Clearance of serum HBsAg by nucleic acid polymers suggests a critical role for HBsAg loss in establishing functional control of HBV and HDV infection

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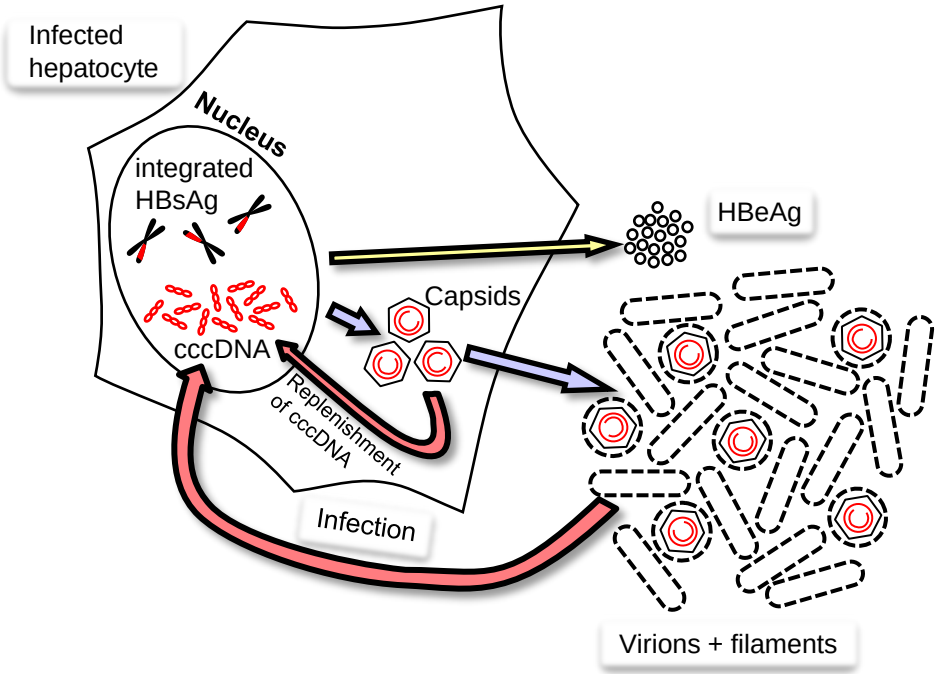
Satellite Symposium
2017 International HBV meeting

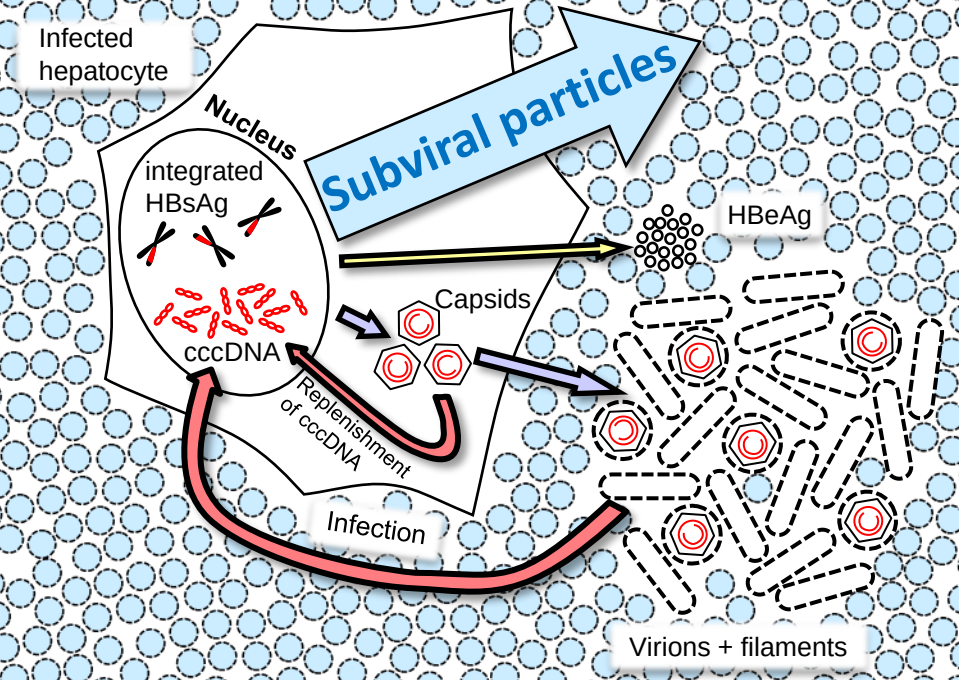
Particle production in HBV

CLASSIC TARGETS FOR HBV:
Virion production (capids and HBV-RT) and cccDNA



Particle production in HBV

THE HBsAg PROBLEM:
almost all HBsAg is derived from subviral particles



HBsAg is an immunosupressor:

- Masks anti-HBs response
- Blocks signalling mechanisms in innate and adaptive immunity
- Blocks the effect of immunotherapies
- **HBsAg clearance is critical to achieving functional cure**

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The HBsAg challenge

HBsAg is the most abundant circulating viral protein in HBV infection

SVPs are produced from hepatocytes:

with active HBV replication
and
HBV DNA integration
(even in the absence of active cccDNA)

Direct targeting of HBsAg synthesis or SVP assembly / secretion will be critical to achieve high rates of functional control that persists after the end of therapy.

What is the contribution by integrated HBV DNA to SVP production?

Gauging the HBV DNA integration problem

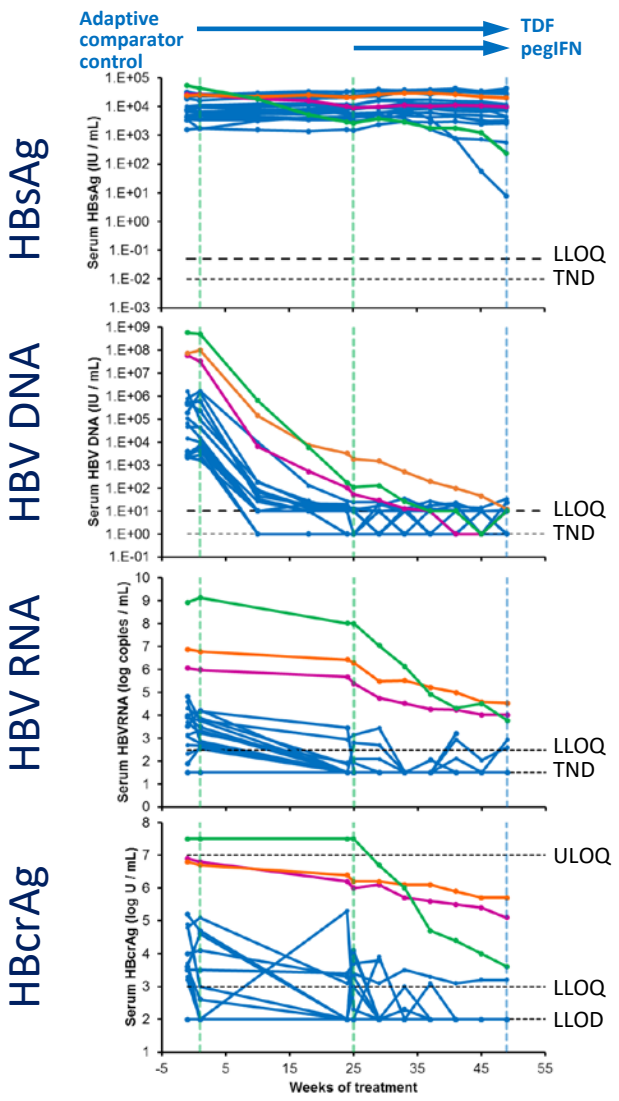
Serum HBV RNA and HBcrAg are novel markers reflecting the activity of intrahepatic cccDNA

Analysis of HBsAg, HBV RNA and HBcrAg during TDF + pegIFN in the REP 401 protocol [NCT02565719]

- During TDF and peg-IFN:
  - HBV RNA TND in 14/20 patients
  - HBcrAg < LLOD in 15/20 patients
  - serum HBsAg reductions > 1 log in 3/20 patients

- weak HBsAg response even in patients with continuous declines from high pre-treatment HBV RNA and HBcrAg (green, pink and orange lines)

Bulk of HBsAg in HBeAg negative patients may be derived from integration



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Restoring functional control of HBV infection

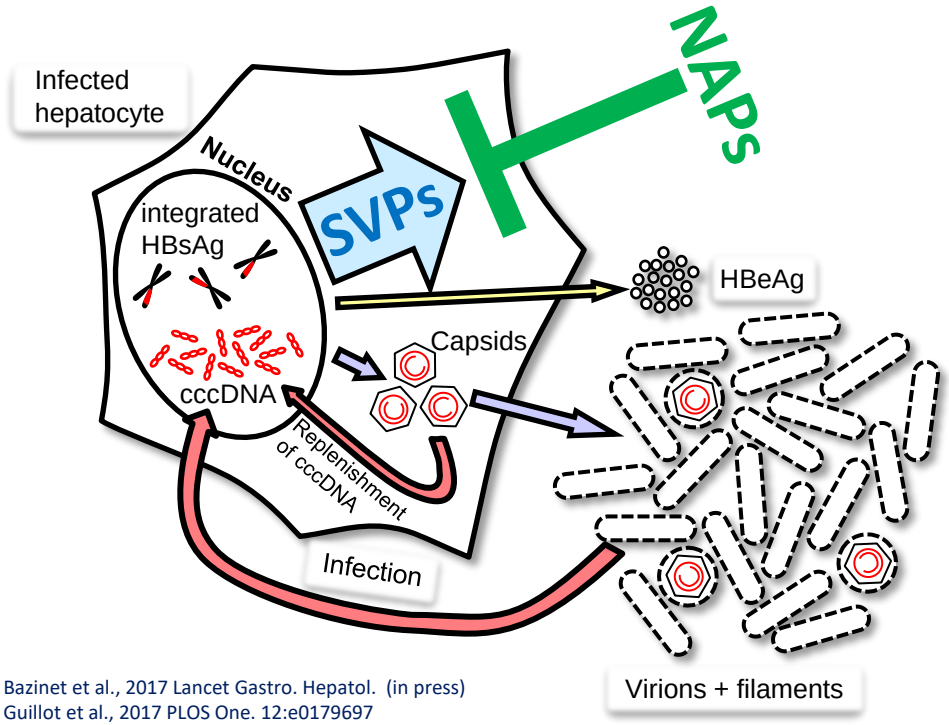
How much HBsAg reduction is required for clinical benefit?

- 1 log HBsAg reduction is common with pegIFN and does not predict off-treatment functional control
- Early multilog reduction and HBsAg loss are rare but predict functional control

Can the clinical database from NAP trials provide clues?

Nucleic Acid Polymers (NAPs)

NAPs block subviral particle release (cccDNA and integration derived) → Efficient HBsAg clearance from the blood



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Critical features of NAPs

- **Target the assembly and or secretion of SVPs**
  - Host factors are targeted
  - Intracellular HBsAg not increased
  - Secretion of virions and HBeAg not affected
- **Establish functional control of hepadnaviral infection *in vivo***
  - Elimination of serum surface antigen
  - Liver replication decreases during NAP monotherapy *in vivo*
  - Clearance of surface and core antigens and control of viral replication in the liver (cccDNA) persists after NAP treatment withdrawal *in vivo*.
- **High potent, validated clinical effect**
  - **Up to 7 log reduction of serum HBsAg**
  - **HBsAg ≤ 0.01 IU/mL achieved in majority of patients during therapy**
  - Effect is not derived from immunostimulation, assay interference, or evolution of immune escape HBsAg.

HBsAg clearance in HBeAg+ CHB

REP 101 and 102 protocols [NCT02646163 / NCT02646189]

20 HBeAg+ patients with documented chronic infection

NAP monotherapy (REP 2055 or REP 2139):

- Serum HBsAg > 1 log reduction in 18 patients, 2-7 log reduction in 15 patients
  - HBsAg < 1 IU/mL in 10 patients
  - HBsAg, < 0.01 IU/ml in 8 patients
- Seroconversion of HBeAg in 14 patients
- Appearance of free anti-HBs > 10mIU/mL (typically 10-50mIU/mL) in 10 patients
- multilog (2-12) log reduction of HBV DNA in 15 patients
- With REP 2055, strong therapeutic transaminase flares and functional control* of infection only occurred in patients achieving HBsAg <1IU/mL

Immunotherapy (12 weeks thymosin α1 or pegIFNα2a) was added to REP 2139:

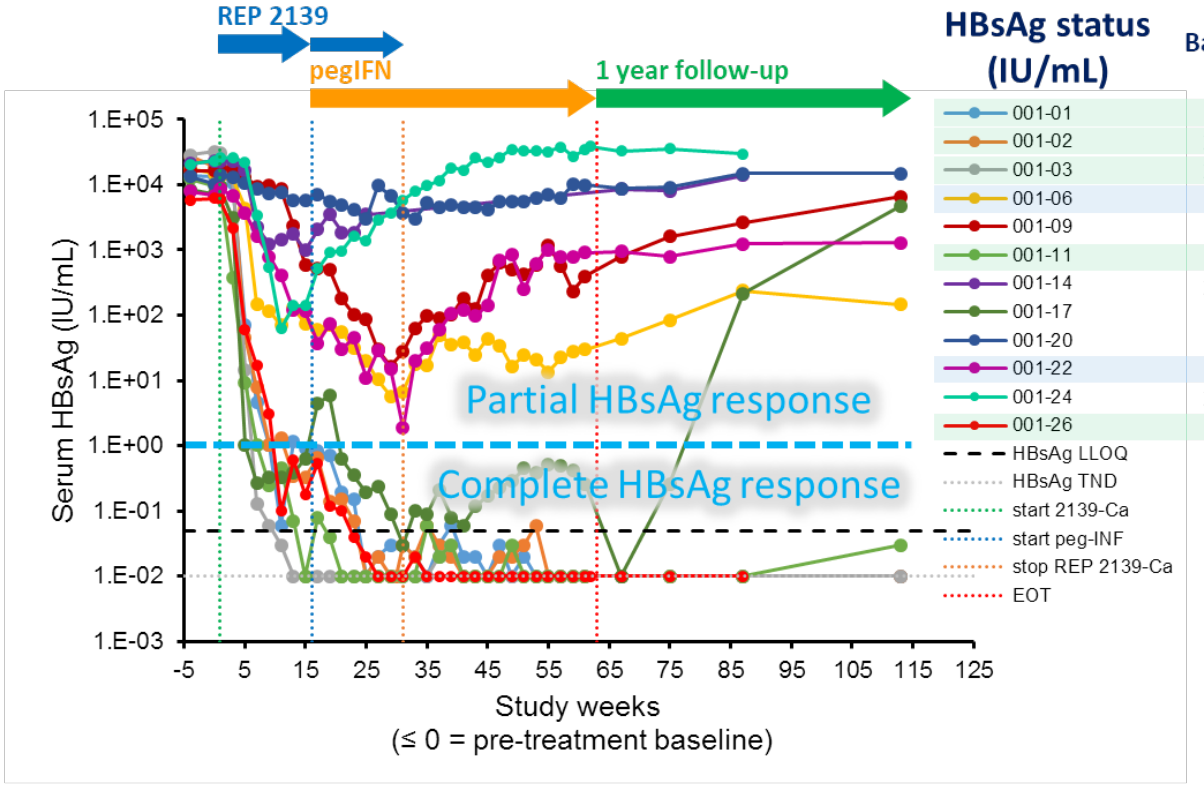
- Restricted to 9 patients with HBsAg <0.01 -180.44 IU/mL at start of immunotherapy (2.45 – 7.09 log reduction from baseline)
- HBsAg became <0.01-0.03 IU/ml in 9/9 patients
- Rapid increase in production of anti-HBs (242-1302 IU/ml) in 9/9 patients
- HBV DNA became LLOQ-2400 copies/mL in 9/9 patients
- Functional control* established in 8/9 patients after therapy (4/9 persisting to 2 years)

Al-Mahtab et al., 2016 PLOS One 11: e0156667

***HBsAg < 1IU/mL, HBV DNA < 1000 copies/mL**

HBsAg clearance in HBV / HDV co-infection

REP 301 protocol [NCT02233075], 12 HBeAg- patients with confirmed chronic HDV coinfection



HBsAg status (IU/mL)	Baseline	24W follow-up	1 year follow-up
001-01	13998	0.00	0.00
001-02	27264	0.00	0.01
001-03	28261	0.00	0.01
001-06	17511	239	146
001-09	16426	2646	6621
001-11	12382	0.00	0.03
001-14	20869	13896	*
001-17	8314	214	4754
001-20	13430	14943	14917
001-22	7836	1225	1281
001-24	20473	29724	pending
001-26	5854	0.00	0.00

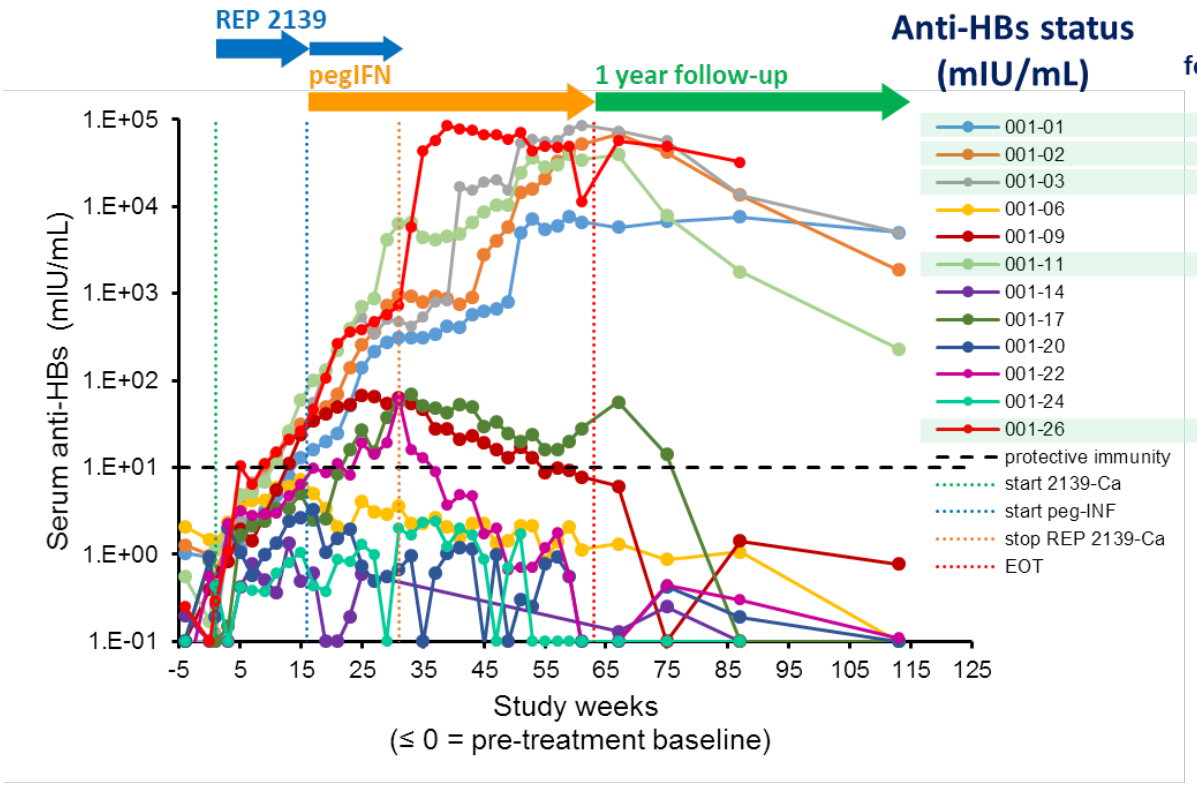
5/12 patients with HBsAg control at 24W – 1 year follow-up.

An additional 2 patients have established a new HBsAg baseline

LLOQ = lower limit of quantification, TND = target not detected (0.00 IU/mL), EOT = end of treatment, * not enrolled in REP 301-LTF

Bazinet et al., 2017 Lancet Gastro. Hepatol. (in press).

HBsAg clearance in HBV / HDV co-infection

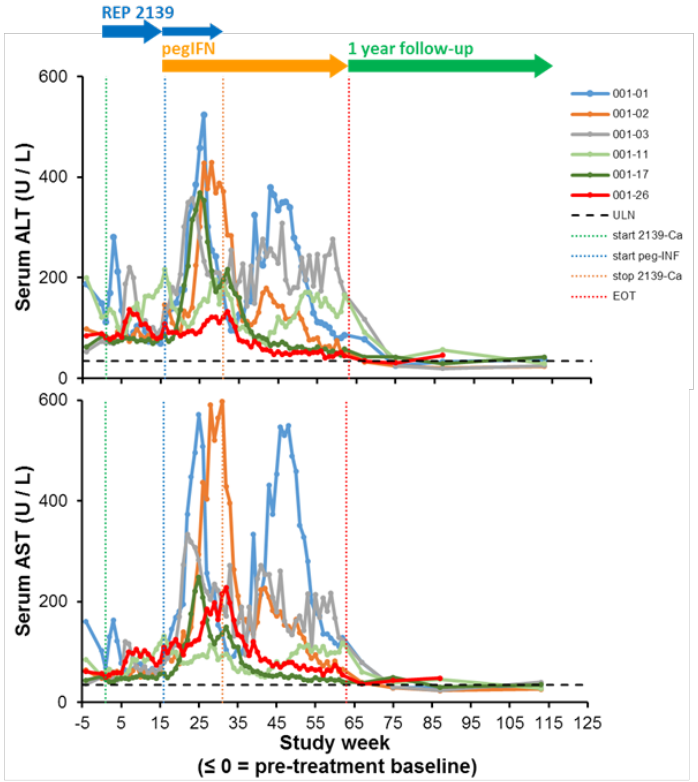


Anti-HBs status (mIU/mL)	1 year follow-up	HBsAg < 1 IU/mL before pegIFN	HBsAg 1 year follow-up
001-01	**5073**	←	**0.00**
001-02	**1873**	←	**0.01**
001-03	**5079**	←	**0.01**
001-06	< 0.1		146
001-09	0.78		6621
001-11	**231**	←	**0.03**
001-14	*		*
001-17	< 0.1	←	4754
001-20	< 0.1		14917
001-22	0.11		1281
001-24	(< 0.1)		(29724)
001-26	**30018**	←	**(0.00)**

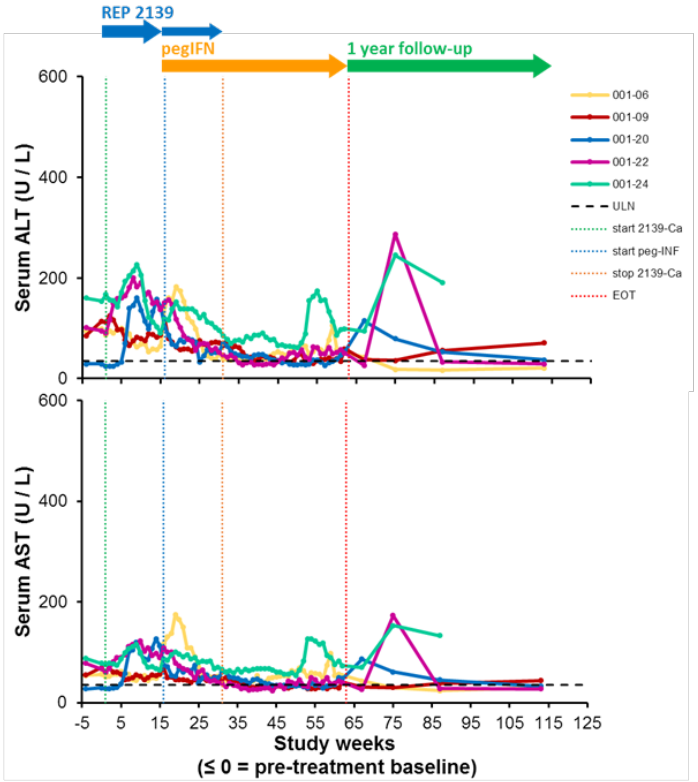
Maintenance of anti-HBs titers at 1 year follow-up is correlated with serum HBsAg < 1 IU / at the start of peg-INF therapy

HBsAg clearance in HBV / HDV co-infection

HBsAg < 1IU/mL prior to pegIFN



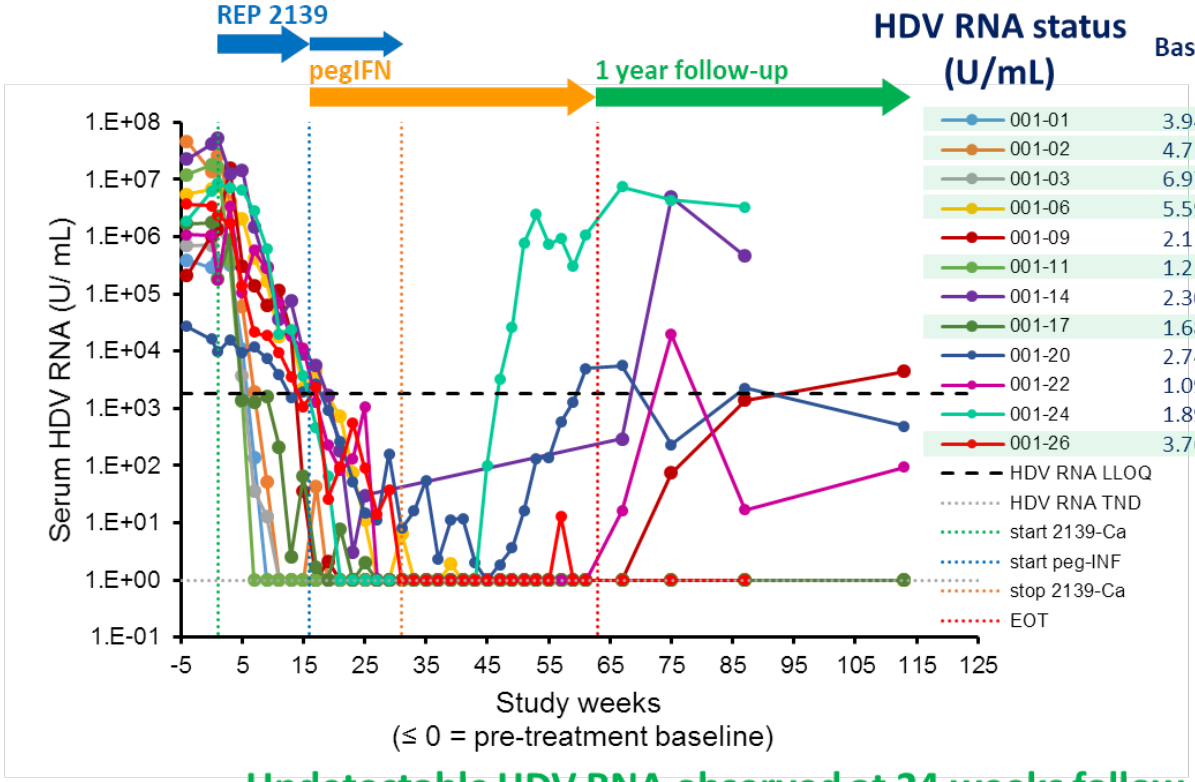
HBsAg > 1IU/mL prior to pegIFN



Transaminase elevations are asymptomatic

Serum transaminases normalize in 8/12 patients during follow-up

HBsAg clearance in HBV / HDV co-infection



HDV RNA status (U/mL)	Baseline	EOT	24 week follow-up	1 year follow-up
001-01	$3.94x10^4$	TND	TND	TND
001-02	$4.71x10^7$	TND	TND	TND
001-03	$6.97x10^5$	TND	TND	TND
001-06	$5.59x10^6$	TND	TND	TND
001-09	$2.11x10^4$	TND	1390	4438
001-11	$1.21x10^7$	TND	TND	TND
001-14	$2.30x10^7$	*	$4.65x10^5$	*
001-17	$1.68x10^6$	TND	TND	TND
001-20	$2.74x10^4$	4920	2250	485
001-22	$1.09x10^6$	TND	17	94
001-24	$1.89x10^6$	$1.09x10^6$	$3.28x10^6$	pending
001-26	$3.76x10^6$	TND	TND	TND

Undetectable HDV RNA observed at 24 weeks follow-up in 7/12 patients is stable at 1 year follow-up

EOT = end of treatment, * early entry into REP 301 follow-up - not enrolled in REP 301-LTF, TND = target not detected

NAPs target S and L forms of HDAg and may inhibit ribozyme activity and RNP assembly

Shamur et al. HBV Int 2017 meeting poster P-145

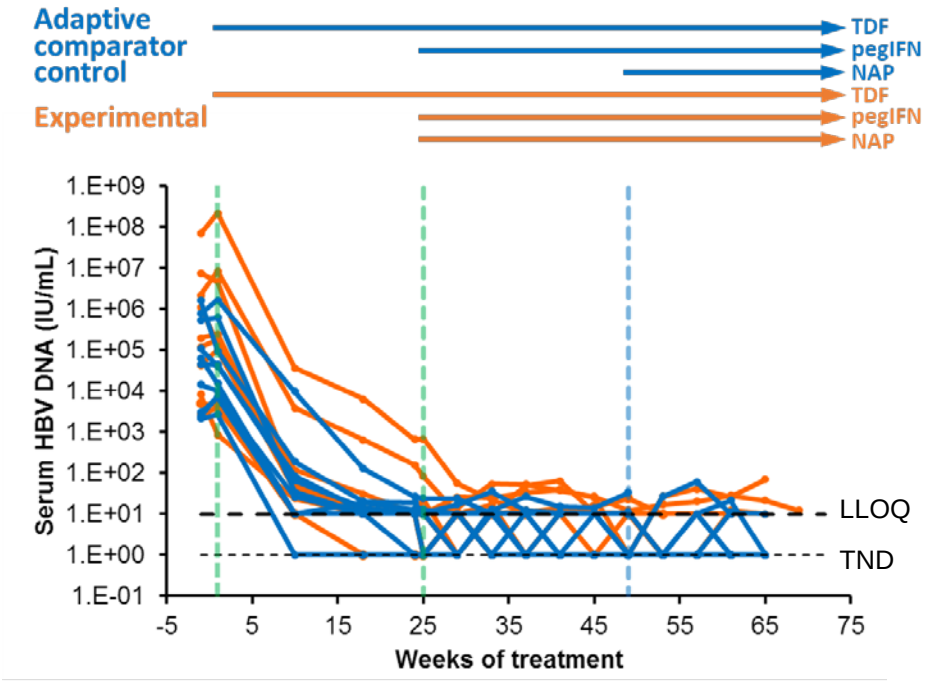
HBsAg clearance in HBeAg- CHB

REP 401 protocol [NCT02565719]

20 HBeAg+ patients (treatment naïve)

(REP 2139 results from EASL 2017)

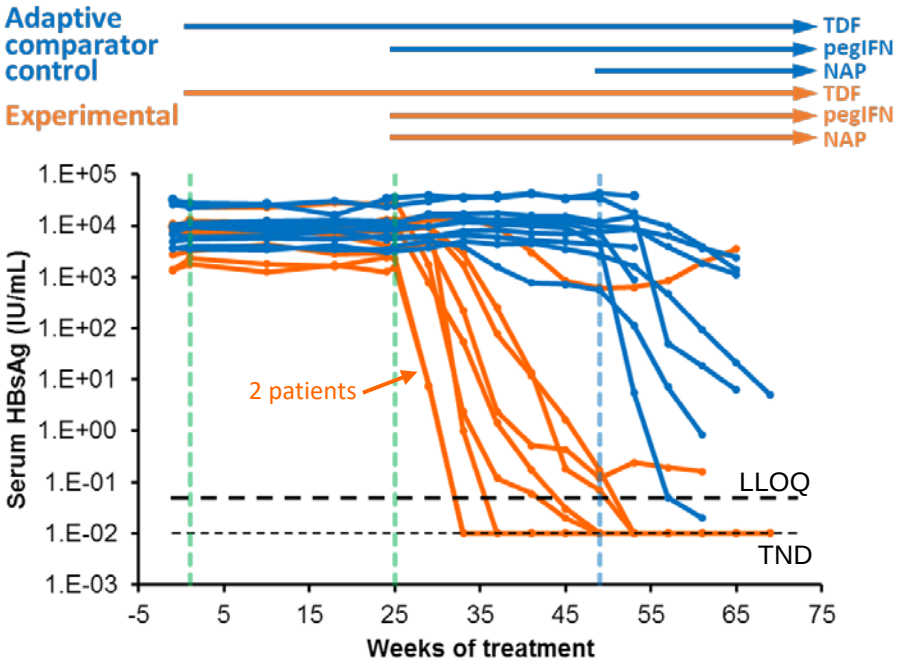
HBsAg clearance in HBeAg- CHB



TDF effect unaltered in triple combination with pegIFN and NAPs

LLOQ = lower limit of quantification (10 IU / ml)
TND = HBV DNA target not detected

HBsAg clearance in HBeAg- CHB

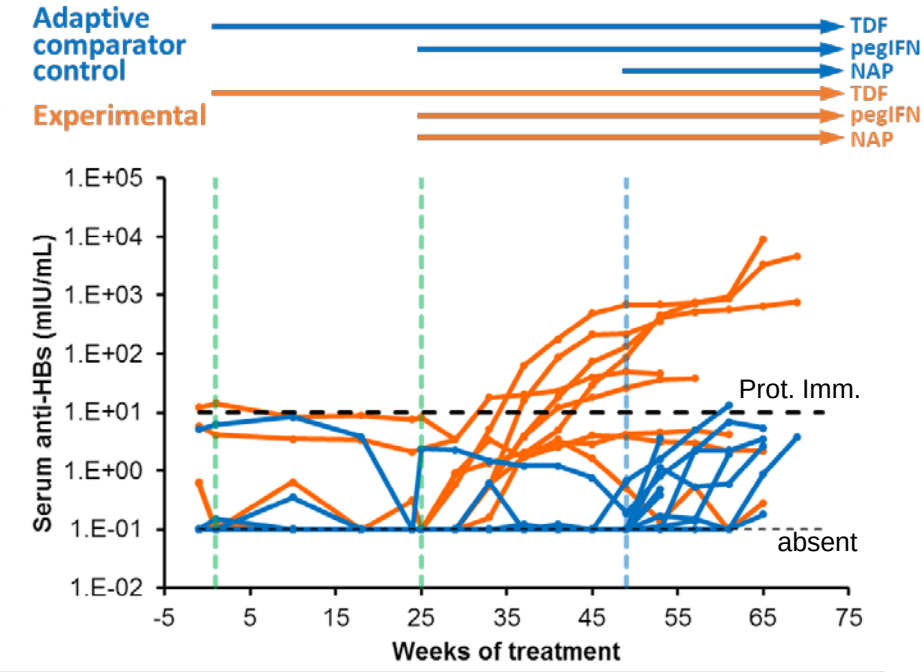


HBsAg response > 4 log: 9/10 2/7

HBsAg loss (≤0.01 IU/mL): 8/10 0/7

LLOQ = lower limit of quantification (0.05 IU / mL)
TND = HBsAg not detected (0.00 IU / mL)

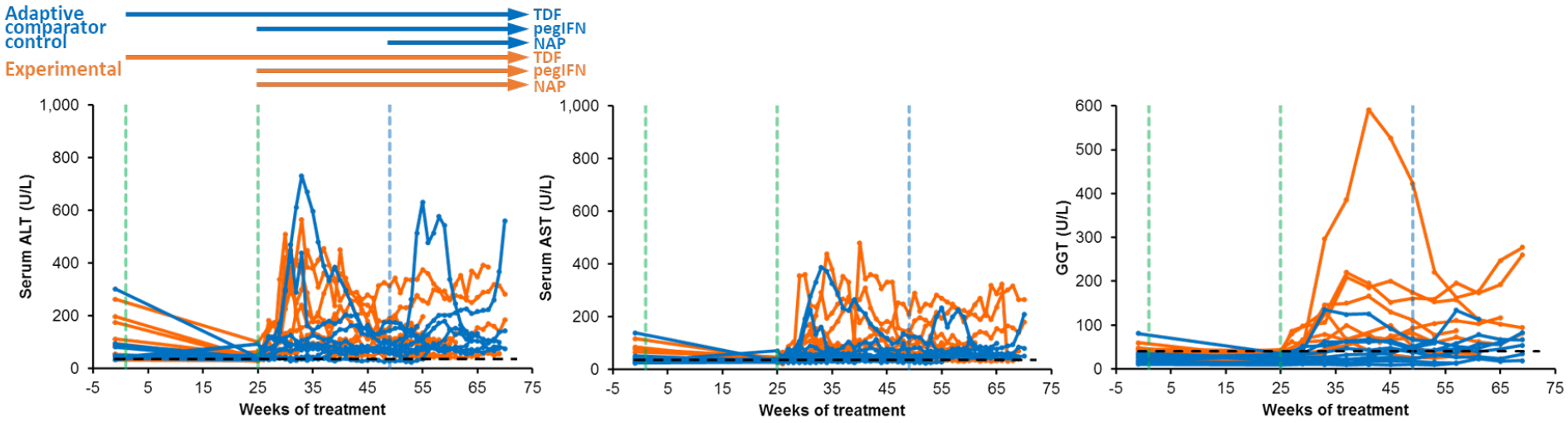
HBsAg clearance in HBeAg- CHB



Peg-IFN mediated elevation in serum anti-HBs restricted to patients with HBsAg < 1IU/mL

Prot. Imm. = threshold for protective immunity (10 mIU / mL)
absent = no significant anti-HBs present (≤ 0.1 mIU / mL)

HBsAg clearance in HBeAg- CHB

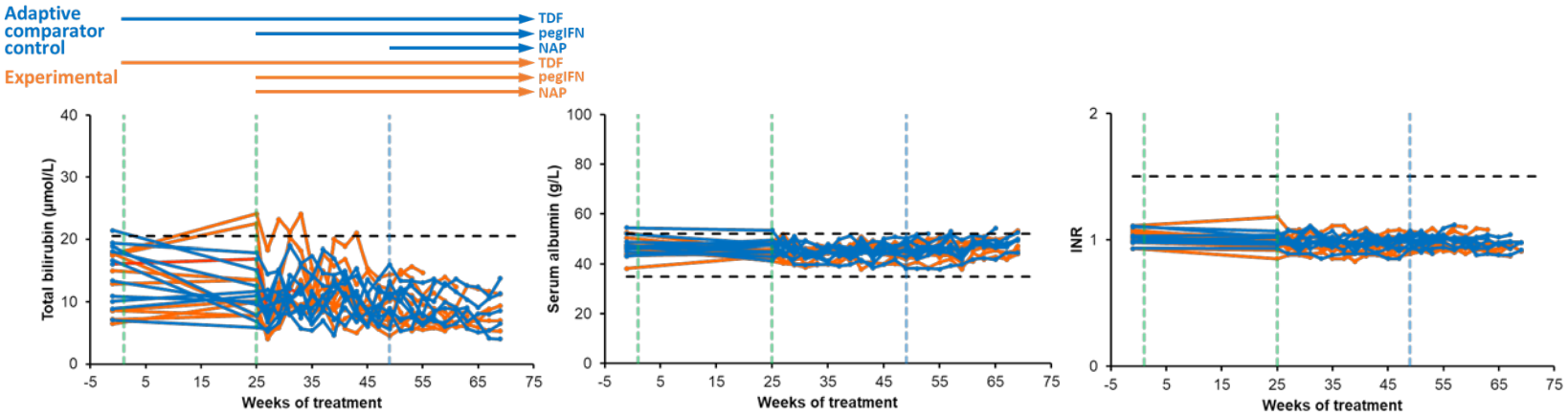


ALT (U/L) **AST (U/L)** **GGT (U/L)**

Peg-IFN mediated transaminase elevations more frequent and stronger in patients with HBsAg < 1IU/mL

- - - - - - - - upper limit of normal

Interim REP 401 Safety Data (liver function)



Bilirubin (µmol / L) **Albumin (g / L)** **INR**

Liver function normal during transaminase flares

- - - - - - - - upper limit of normal / normal range

Summary

SVP-derived HBsAg inhibits the immune response to HBV infection

- maintains chronic HBV infection
- blocks activity of immunotherapeutic agents

SVPs may be derived mainly from integrated HBV DNA in HBeAg negative patients

Achieving HBsAg <1IU/mL is reliably achieved with REP 2139

- SVP assembly/ secretion derived from cccDNA or integrated HBV DNA is inhibited

HBsAg clearance to levels <1IU/mL may be required for clinical benefit

- With HBsAg as low as 6 IU/mL, response to immunotherapy is absent

- With HBsAg <1IU/mL, response to immunotherapy is universally potentiated
  - Increased anti-HBs production
  - Strong, therapeutic transaminase flares
  - **Increased incidence of functional control persisting after the end of therapy**