunoactive phase
AgHBeAg+, VL > 2000 UI/mL, elevated ALT
HBeAg-, VL > 2000 UI/mL, elevated ALT (flucutating)

EASL CPG 2012
AASLD CPG 2015

Inactive carriers
HBeAg-, VL < 2000 UI/mL, normal ALT
If immune suppressive therapy / prevention of viral reactivation

Huang et al JAMA 2014
Perrillo et al JAMA 2015

Immune tolerant patients
HBeAg+, VL > 6log UI/mL, normal ALT
Familial history of cirrhosis or HCC
Why not all ?

Chan et al, Gastroenterology 2014

Pregnant women
If VL > 6 log UI/mL
Last trimester of pregnancy to prevent MTCT
With HBIG and vaccine in the newborn

Chen et al Hepatology 2015
Brown et al Hepatology 2016
Visvanathan et al, Gut 2016

Current treatments: virus suppression and sustained disease control

	Entecavir[1,2]	Tenofovir[3]	PEG-IFN α-2a[4,5]
HBeAg positive	n = 354	n = 176	n = 271
HBV DNA undetectable	67%	76%	25%[a]
HBeAg seroconversion	21%	21%	27%
ALT normalisation	68%	68%	39%
HBsAg loss	2%	3.2%	2.9%[b]
HBeAg negative	n = 325	n = 250	n = 177
HBV DNA undetectable	90%	93%	63%[a]
ALT normalisation	78%	76%	38%
HBsAg loss	0.3%	0%	0.6%[b]

Results at 48 weeks
[a] HBV DNA < 400 copies/mL; [b] At 72 weeks

1. Chang T-T, et al. N Engl J Med 2006;354:1001–10.
2. Lai C-L, et al. N Engl J Med 2006;354:1011–20.
3. Marcellin P, et al. N Engl J Med 2008;359:2442–55.
4. Lau GKK, et al. N Engl J Med 2005;352:2682–95.
5. Marcellin P, et al. N Engl J Med 2004;351:1206–17.

TDF administration: Virologic Suppression at Year 6

Response	HBeAg- Patients (Study 102)		HBeAg+ Patients (Study 103)	
	Year 5	Year 6	Year 5	Year 6
HBV DNA < 400 copies/mL Intent-to-treat*, % (n/N)	**83** (291/350)	**81** (281/345)	**65** (160/248)	**63** (157/251)
HBV DNA < 400 copies/mL On treatment†, % (n/N)	**99** (292/295)	**99.6** (283/284)	**97** (170/175)	**99** (167/169)

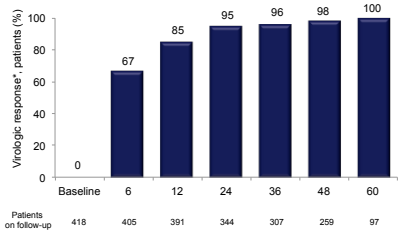
* LTE-TDF (missing = failure/addition of FTC = failure)
† Observed (missing = excluded/addition of FTC = included)

- 80% of 585 patients entering the open-label phase remained on study at Year 6; 73% of enrolled patients remained on study
- HBeAg loss/seroconversion rates of 50% and 37%, respectively, through 6 years
- 11% of HBeAg+ patients had confirmed HBsAg loss (8% with seroconversion)
- No resistance to TDF was detected through 6 years

Neither Truvada (TVD = TDF + FTC) or emtricitabine (FTC) are licensed for use to treat CHB

Marcellin P, et al. AASLD 2012; Boston. #374.

Italian ETV cohort: 100% of naive patients achieved HBV-DNA undetectability at 60 months



Resistance
- One patient (0.2%) developed resistance

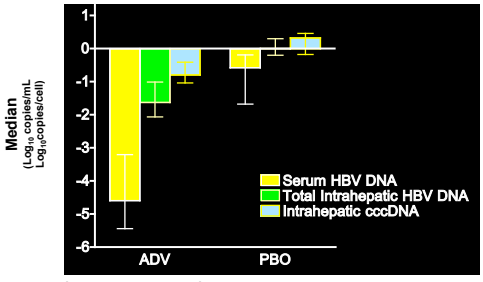
Safety
- Favourable safety profile after 53 months of treatment
- Renal safety profile: two patients reduced ETV dose due to eGFR decline†

*Undetectable HBV-DNA
† A 78-year-old woman with AH and a 48-year-old renal-transplanted woman with compensated cirrhosis

ANRS

Adapted from Lampertico P, et al. AASLD 2012, poster 366. Available at http://liverlearning.aasld.org/aasld/2012/thelivermeeting/22910/pietro.lampertico.entecavir.treatment.for.nuc.na.field.practice.patients.with.html?history_id=78126. [Accessed April 2013].

Reductions in Serum HBV DNA, Total Intrahepatic HBV DNA and cccDNA During Adefovir Therapy

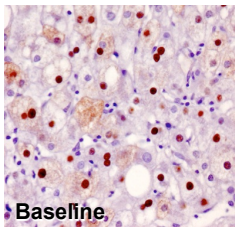


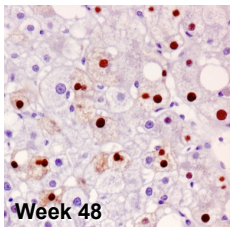
- **48 weeks of ADV resulted in significant reductions in : serum HBV DNA > total intrahepatic HBV DNA > cccDNA**

> 14 years of therapy to clear completely viral cccDNA

Werle et al, Gastroenterology 2004

Immunohistochemical Staining of Patient Biopsies at Baseline and After 48 Weeks ADV Therapy

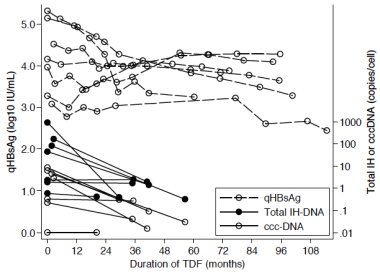




- 0.8 $\log_{10}$ (84%) decline in cccDNA, not paralleled by a similar decline in the number of HBcAg+ cells
- Suggests cccDNA depleted primarily by non-cytopathic mechanisms or new rounds of hepatocyte infection occurred during therapy

Werle et al, Gastroenterology 2004

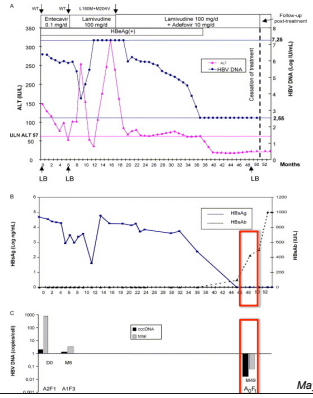
Persistence of intrahepatic viral DNA synthesis during Tenofovir therapy (HIV-HBV cohort)



New round of infection and/or replenishment of the cccDNA pool occur despite « viral suppression »

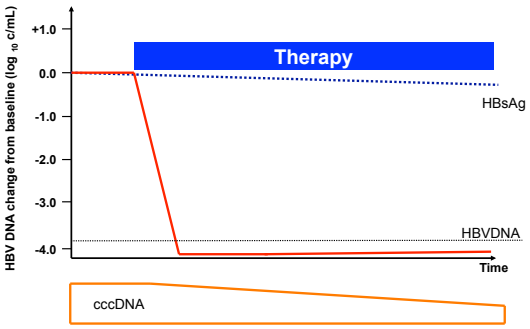
Boyd et al, in revision

Persistence of cccDNA after HBs seroconversion

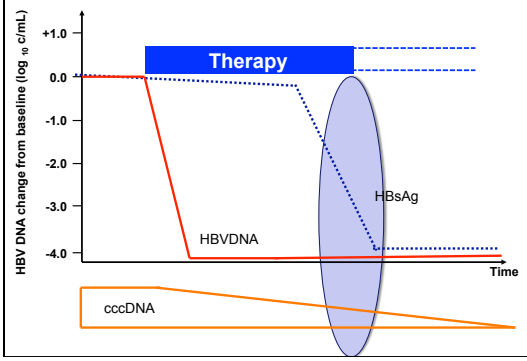


Maynard et al, J Hepatol 2005

Long-term therapy is required to maintain viral suppression



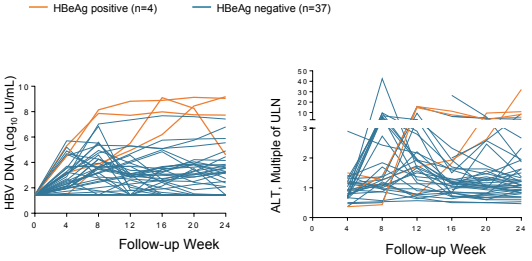
New treatment concepts for a functional cure of HBV infection



Can we do more with currently approved antivirals ?

Stopping TDF therapy after long-term viral suppression

High rates of viral replapse & ALT elevations

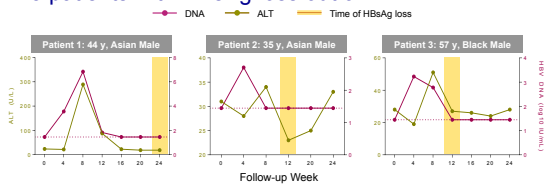


24-week TFFU Completers (n=41)

Buti et al AASLD 2015

Stopping TDF therapy after long-term viral suppression

3 patients with HBsAg loss out of 41



	Patient 1	Patient 2	Patient 3
HBV-GT	D	B	A
Baseline viral load, log_{10} IU/mL	5.97	6.79	6.33
Year 6 HBsAg, IU/mL	1145	74	785

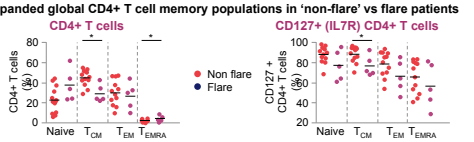
- No patients with HBeAg loss (0/4)

24-week TFFU Completers (n=41)

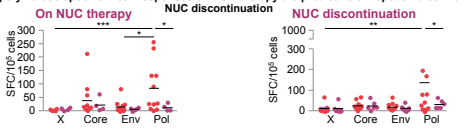
Buti et al AASLD 2015

Characterisation of the immune profile in Chronic Hepatitis B, with CyTOF, to identify biomarkers of immune control following NUC therapy discontinuation

Expanded global CD4+ T cell memory populations in 'non-flare' vs flare patients



HBV polymerase specific T cell responses on NUC therapy are predictive of hepatic flares with NUC discontinuation



- Patients with detectable frequencies of circulating HBV-specific T cells controlled HBV replication / no hepatic flare following NUC discontinuation

Gill US, et al. AASLD 2015, San Francisco. #167

BE-LOW (ETV-110): study design

RANDOMIZATION 1:1

- ETV 0.5 mg, once daily (N=182)*
- ETV 0.5 mg + TDF 300 mg, once daily (N=197)*

Week 96

Randomized, open-label, Phase IIIb trial

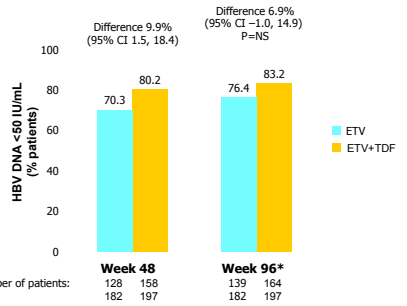
NA-naïve CHB, HBeAg(+) or HBeAg(–)

HBsAg levels were quantified (Abbott Architect assay at a central laboratory)

*Modified intent-to-treat population: received at least one dose of study medication
†HBV DNA assayed by Roche COBAS® TaqMan HPS assay

. Lok A, et al. *Gastroenterology* 2012

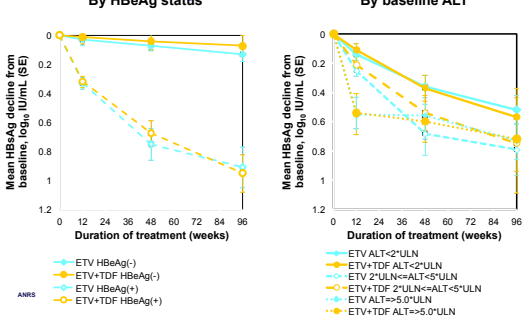
HBV DNA <50 IU/mL at Weeks 48 and 96: overall



Non-completer = failure

*Primary endpoint

HBsAg decline through Week 96 by baseline HBeAg status and baseline ALT



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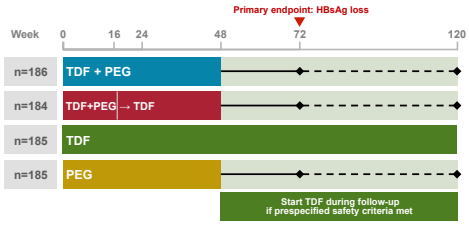
Study GS-US-174-0149 Design



Randomized, controlled, open-label study (N=740)

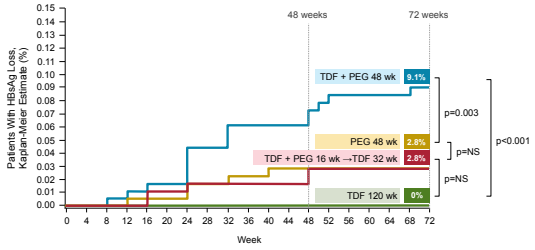
- **Stratified by screening HBeAg status and HBV genotype**

Inclusion criteria

- **HBeAg+ and HBV DNA ≥20,000 IU/mL; HBeAg- and HBV DNA ≥2,000 IU/mL**
- **ALT >54 and ≤400 U/L (men); ALT >36 and ≤300 U/L (women)**
- **No bridging fibrosis or cirrhosis on liver biopsy or by transient elastography**

24

Results: HBsAg Loss Over Time (Week 72)

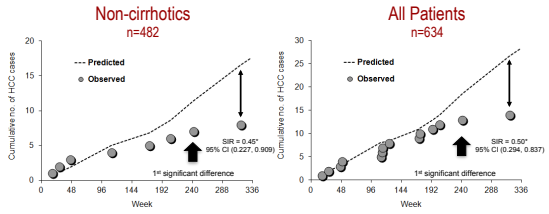


7 patients had HBsAg seroreversion on or after Week 48 (n=4 treated with TDF + PEG 48 wk; n=3 with TDF + PEG 16 wk →TDF 32 wk)

Marcellin P, et al. *Gastroenterology*. 2016

Prevention of HCC by antiviral therapy

Tenofovir Studies 102/103
Observed vs. Predicted HCC Cases

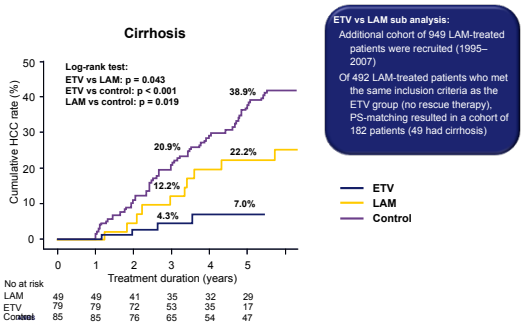


- Incidence of HCC in patients on TDF in Studies 102/103 was lower than predicted by the REACH-B model
- In non-cirrhotic patients, the effect of TDF becomes noticeable between 2–3 years of therapy and became statistically significant (55% reduction) at 6 years of therapy

*Statistically significant at nominal α-level of 0.05.
Kim WR, et al. J Hepatol 2013 Supp 1;58(43):S19 - Oral#43

REACH-B is a risk calculator developed in non-cirrhotic pts so it may underestimate the risk

Japanese cohorts: HCC incidence lower with Entecavir than with Lamivudine in cirrhotic patients



ETV vs LAM sub analysis:
Additional cohort of 949 LAM-treated patients were recruited (1995–2007)
Of 492 LAM-treated patients who met the same inclusion criteria as the ETV group (no rescue therapy), PS-matching resulted in a cohort of 182 patients (49 had cirrhosis)

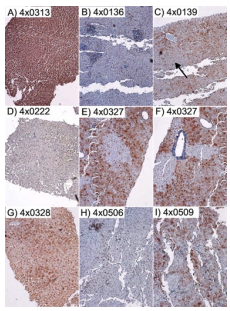
Adapted from Hosaka T, et al. Hepatology 2013 [Epub ahead of print]. doi: 10.1002/hep.26180.

Devons nous redéfinir la tolérance immunitaire et repenser les indications thérapeutiques ?

Clonal expansion of hepatocytes
- Cells that do not express viral antigens
- Decrease in viral load despite the absence of detectable liver damage
- One of the first steps of HCC development

« Immune tolerance »
- Almost all hepatocytes are infected
- Viremia > 10^E9 copies/mL

Should we do a liver biopsy when VL declines in the absence of ALT elevation ?
And consider antiviral treatment ?



Zoulim & Mason, W. S. Gut 2012

Mason, W. S. et al. 2009 / 2010. J. Virol

Conclusions

- Potent and safe antivirals
- High barrier to resistance
- Histological and clinical improvement
- Decreased risk of HCC development
- First results in immune tolerant patients
- Strong arguments for:
  - screening of Hepatitis B
  - antiviral Tx for all patients meeting the criteria
  - considering treatment in patients with minimal hepatitis and immune tolerant patients (beyond guidelines)

Why a need for new antiviral targets for hepatitis B ?

Current antivirals achieve viral suppression in the majority of patients (in western countries)

Issues with antiviral drug resistance in developing countries (use of low barrier to resistance antivirals)

The cure rate (cccDNA / HBsAg loss) remains very low

Life-long therapy is needed in the majority of cases

Treatment with finite duration if:
- **cccDNA control or loss**
- **HBsAg loss**

HBsAg clearance is associated with a lower risk of HCC development

Zoulim, Antiviral Research 2012

Definition of HBV cure

Virologic definition

- Functional cure

- Situation where antiviral therapy could be stopped with a minimal risk of viral reactivation
- HBsAg loss with anti-HBsAb seroconversion
- cccDNA inactivation and/or control by host mechanisms

- Complete cure

- HBsAg clearance and cccDNA eradication

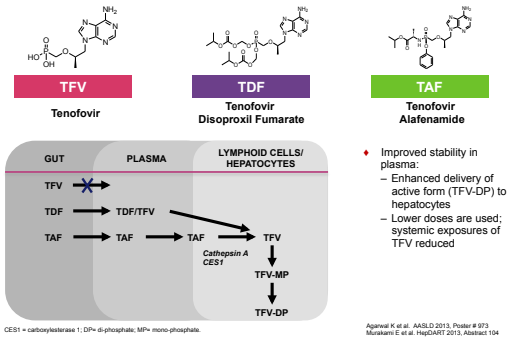
Clinical definition

- Functional cure associated with a regression in the risk of progression of fibrosis and HCC

Zeisel, Lucifora et al, Gut 2015

Improvement of already existing drugs

Improvement of existing drugs Example of TAF for tenofovir

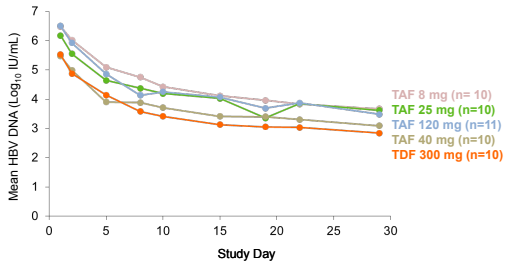


CES1 = carboxylesterase 1; DP= di-phosphate; MP= mono-phosphate.

- Improved stability in plasma:
  - Enhanced delivery of active form (TFV-DP) to hepatocytes
  - Lower doses are used; systemic exposures of TFV reduced

Agarwal K et al. AASLD 2013, Poster # 973
Murakami E et al. HepDART 2013, Abstract 104

Phase 1B results: HBV DNA kinetics on 28 days

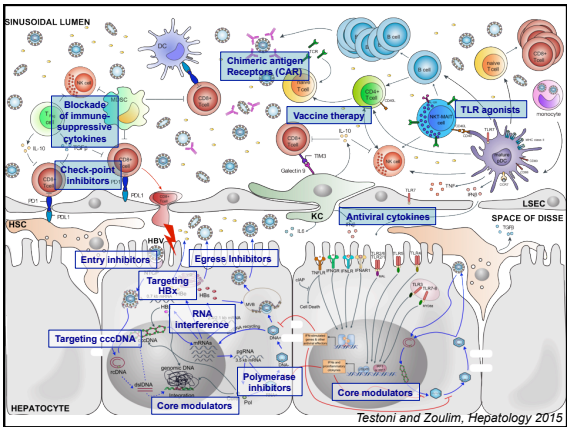


♦No differences in viral declines over range of TAF 8 mg to 120 mg

♦Viral suppression over 4 weeks with TAF was similar to TDF

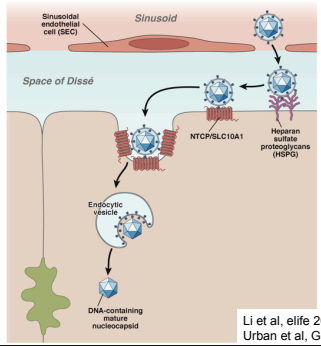
Agarwal K et al. AASLD 2013, Poster # 973
GS-US-320-0101 - Clinicaltrials.gov NCT01671787

New targets for HBV therapy



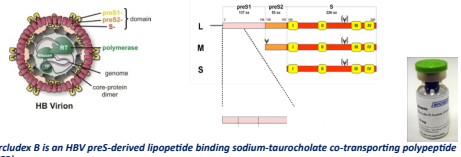
Testoni and Zoulim, Hepatology 2015

Model for HBV entry in hepatocytes and development of entry inhibitors



Li et al, elife 2012
Urban et al, Gastroenterology 2014

Myrcludex B, a peptidic inhibitor of NTCP-mediated entry of HBV and HDV



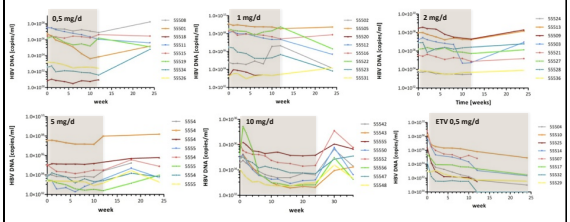
- *Myrcludex B is an HBV preS-derived lipopetide binding sodium-taurocholate co-transporting polypeptide (NTCP).*
- *Myrcludex B inhibits HBV and HDV receptor function of NTCP in vitro and in animal models (IC_{50} ~80 pM).*
- *Myrcludex B inhibits NTCP-mediated bile salt uptake into hepatocytes (IC_{50} ~100 nM).*
- *Myrcludex B specifically targets liver hepatocytes after subcutanous administration.*
- *Myrcludex B showed safety in Phase I clinical trials.*

⇒ (A) Proof of safety and efficacy in chronically HBV infected individuals.

⇒ (B) Proof of safety and efficacy in chronically HBV/HDV co-infected individuals.

S Urban Heidelberg U & MyrGmbH

HBV Serum DNA-levels decline during Myrcludex B treatment



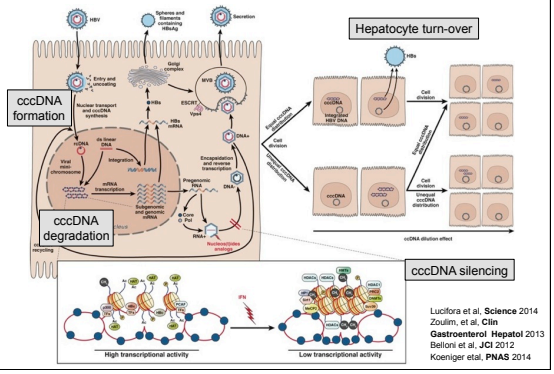
⇒ HBV DNA levels decline significantly during Myrcludex B treatment in all groups.

⇒ Pronounced effects by > 1log in 6/8 patients were observed in the 10 mg dosing group.

⇒ 7/40 showed > 1log HBV reduction in lower dosing groups.

S Urban Heidelberg U & MyrGmbH

Targeting cccDNA

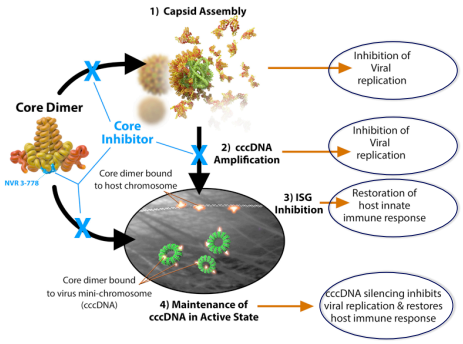


Lucifora et al, **Science** 2014
Zoulim, et al, **Clin Gastroenterol Hepatol** 2013
Belloni et al, **JCI** 2012
Koeniger etal, **PNAS** 2014

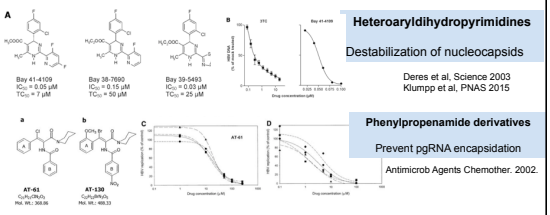
Challenges in targeting cccDNA

- cccDNA formation: involves nuclear enzyme / DNA repair machinery
- cccDNA degradation: is the whole pool of cccDNA susceptible to degradation ? will all infected cells be susceptible ?
- cccDNA damage: CRISPR/cas9 technologies and others. Issues with delivery ?
- cccDNA silencing: targeting virus-specific mechanisms to avoid safety issues
- Hepatocyte turn-over: may trigger the clonal selection of hepatocytes in the context of an oncogenic virus
- Small molecules needed !

Targeting the HBV capsid



Targeting HBV nucleocapsids



Heteroaryldihydropyrimidines

Destabilization of nucleocapsids

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

Phenylpropenamide derivatives

Prevent pgRNA encapsidation

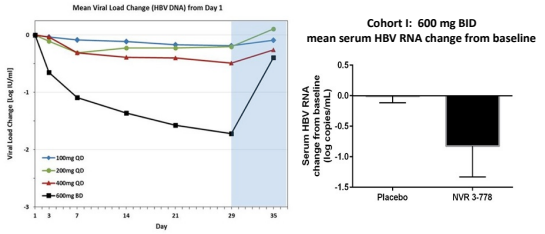
Antimicrob Agents Chemother. 2002.

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

Phase 1b clinical trial: NVR 3-778 reduces serum HBV DNA and RNA (Novira)

Serum HBV DNA: mean 1.7 log reduction (600 mg BID)

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



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

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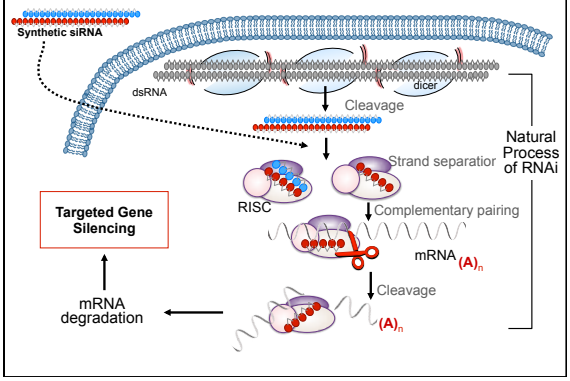
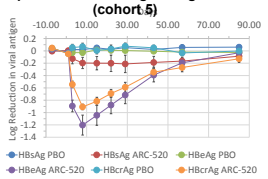
RNA Interference

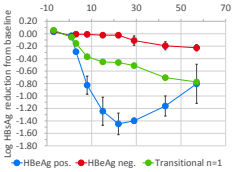


ARC-520 produces deep and durable knockdown of viral antigens and DNA in a phase II study in patients with chronic hepatitis B

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)



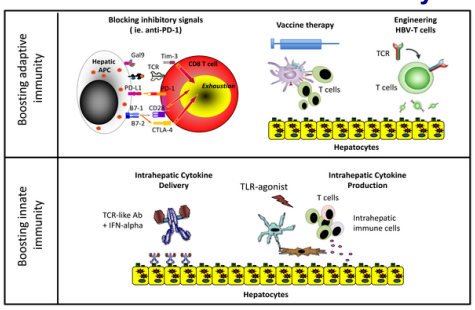
Direct antiviral effect lasted up to 57 days after a single dose of ARC-520, delayed response duration >85 days

Small dose-related reduction in HBsAg

Maximum effective dose not reached

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

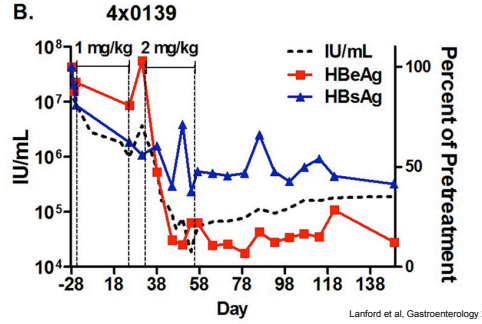
Restoration of antiviral immunity



Bertoletti A, Gehring AJ (2013) Immune Therapeutic Strategies in Chronic Hepatitis B Virus Infection: Virus or Inflammation Control?. PLoS Pathog 9(12): e1003784. doi:10.1371/journal.ppat.1003784
http://www.plospathogens.org/article/info:doi/10.1371/journal.ppat.1003784

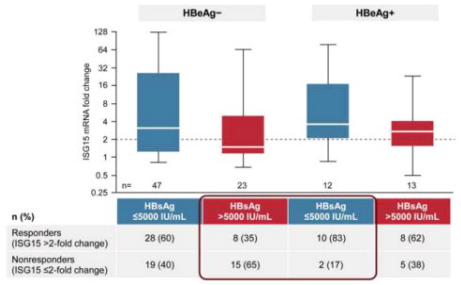
PLOS | PATHOGENS

Antiviral activity of a TLR7 agonist in HBV infected chimpanzees



Lanford et al, Gastroenterology 2013

The Oral Toll-Like Receptor-7 Agonist GS-9620 in Patients with Chronic Hepatitis B Virus Infection

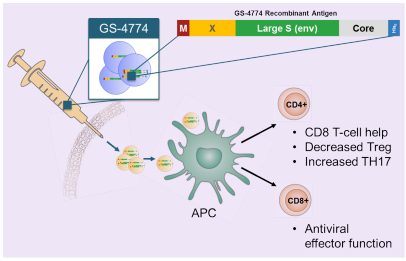


n (%)	HBsAg ≤5000 IU/mL	HBsAg >5000 IU/mL	HBsAg ≤5000 IU/mL	HBsAg >5000 IU/mL
Responders (ISG15 ≥2-fold change)	28 (60)	8 (35)	10 (83)	8 (62)
Nonresponders (ISG15 ≤2-fold change)	19 (40)	15 (65)	2 (17)	5 (38)

Gane et al, Journal of Hepatology, 2015

Therapeutic vaccine: GS-4774

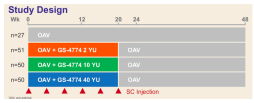
- Recombinant antigen containing X, Large S (env) and Core epitopes
- GS-4774 activates dendritic cells after phagocytosis
- Recombinant antigen epitopes are displayed via MHC class I and II and stimulate CD4⁺ and CD8⁺ T cells

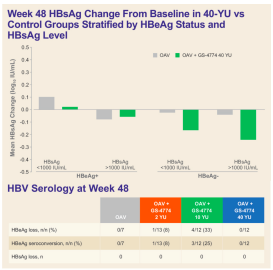


Stubbs AC, et al. Nat Med 2001; Cereda V, et al. Vaccine 2011

Safety and efficacy of GS-4774 in patients with chronic hepatitis B on oral antiviral therapy—Phase II

Phase II randomized, open-abel study (GS-US-330-0101; NCT02174276)
All patients on OAV with undetectable HBV DNA for at least 1 years prior to screening
GS-4774 administered sc every 4 weeks x 6 doses

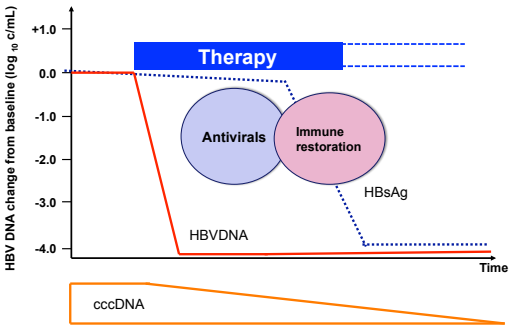




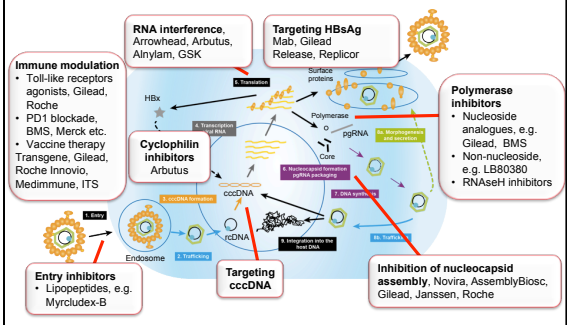
- No clinical significant decline in HBsAg was observed during and after treatment with GS-4774, no HBsAg loss.
- HBeAg loss was only achieved with GS-4774 treatment.

Lok AS, et al AASLD 2015

New treatment concepts for a functional cure of HBV infection



Target & drug discovery to cure HBV infection



Zoulim F, et al. Antiviral Res 2012;96(2):256–9; HBF Drug Watch, Available at: http://www.hepb.org/professionals/hbf_drug_watch.htm

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INSERM U1052

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anRS France Recherche Nord & sud Sida-hiv Hépatites
Agence autonome de l'Inserm

"Save the date"

Third ANRS "HBV cure" Workshop
HBV pathobiology and target discovery

Scientific coordination: Fabien Zoulim

Tuesday, May 31st, 2016
Union internationale des chemins de fer (UIC)
16, rue Jean Rey - 75015 PARIS

HBV cure 2014: Zeisel, M. B. *et al.* Towards an HBV cure: state-of-the-art and unresolved questions-report of the ANRS workshop on HBV cure. *Gut*, doi:10.1136/gutjnl-2014-308943 (2015).

HBV cure 2015: http://www.anrs-hbvcure2015.com/