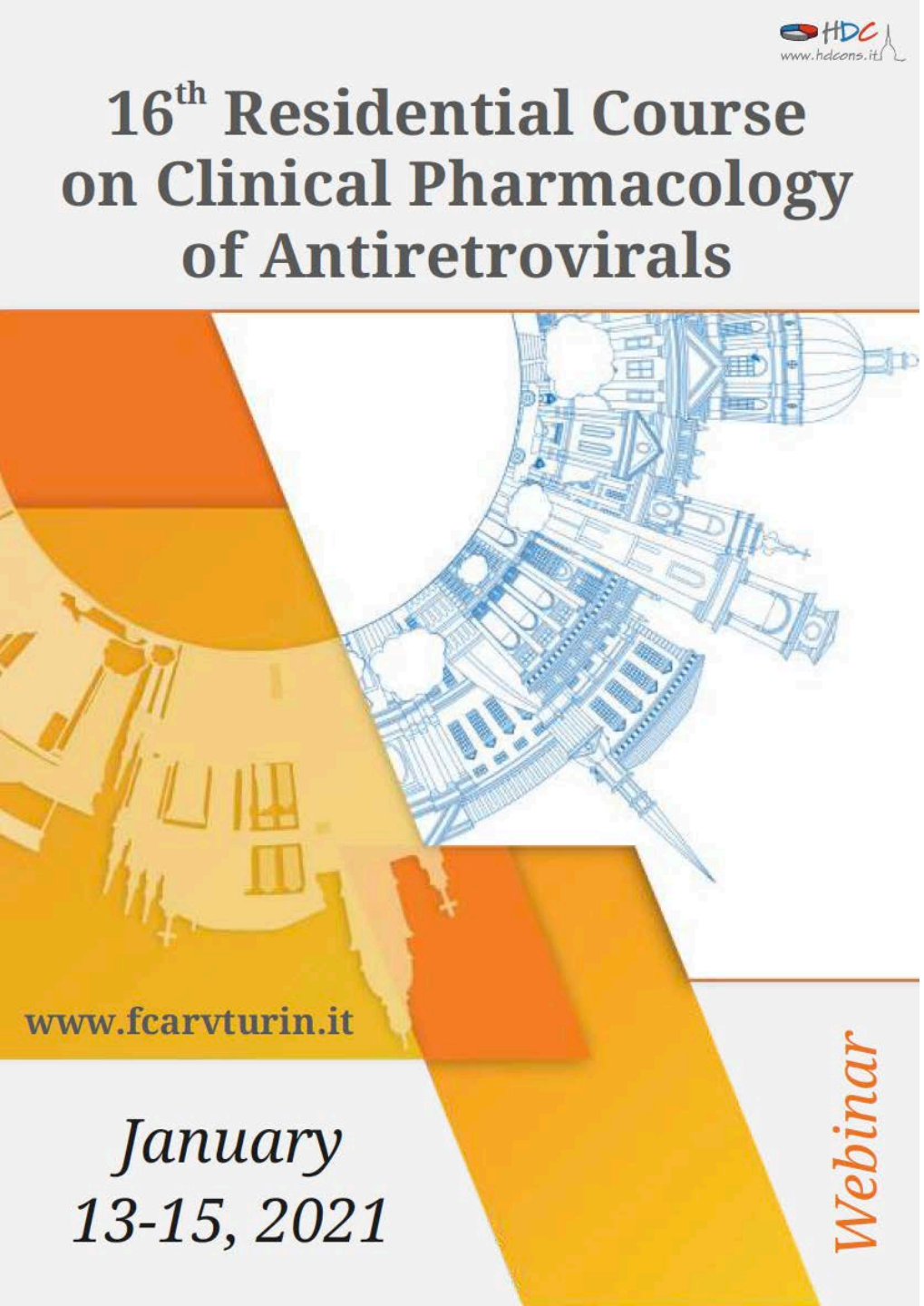

Novel targets and strategies to cure HBV

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Towards a functional cure of chronic Hepatitis B

At present

- The control/cure of chronic hepatitis B is achieved in the majority of patients by a continuous pharmacological suppression of viral replication

- In a small proportion of patients the transition to HBeAg negative infection or the HBsAg clearance can be obtained by a treatment of limited duration (usually, with Peg-IFN treatment, occasionally with NA)

The criteria to define the virological response to treatment vary according to the type of treatment and the timing

- *during NA* **undetectable HBV DNA** by a sensitive PCR (LoD 10 IU/ml)
- *after NA*, sustained off-therapy virological response, **HBV DNA <2,000 IU/ml** for at least 12 months

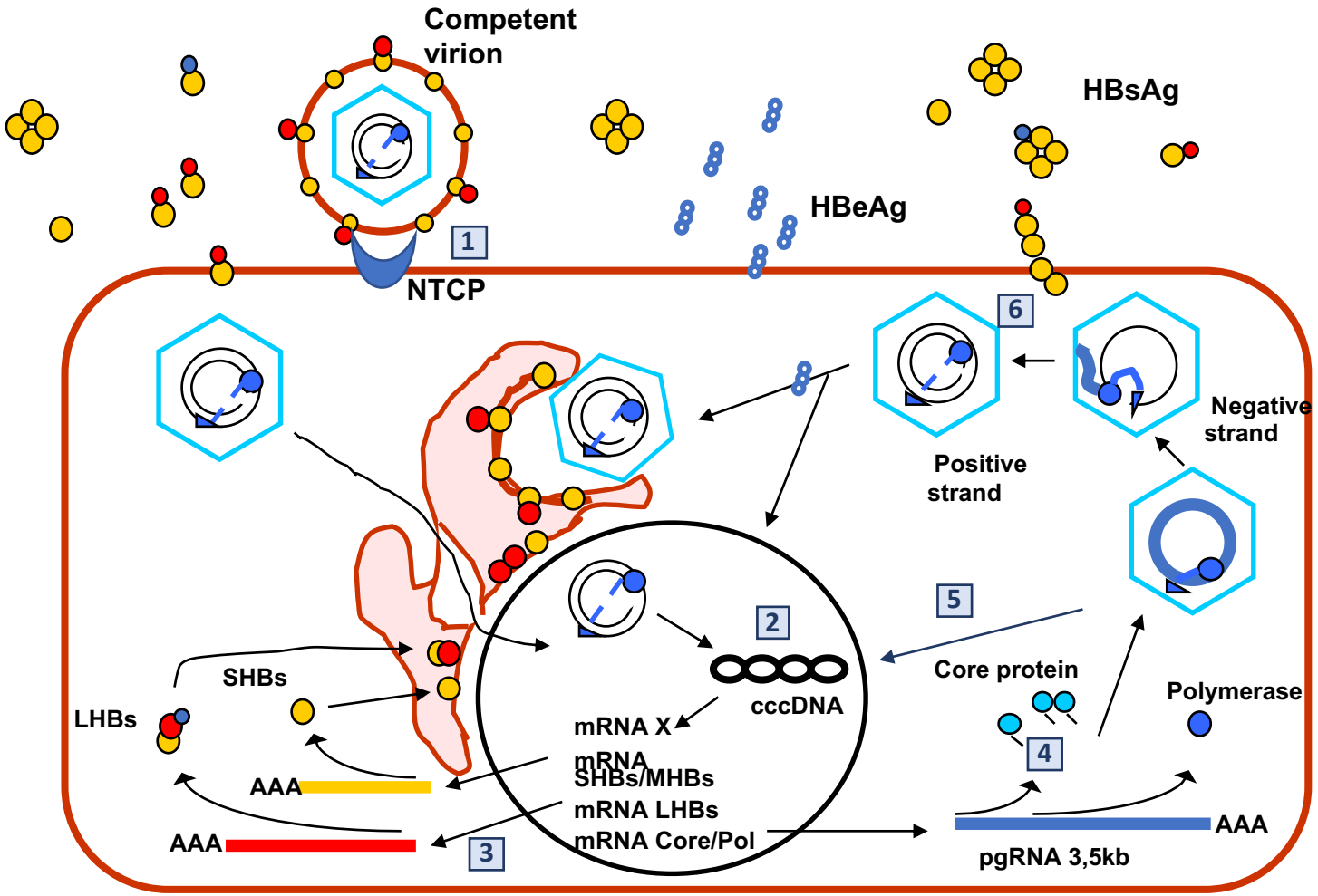
- *during PegIFNa* **HBV DNA <2,000 IU/ml** at 6 months and at the end of therapy.
- *after PegIFNa* **HBV DNA <2,000 IU/ml** for at least 12 months

Serological responses

- **HBeAg** are HBeAg loss and HBeAg seroconversion
- **HBsAg** are HBsAg loss and HBsAg seroconversion

EASL, HBV CPG 2017

Towards a functional cure of chronic Hepatitis B



Viral targets

1. Inhibition of viral entry via NTCP
2. Targeting cccDNA (destabilizer, epigenitic regulators, endonucleases)
3. Inhibition of viral proteins syntesis by interfering with mRNA
4. Inhibition of capsid synthesis/formation
5. Interfering with HBcAg-mediated cccDNA replenishment
6. Inhibition of the HBsAg release

Immunologic Targets

a. Modulation of innate Immunity (TLR agonist)

b. Modulation of adaptive immunity (anti-PD1, anti-PD-L1, anti-CTLA4, vaccine)

Brunetto MR, Asian Pacific Digestive Week 2017, Cornberg M et al J Hep 2020,, Yuen MF AASLD-HBV SIG 2020

New HBV antiviral drugs

Compound	Phase of development	Comments / Data
HBV entry inhibitors		
NTCP inhibitor, Myrcludex B (Myr Pharmaceuticals)	Phase III (Hepatitis D) Phase I/II (Hepatitis B)	Strong effect on serum HDV RNA levels, induced ALT normalisation under monotherapy.[111]
Cyclosporine analogues	Preclinical	Several cyclosporine derivatives inhibited HBV infection with a sub-micromolar IC_{50} with no inhibition of bile acid uptake.[112]
Targeting cccDNA (Destabiliser, epigenetic regulators, endonucleases)		
cccDNA destabiliser, ccc_R08 (Roche)	Preclinical	First-in-class orally available HBV cccDNA destabiliser achieved sustained HBsAg and HBV DNA suppression in a mouse model.[64]
Targeted endonuclease, CRISPR/CAS9	Preclinical	Cleavage of cccDNA by Cas9 showed reduction in both cccDNA and other parameters of viral gene expression and replication *in vitro*.[66]
Targeting HBx		
CRV431 (ContraVir)	Phase I	Cyclophilin inhibitor that prevents Cyclophilin A-HBx complex formation and HBV replication.[110]
Nitazoxanide (Romark)	Phase II	First-in-class thiazolide originally developed as antiprotozoal agent. Inhibits HBV transcription from cccDNA by targeting the HBx-DDB1 interaction.[70] A pilot trial showed antiviral efficacy.[114]
Inhibition of gene expression / gene silencing		
Antisense oligonucleotides and locked nucleic acids		
GSK3389404 (GlaxoSmith Kline)	Phase II	Methoxyethyl antisense oligonucleotide conjugated to N-acetylgalactosamine moieties. Acceptable safety and pharmacokinetic profile in phase I.[72]
locked nucleic acid platform-based single-stranded oligonucleotides (Roche)	Preclinical	Liver-targeted single-stranded oligonucleotide therapeutics based on the locked nucleic acid platform. Rapid and long-lasting reduction of HBsAg in a mouse model.[71]
RNA interference		
ARC-520 (Arrowhead)	Development discontinued	Decrease in HBsAg level in HBeAg-positive but not in HBeAg-negative patients.[29]
JNJ-3989 (Janssen) formerly ARO-HBV-1001 (Arrowhead)	Phase I/II	HBsAg reduction in HBeAg-positive and HBeAg-negative patients. Majority of patients achieved HBsAg <100 IU/ml.[73]
AB-729 (Arbutus)	Preclinical	Activity *in vitro* and strong HBsAg reduction in mice.[115]
ALN-HBV (Alnylam)	Preclinical	Profound and durable HBsAg silencing *in vitro* and *in vivo*.[116]
Targeting the viral RNA post-transcriptional regulatory element		
Dihydroquinolizinone compounds RG7834 (Roche) AB-452 (Arbutus)	Preclinical	Specific blockage of the production of HBV DNA and viral antigens.[76,117]
Core protein (Capsid) assembly modulators (CpAMs)		
NVR 3-778 (Novira, Janssen Pharmaceutica)	Development discontinued	First in-class CpAM showed reduction of HBV DNA and HBV RNA, greater effect in combination with PEG-IFN.[79]
ABI-H0731 (Assembly Bioscience)	Phase IIa	CpAMs showed high antiviral efficacy in phase I and IIa studies with >2 log decline of HBV DNA. HBV RNA decline is stronger with CpAM (ABI-H0731) compared to NA therapy.[118–124]
RO7049389 (Roche)	Phase II	
JNJ-56136379 (Janssen)	Phase II	
AB-506 (Arbutus)	Phase I	
ABI-H2158 (Assembly Bioscience)	Phase I	
GLS4JHS (Jilin University)	Phase I/II	
EDP-514 (Enanta)	Preclinical	
GLP-26 (Emory University)	Preclinical	
ABI-H3733 (Assembly Bioscience)	Preclinical	
HBsAg release inhibitors		
Nucleic acid polymers (REP compound series) (Replicor)	Phase II	Small studies with REP compounds (i.v. application) in combination with TDF and PEG-IFN in HBV monoinfected and HBV/HDV co-infected patients show strong HBsAg decline.[81,82]

Immunomodulatory drugs targeting HBV

Compound	Phase of development	Comments / Data
Targeting cell intrinsic and innate immune responses		
RO7020531 (Roche) TLR 7 agonist	Phase I	Combination with the CpAM RO7049389 achieved sustained HBV DNA suppression and HBsAg loss in a mouse model.[125]
Vesatolimod, GS-9620 (Gilead) TLR 7 agonist	Phase II	Dose-dependent pharmacodynamic induction of ISG15 and host NK and HBV-specific T cell responses but no HBsAg reduction in patients.[87,88] Lack of effect for cccDNA *in vitro*.[126]
JNJ-4964 (Janssen) TLR 7 agonist	Preclinical	Antiviral efficacy (HBV DNA, HBsAg, liver HBV DNA, HBV RNA) in a mouse model.[127]
GS-9688 (Gilead) TLR 8 agonist	Phase I	Induced IL-12 and IL-RA1 in humans. Short duration did not result in HBsAg decline.[128]
AIC649 (AiCuris) TLR 9 agonist	Phase I	Increased IL-1β, IL-6, IL-8 and IFN-γ and reductions in IL-10 levels.[129]
Inarigivir soproxil (Spring Bank) RIG-I agonist	Phase II	Dual mode of action: RIG-I Agonist and interference with the interaction of the viral polymerase and pgRNA. The ACHIEVE trial showed dose-dependent antiviral response on HBV DNA and HBV RNA.[92]
Targeting adaptive immune responses		
Checkpoint Inhibitors Nivolumab (Opdivo, Bristol-Myers Squibb)	Phase I	Single dose of Nivolumab (with or without GS4774) showed HBsAg reduction >0.5 log in some patients.[90]
TG1050/TI01 (Transgene/Talsy) Non-replicative adenovirus serotype 5 encoding 3 HBV proteins (Therapeutic Vaccine)	Phase I	Induction of T cell responses in mouse models and reduction of viral parameters.[130] Dose-related immunogenicity in patients but so far only preliminary data on clinical effects.[131]
CPmutS (Vaccitech) Adjuvanted ChAd and MVA vectored therapeutic HBV vaccines	Phase I	Robust T cell and anti-HBs response in mice.[132]
HepTcell (Altimmune) HBV Peptide therapeutic vaccine with TLR9 adjuvant IC31	Phase I	Human T cell responses against HBV markedly increased over baseline compared to placebo but no effect on HBsAg.[133]
JNJ-64300535 (Janssen) Electroporation of DNA vaccine	Phase I	No clinical data (NCT03463369).
INO-1800 (Inovio) DNA plasmids encoding HBsAg and HBcAg) plus INO-9112 (DNA plasmid encoding human interleukin 12)	Phase I	Activated and expanded CD8+ killer T cells (www.inovio.com).
GS-4774 (Globeimmune, Gilead) Heat-inactivated, yeast-based, T cell vaccine	development discontinued	No significant reductions in serum HBsAg in phase II.[134]
Genetically engineered T cells / Monoclonal or bispecific antibodies	Preclinical	Reductions in HBsAg and HBV DNA in mouse models.[101,102]

Most of the drugs or combinations are in the early phases of clinical development

Cornberg M et aJ Hep 2020

Unmet need for a personalized management of current and future antiviral treatments

- Development and standardization of specific assays with high sensitivity to detect the viral targets of antiviral drugs
- Development and standardization of assays unrevelling the impact of antiviral treatment on the biology of HBV infection
- Identification of viral markers associated with cure/control of HBV infection and not simply with target engagement
- Identification of the immunological profiles associated with an effective control of HBV infection
- Availability of biomarkers with high diagnostic accuracy in the identification of the carriers who achieved an effective and persistent control of HBV infection

Brunetto MR, Asian Pacific Digestive Week 2017; Brunetto MR, ILC 2019

Towards a functional cure of chronic Hepatitis B, starting from a new nomenclature

Guidance for design and endpoints of clinical trials in CHB – Report from 2019 EASL-AASLD HBV treatment endpoints Conference

In the context of the emergence of novel therapies, **representatives from academia, industry, regulatory agencies, and patient groups** convened in March 2019 in London **to develop an agreement on HBV treatment endpoints to guide the design of clinical trials aiming to 'cure' HBV.**

- **Functional** but not sterilising **cure** is achievable and should be defined as **sustained HBsAg loss in addition to undetectable HBV DNA 6 months post-treatment**.
- The primary endpoint of phase III trials should be functional cure: **HBsAg loss in > 30% of patients** after 1 year of treatment was suggested as an acceptable rate of response in these trials.
- **Sustained virologic suppression** (**undetectable serum HBV DNA**) without HBsAg loss **6 months after discontinuation of treatment would be an intermediate goal.**
- Clinical trials aimed at HBV functional cure should initially focus on patients with **HBeAg-positive or negative chronic hepatitis**, who are treatment-naïve or virally suppressed on nucleos(t)ide analogues.

Cornberg M et aJ Hep 2020

Towards a functional cure of chronic Hepatitis B

At present, in spite of the preliminary data showing different extent of target engagement, inhibition of viral proteins, pgRNA and HBV-DNA production, we are still awaiting the evidence that functional cure can be achieved with the new drugs

- HBV infection is difficult to be eradicated because of the viral genetic reservoir (cccDNA) and of the integration of HBV-DNA fragments
- The HBV interaction with the hepatocyte is pervasive and complex and could make it difficult to identify molecules acting exclusively on the virus machinery
- An effective and specific immune control is required to achieved a functional cure of HBV infection

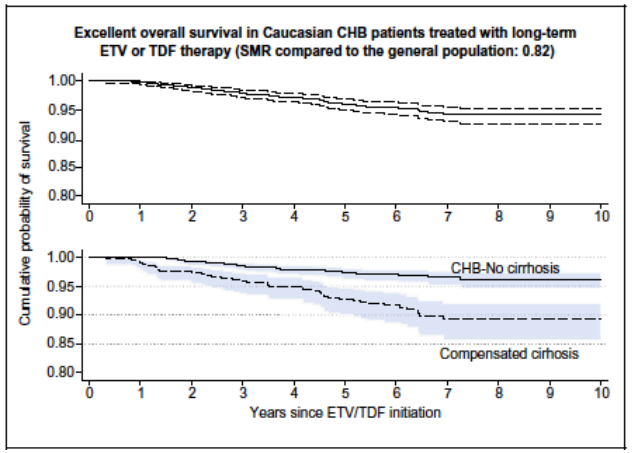
Bruegel il Vecchio, 1559, Proverbi

CPG EASL 2017, Brunetto MR, Asian Pacific Digestive Week 2017, AASLD-HBV SIG 2020

Will the functional cure have an impact on chronic liver disease?

➢ Eight-year survival in CHB patients under long-term ETV and TDF therapy is similar to the general population

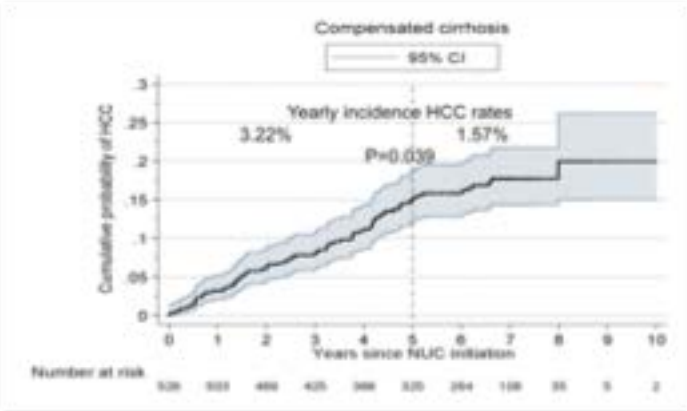
Papatheodoridis G et al, J Hep 2018



- 1951 adult Caucasian with CHB with (27%) /without cirrhosis, without HCC at BL on NUCs for at least 12 months /median 6 years)
- Overall mortality rate was 0.77 (95%CI 0.62-0.96) comparable to that of the general population, 0.82 (95%CI 0.66-1.039)

➢ The PAGE B cohort study: 10-year cumulative probability of HCC during NUC therapy

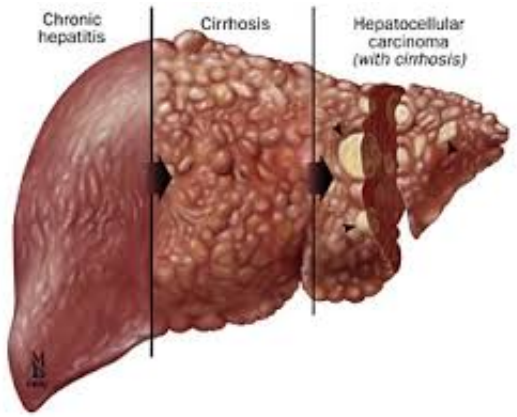
Papatheodoridis G et al, Hepatology 2017



- After the first 5 years of ETV or TDF therapy in CHB patients, the HCC incidence is decreasing, with the decrease being more evident in patients with baseline cirrhosis

Will the functional cure have an impact on chronic liver disease?

Liver disease



Cure: resolution of the chronic liver disease

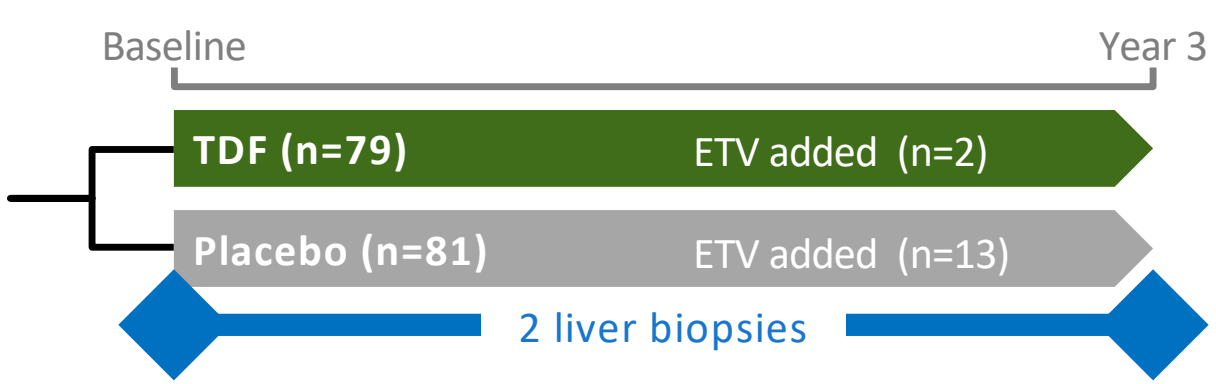
Control: halting progression of the disease, without complete resolution, but with reduction/ elimination of the complications

- **Currently, we do not know whether the new therapeutic approaches will further improve the outcome of CHB**
- **However, a more profound and early inhibition of viral replication could halt events that may have oncogenetic potential**

Tenofovir Disoproxil Fumarate Treatment Reduces the Number of Transcriptionally Active Viral Integrations in Chronically Infected HBV patients

CHB patients (N=160)

- Serum ALT 1–2x ULN
- No clinical liver cirrhosis or decompensation

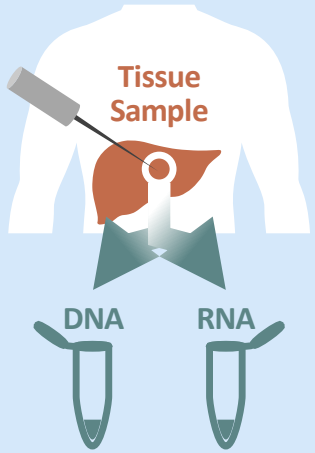


Paired biopsies available in *

TDF (n=66)

Placebo (n=61)

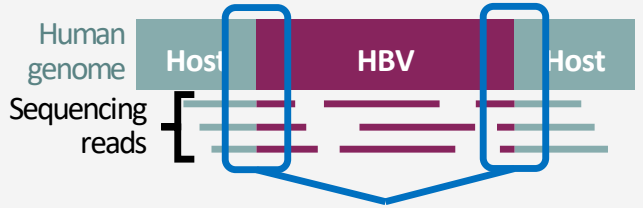
WORKFLOW



Analytics

Align reads to human (gencode.v29) and HBV genomes

Extract chimeric read-pairs and reads



Chimeric reads: evidence of viral integration

Split chimeric reads

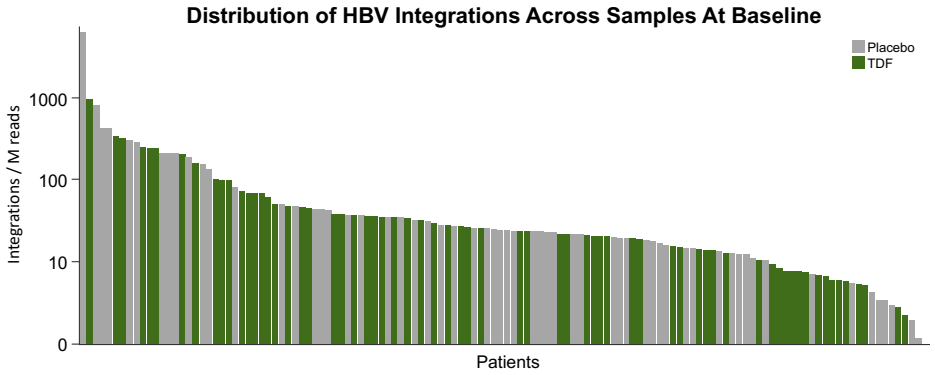
Cluster breakpoints by human & HBV coordinates and strand

Catalogue of expressed integration loci
(Units Log_{10} Expressed integrations per million reads)

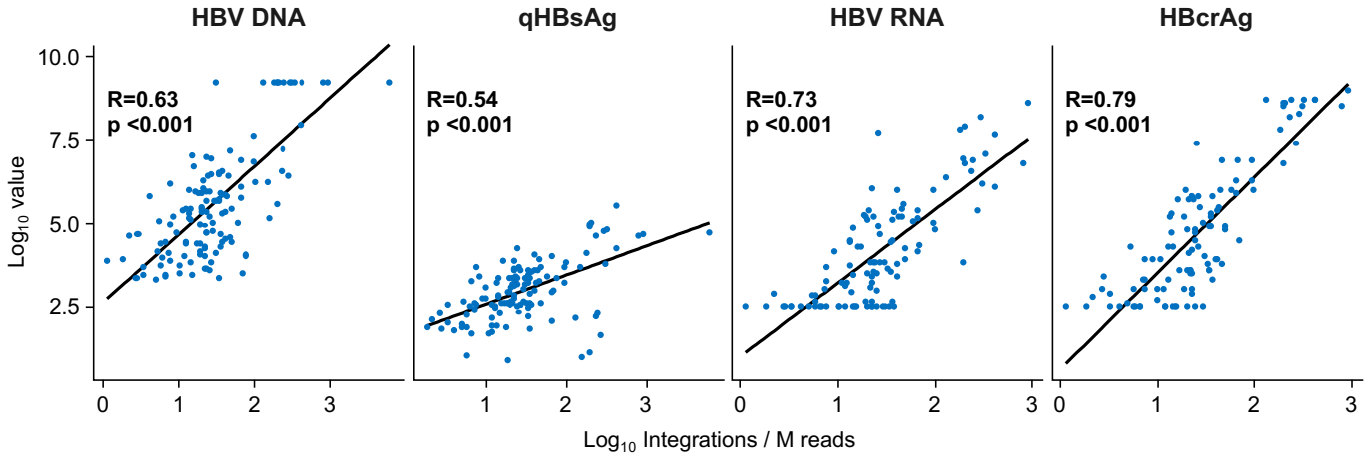
ALT, alanine aminotransferase; ETV, entecavir; ULN, upper limit of normal. *subset of original study.

Tenofovir Disoproxil Fumarate Treatment Reduces the Number of Transcriptionally Active Viral Integrations in Chronically Infected HBV patients

Demographics and virology	TDF n=66	Placebo n=61
Baseline		
Age, years	45 (38, 53)	43 (37, 50)
Women, n (%)	11 (17)	13 (21)
HBV DNA, log_{10} IU/mL	5.3 (4.3, 6.2)	5.3 (4.4, 6.4)
HBsAg, log_{10} IU/mL	2.9 (2.3, 3.5)	2.9 (2.5, 3.4)
HBeAg negative status, n (%)	57 (86)	45 (74)
HBV RNA, log_{10} IU/mL	3.8 (2.8, 5.1)	3.8 (2.6, 5.3)
HBcrAg, log_{10} IU/mL	4.4 (3.0, 6.0)	4.3 (3.3, 5.7)
Year 3		
HBV DNA, log_{10} IU/mL	ND	4.2 (3.3, 5.4)
Virally suppressed, n (%)	9/62 (15)	1/56 (2)

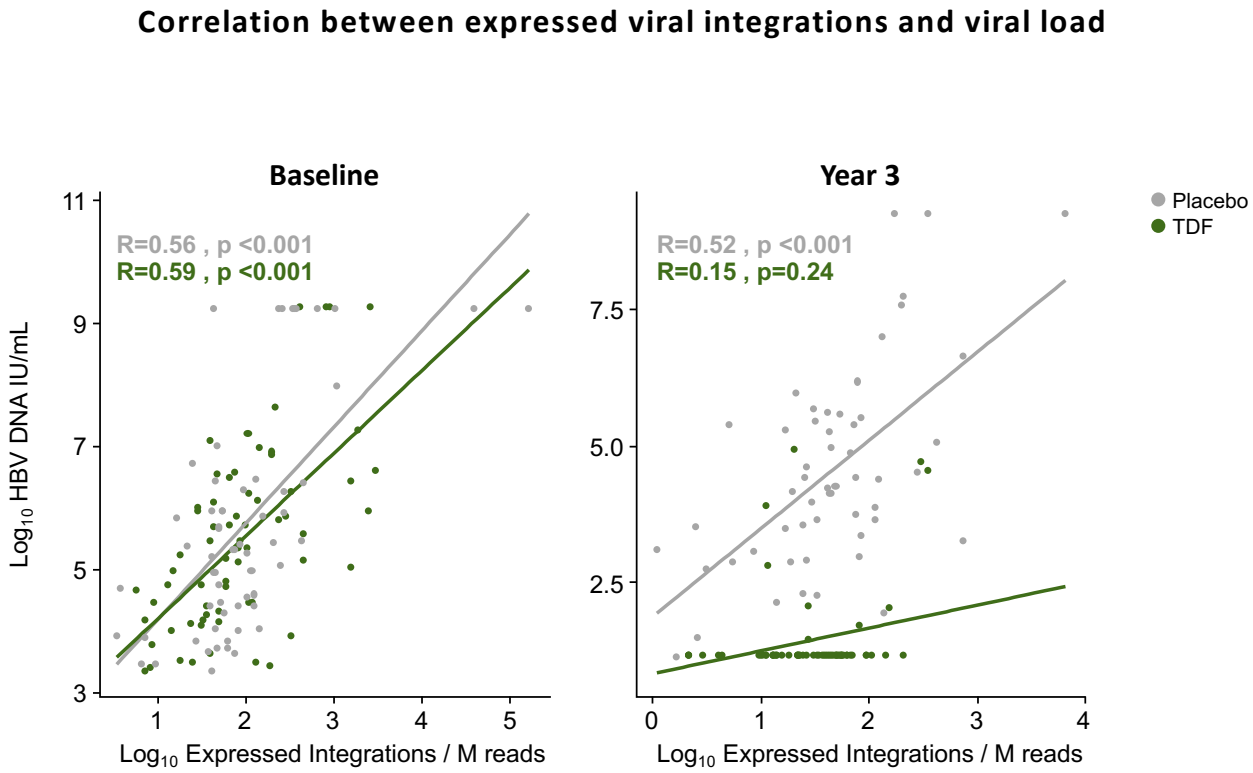


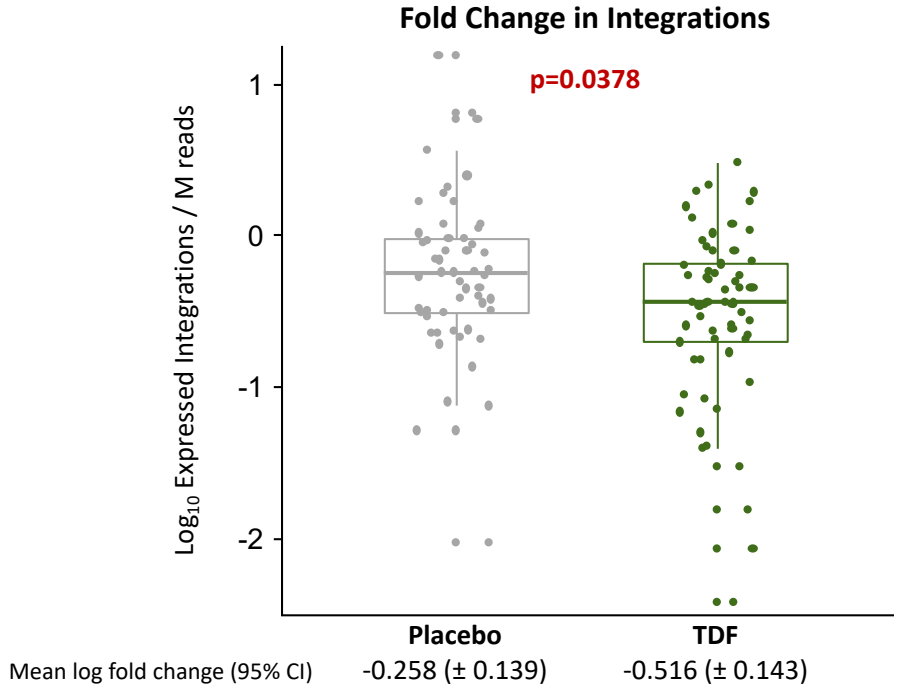
High-confidence expressed integrations detected in 88% of patients at baseline



HBV integrations correlate with HBV DNA, HBcrAg, and HBV RNA serum levels

HBV Integrations Correlate With Baseline Viral Markers and TDF Reduces Number of Expressed Viral Integrations

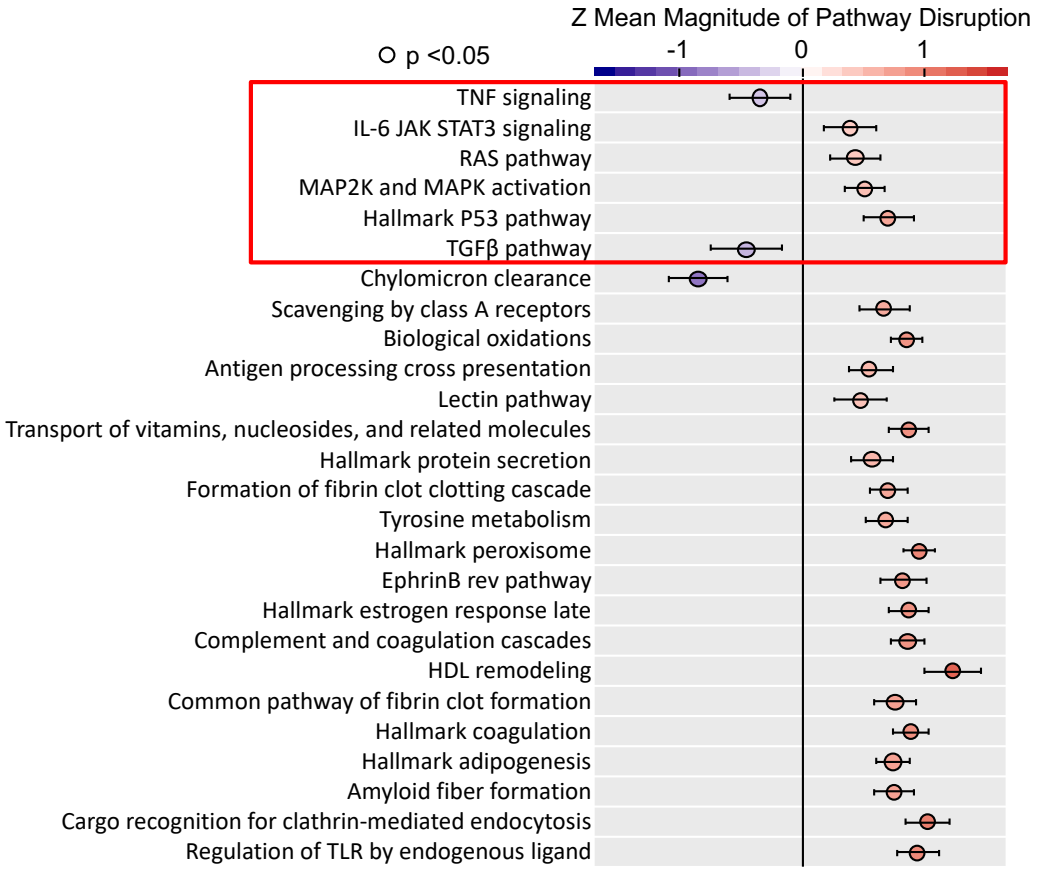


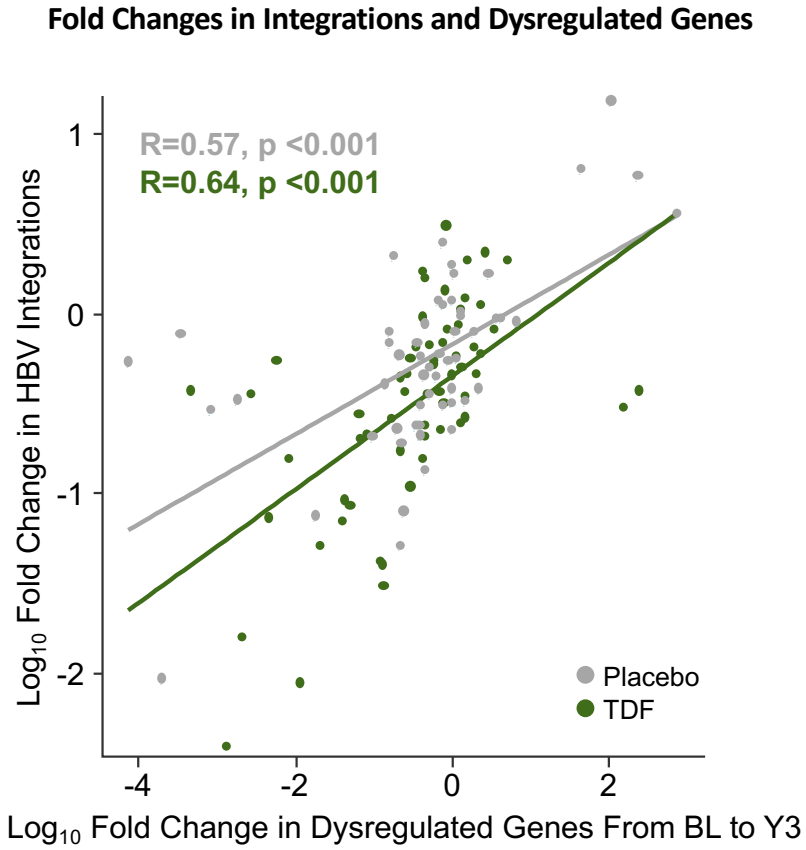


Correlation between expressed viral integrations and viral load lost in TDF arm at Year 3 due to effective viral suppression

A significant decline in expressed integrants is observed in TDF treated patients as compared to Placebo

HBV Integrations and Gene Dysregulation



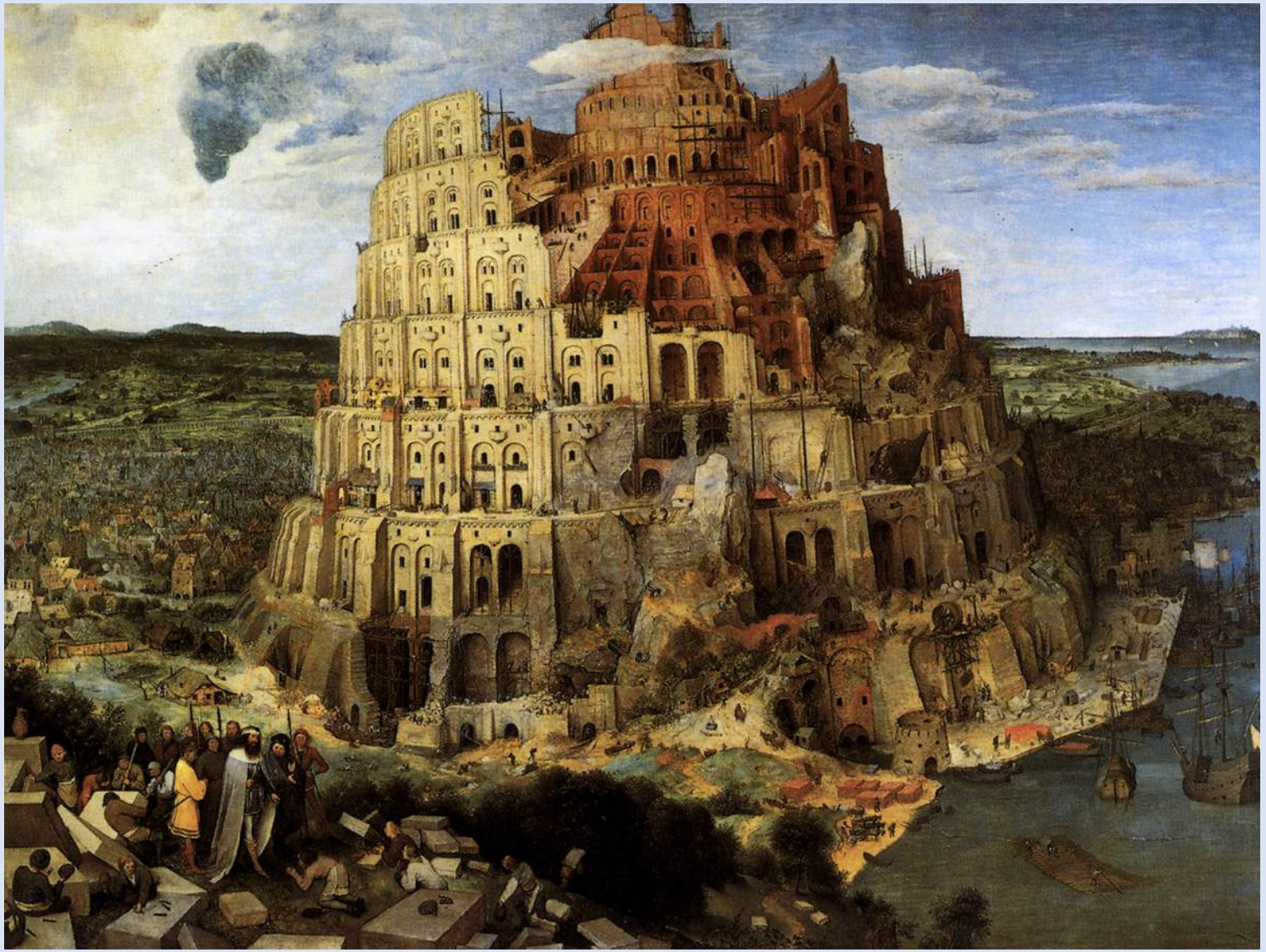


HBV integration is associated with gene dysregulation, affecting important pathways

TDF treatment tends to reduce the number of dysregulated genes more significantly than placebo

Conclusions

- Novel sequencing and analysis methods can precisely identify viral transcripts expressed from integrated viral DNA
- In CHB patients with serum ALT 1~2 folds ULN, expressed viral integrations can be detected in the majority of patients and are highly associated with viral load.
- **Integrations also affected host transcripts, resulting in significant gene dysregulation**
- **Reduction in viral load** with TDF treatment or as a part of natural history is associated with a **reduction in expressed viral integrations and dysregulated genes**
- These data suggest that patients with elevated HBV DNA, regardless of ALT level, may have significant levels of viral integrations impacting host gene transcription
- **Larger studies with longer follow-up required to quantify the impact on HCC incidence.**



What about the optimization of the current treatments?

Bruegel il Vecchio, 1563, La Torre di Babele

EASL | JOURNAL OF HEPATOLOGY

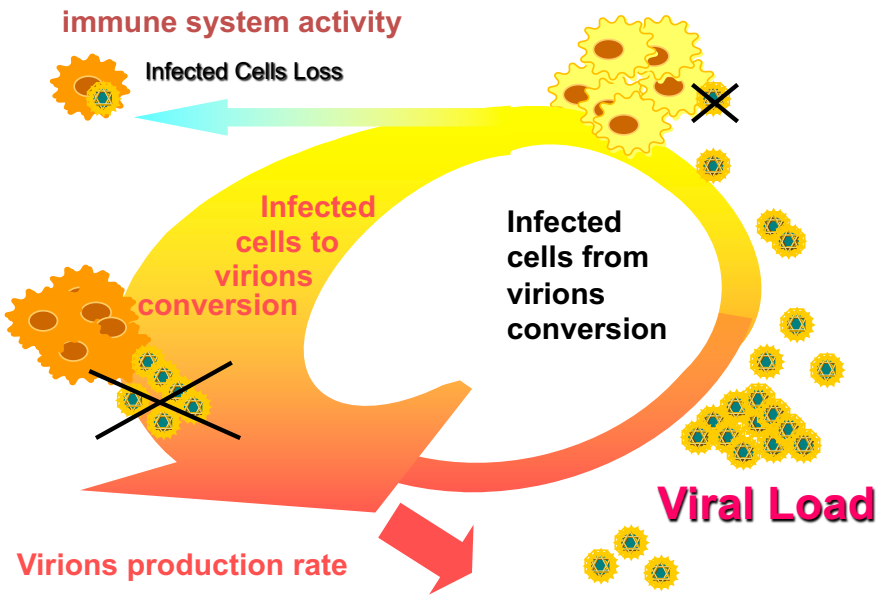
EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection☆

- NAs should be discontinued after confirmed HBsAg loss, with or without anti-HBs seroconversion (Evidence level II-2, grade of recommendation 1).
- NAs can be discontinued in non-cirrhotic HBeAg-positive CHB patients who achieve stable HBeAg seroconversion and undetectable HBV DNA and who complete at least 12 months of consolidation therapy. Close post-NA monitoring is warranted (Evidence level II-2, grade of recommendation 2).
- Discontinuation of NAs in selected non-cirrhotic HBeAg-negative patients who have achieved long-term (⩾3 years) virological suppression under NA (s) may be considered if close post-NA monitoring can be guaranteed (Evidence level II-2, grade of recommendation 2).

- According to the existing data, virological remission (HBV-DNA <2000-20000 IU/ml) will be maintained in approximately 50% of HBeAg negative patients 3 years after NA discontinuation, if thay have remained for more than 2 years on virological remission during therapy
- The optimal duration of on therapy virological remission before discontinuation remain unclear
- No reliable predictors of post-treatment remission has been identified to date

The achievement of a functional cure with NUCs is influenced by several factors

- The effectiveness of the antiviral
- The burden of the infection at the time of treatment start
- The modulation of both innate and adaptive immune response induced by the viral suppression

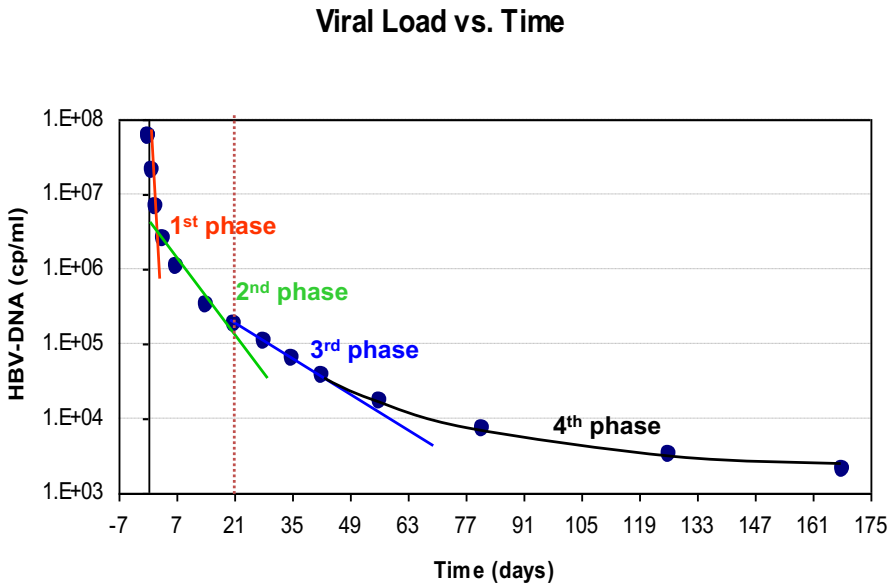


During NUCs treatment the burden of intrahepatic HBV infection declines progressively

but the decay is function of the direct antiviral effectiveness of the drug

The very early decline of viral load is due to the block of virus production → ε defines **fraction of viral production inhibited by the drug**

- **The value ranges between 0.81 to 0.99, but it is not 1.**
- **Therefore, during NUCs therapy the viral production is not completely blocked**



Sypsa et al Hepatology 2005, Colombatto P et al Ant Ther 2006

The achievement of a functional cure with NUCs is influenced by several factors

- The effectiveness of the antiviral
- The burden of the infection at the time of treatment start
- The modulation of both innate and adaptive immune response induced by the viral suppression

- We studied the **HBsAg serum levels kinetics during NUCs** treatment in a cohort of **92 HBeAg negative patients** to develop a diagnostic algorithm to identify patients who will achieve HBsAg serum levels < 100 IU/ml

- During a median treatment of 77,5 months (range, 30,0-204,6), in 20/90 (**22,2%**) patients **HBsAg decline < 100 IU/ml**

- We identify 2 kinetics of HBsAg:
  - **monophasic** in 62/92 (67,4%) pts, 8 (13%) with HBsAg decline <100 IU/ml
  - **biphasic** in 20/92 (21,7%) pts, 11 (55%) with HBsAg decline < 100 IU/ml

- The **Delta variation of HBsAg serum levels from BL to month 36** was independently associated with the decline of HBsAg < 100 IU/ml (OR 3,549, 95% CI 1,587-7,938, P= 0.002) and showed a diagnostic accuracy of 93.3% in the identification of the patients who will achieve it

P.Colombatto et al DILC 2020

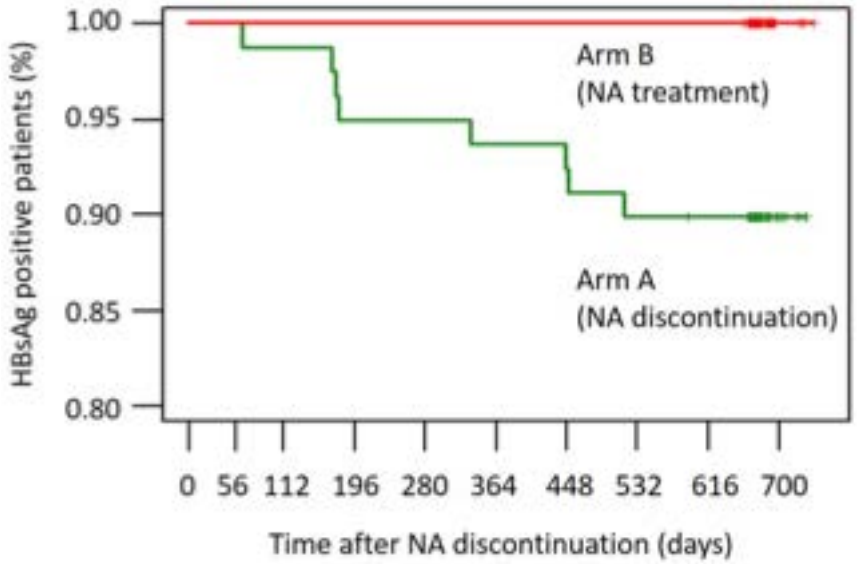
Response to discontinuation of long-term nucleos(t)ide analogue treatment in HBeAg-negative patients: Results of the Stop-NUC trial

- **166 HBeAg-negative patients without cirrhosis** with HBV-DNA suppression for ≥4 years were randomized to continue or stop treatment
- All patients were followed up for 96 weeks
- **Primary endpoint:** sustained HBsAg loss at Week 96; **secondary endpoints:** HBsAg seroconversion, virologic response (HBV-DNA ≤20 IU/ml), number of ALT flares, time to re-therapy in pts who discontinued NUC

At week 96 after NUC discontinuation:

- HBsAg loss in 8/79 (10%)
- HBsAg seroconversion in 6/79 (8%) patients
- no patients in NUC had HBsAg loss

p=0.006 and p=0.028, respectively



Predictive value of HBsAg levels at discontinuation

HBsAg loss	Baseline HBsAg <1,000 U/mL	Baseline HBsAg ≥1,000 U/mL	p value
No	18 (72%)	53 (98.1%)	0.001
Yes	7 (28%)	1 (1.9%)	

van Bömmel F, et al. DILC 2020; LBO06

- All patients with NUC discontinuation, but none of those who remained on treatment experienced an HBV DNA flare >20 IU/mL after NA discontinuation

Parameter at Week 96	ARM A	ARM B	p value
HBV DNA ≤20 IU/mL	24/79 (31%)	79/79 (100%)	<0.001
ALT flare	28/79 (35%)	0	–
NA re-installed	11/79 (14%)	N/A	–
No NA indication[†]	54/79 (68%)	N/A	–

- No patient in ARM A had a severe SAE possibly related to NA discontinuation

The STOP-NUC study demonstrates the potential of discontinuation of long-term NA treatment for inducing durable immune control and functional cure in patients with HBeAg-negative CHB

van Bömmel F, et al. DILC 2020; LBO06

Randomized Trial of 192 weeks of TDF with or without Peg-IFN for the first 24 weeks followed by protocolized withdrawal

LIVE

Study Design

281 screening eligible

201 enrolled and randomized

102 TDF

99 TDF+ Peg-IFN

Day 0 to Week 192 Treatment Phase

1:1 Random-ization

- TDF for 192 wks
- TDF for 192 wks + Peg-IFN α2a 24 wks

Stratified by:
- HBeAg status
- HBV genotype (A vs non-A)
- Cirrhosis

Week 192 to 240 Withdrawal Phase

96 Evaluated
51 Withdrawal

Withdrawal of TDF if eligible / Continue TDF if ineligible

- TDF retreatment for flares
- TDF to week 240

92 Evaluated
62 Withdrawal

Withdrawal Criteria
- **HBV DNA <1000 IU/mL** for previous 24 wks
- **Absence of cirrhosis**

Safety Amendment: (02/2017)
- **HBeAg negative** by wk 144

Safety Amendment: (10/2018)
- **Anti-HBe positive** by wk 180

Week 240 End of Follow-up

Outcomes

Primary: HBsAg loss

Secondary:
- HBeAg loss and seroconversion
- ALT <1.25 X ULN and normalization (M<30, F:<20 U/L)
- HBVDNA <20 IU/mL and <1000 IU/mL
- Frequency of ALT flares
- Frequency of adverse events (AE)/serious adverse events (SAE)

Intent to treat analysis

53% & 49% HBeAg positive

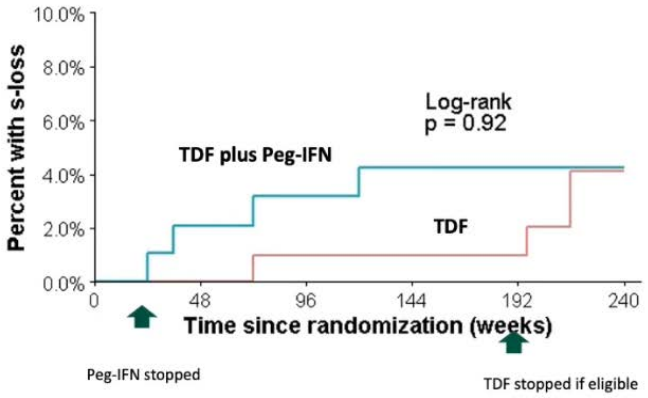
Randomized Trial of 192 weeks of TDF with or without Peg-IFN for the first 24 weeks followed by protocolized withdrawal

LIVE

Primary Outcome: HBsAg Loss

ITT Analysis	TDF N=102	TDF plus Peg-IFN N=99	P Value
Week 192 (EOT)	1 (1.0%)	4 (4.3%)	0.21
Week 240 (End of follow-up)	4 (4.5%)	5 (5.7%)	0.74

Cumulative incidence of HBsAg Loss by Treatment Group



Outcomes at week 240 by withdrawal status

TDF withdrawal	TDF n. 51	TDF +Peg-IFN n. 60	P Value
HBsAg loss	3 (6.5%)	4 (7.3%)	>0.99
HBV-DNA <1000 IU/ml + normal ALT	13 (30%)	20 (39%)	0.39
Ineligible to TDF withdrawal	**TDF n. 45**	**TDF+Peg-IFN n. 32**	**P Value**
HBsAg loss	1 (2.4%)	1 (3.2%)	<0.99
HBV-DNA <1000 IU/ml + normal ALT	26 (63%)	20 (67%)	0.8

- HBsAg loss was similar in TDF vs TDF+Peg-IFN, but with different timing
- HBeAg loss was not statistically different in the 2 groups (41% vs 61%, P=0.06)
- About 1/3 of patients who discontinued TDF withdrawal met criteria for HBeAg negative infection
- 24-31% of the pts had ALT flares (early on treatment with TDF+Peg-IFN; later in withdrawal phase with TDF)

- **Withdrawal of TDF after 4 years of therapy can be safely achieved in most patients meeting protocolized criteria**
- **HBsAg loss was not enhanced by Peg-IFN for 24 weeks, longer follow is desirable to capture late effects of Peg-IFN and TDF withdrawal**

Terrault N, AASLD 2020, 19

Currently, it is possible to personalize the management of chronic HBsAg carriers, provided that the relevant scientific evidences are applied with the guide of the individual clinical expertise according to the specific need of each patient

Bruegel il Vecchio, La parabola dei ciechi,1568

Sackett D et al. Evidence-Based Medicine: How to Practice and Teach EBM, 2000