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HBV Forum 2
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Hilton Amsterdam

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Mapping and Modulation

Doherty Institute
A joint venture between the University of Melbourne and Melbourne Health

GILEAD

Abbott
A Promise for Life

VIDRL
Victorian Infectious Diseases Reference Laboratory

Epitope Mapping the HBsAg to Identify Biomarkers Associated with HBsAg Loss /seroconversion

Renae Walsh, Rachel Hammond, Lilly Yuen, Tom Leary, Anuj Gaggar, Mani Subramanian, Kathryn Kitrinos, Alexander Thompson, Stephen Locarnini

Functional CHB Cure

Seroclearance of HBsAg and seroconversion to anti-HBs antibody

Cure markers off-treatment

no HBV cccDNA
no HBV RC/DSL DNA
HBcAg staining negative
± HBsAg (occasional)

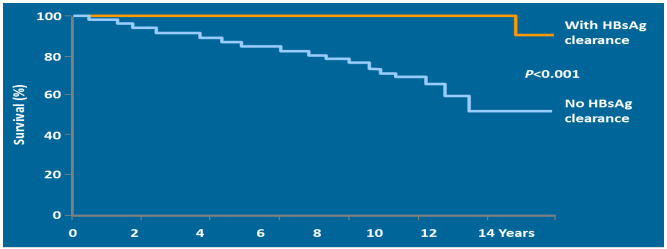
HBV DNA/HBsAg negative
anti-HBs positive

- Antiviral therapy could be stopped with a minimal risk of viral reactivation
- Is linked to improved clinical outcome if achieved before 50 years of age (significantly reduced HCC risk)
- Is achieved rarely (1-2% per annum) in the natural history of chronic hepatitis B
- Current antiviral therapies supress HBV DNA, but are not curative; HBsAg clearance is a major goal for new CHB therapies

There is a need for diagnostic biomarkers predictive of HBsAg clearance and anti-HBs development

HBsAg Clearance & Functional Cure

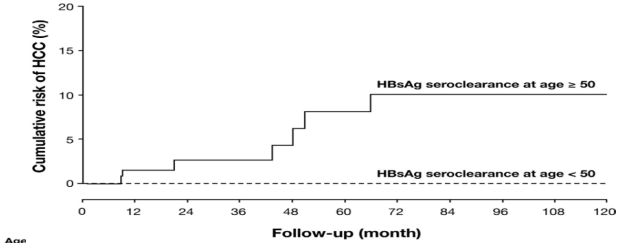
HBsAg clearance improves survival rates and reduces risk of HCC



Retrospective study of 309 cirrhotics, mean follow-up of 5.7 years

Fattovich et al. Am J Gastroenterol 1998

Cumulative risk of HCC



Need to achieve clearance < age 50 to reduce HCC risk

Yuen M-F, et al. Gastroenterology 2008; 135:1192

There is no biomarker to predict HBsAg loss/seroconversion

HBsAg Clearance & Functional Cure

Rate of spontaneous HBsAg loss/seroconversion ~2%

Rate of HBsAg loss/seroconversion on NA therapy (HBeAg+) at 1 year:

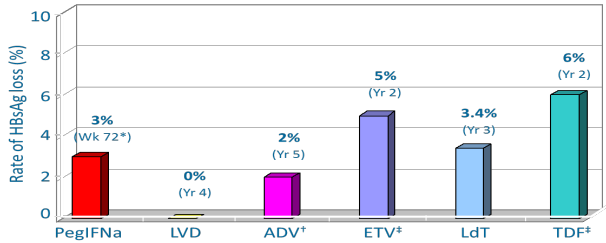
- telbivudine 0%
- adefovir 0%
- lamivudine 1%
- entecarvir 2%
- tenofovir 3%

EASL Clinical Practice Guidelines Panel. J Hepatol. 2009;50:227-42

Median number of years to clear HBsAg on NA therapy **52.2 years**

Chevaliez, et al. J Hepatol (2013) 58:676-683

Rate of HBsAg loss/seroconversion on NA therapy (HBeAg+) at 2 years:



Lok ASF & McMahon BJ. Hepatology 2007;45:507–539. Lau GKK et al. N Engl J Med 2005;352:2682–2695. Chang T-T et al. J Gastroenterol Hepatol 2004;19:1276–1282. Marcellin P et al. Hepatology 2008;48:750–758. Gish RG et al. Gastroenterology 2007;133:1437–1444. Gane E et al. Presented at: 59th Annual Meeting of the American Association for the Study of Liver Diseases, San Francisco, USA, 31 October–4 November 2008; Poster 942. Heathcote E et al. Presented at: 59th Annual Meeting of the American Association for the Study of Liver Diseases, San Francisco, USA, 31 October–4 November 2008; Abstract 158.

There is no biomarker to predict HBsAg loss/seroconversion

HBsAg Clearance & Functional Cure

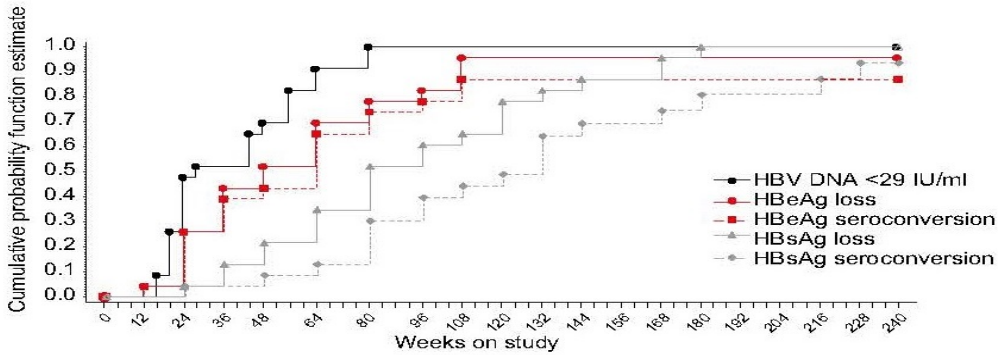
Research Article

EASL JOURNAL OF HEPATOLOGY

Kinetics of hepatitis B surface antigen loss in patients with HBeAg-positive chronic hepatitis B treated with tenofovir disoproxil fumarate

Patrick Marcellin[1,*], Maria Buti[2], Zahari Krastev[3], Robert A. de Man[4], Stefan Zeuzem[5], Lillian Lou[6], Anuj Gaggar[6], John F. Flaherty[6], Benedetta Massetto[6], Lanjia Lin[6], Phillip Dinh[6], G. Mani Subramanian[6], John G. McHutchison[6], Robert Flisiak[7], Selim Gurel[8], Geoffrey M. Dusheiko[9], E. Jenny Heathcote[10]

2014. Vol. 61. Pages 1228-1237



HBV DNA <29 IU/ml	N = 23	23	17	11	8	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0
HBeAg loss	N = 23	23	22	17	13	11	7	5	4	1	0	0	0	0	0	0	0	0	0	0
HBeAg seroconversion	N = 23	23	22	17	14	13	8	6	5	3	2	2	2	2	2	2	2	2	2	2
HBsAg loss	N = 23	23	23	22	20	18	15	11	9	8	5	4	3	3	1	0	0	0	0	0
HBsAg seroconversion	N = 23	23	23	22	22	21	20	15	13	12	10	7	6	6	4	3	3	3	2	1

Study Cohort

Analysis of HBsAg and anti-HBs responses in functional CHB cure patients :

GS-US-174-0103 (G103) study cohort (n=142 patients)

- Phase 3 clinical trial of tenofovir disoproxil fumarate (TDF) monotherapy

A subset of 25 genotype A patients were analysed to evaluate the predictive value of HBsAg CP for HBsAg loss:

HBsAg Loss/seroconversion (SL) ***n=14*** patients on-TDF by W192

HBsAg Non-Responder (NR) ***n=11*** patients on-TDF with <0.5 $\log_{10}$ HBsAg decline by W192

Hypothesis

- Immune pressure applied to the epitope occupancy of HBsAg due to a recovering anti-HBs response during TDF antiviral therapy influences the HBsAg epitope profile.
- This influences the development of a CP (versus NCP) predictive of HBsAg loss on-therapy

Study Aim:

To map HBsAg epitope profiles (multiplex immunoassay) to assess the predictive value of an HBsAg CP for HBsAg loss (SL) among GT A CHB patients on TDF

HBsAg Loss/seroconversion (SL) ***n=14*** GT A patients on-TDF by W192

HBsAg Non-Responder (NR) ***n=11*** GT A patients on-TDF with <0.5 $\log_{10}$ HBsAg decline

HBsAg Clearance Profile (CP): reduced anti-HBs epitope availability/recognition at BOTH Loop1 AND Loop2 HBsAg epitopes

HBsAg Non-Clearance Profile (NCP): unchanged anti-HBs epitope binding, or loss of availability/recognition at one epitope loop only

Methods

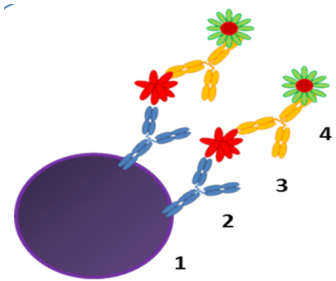
HBsAg epitope mapping 19plex immunoassay to identify an HBsAg Clearance Profile (CP)

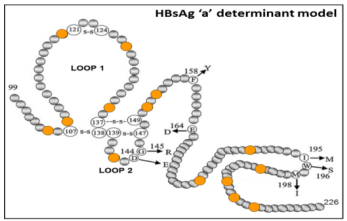
HBsAg profile reported based on epitope display (antigen conformation) and occupancy ('native' anti-HBs recovery), as fold change (95% CI: +/- 0.5 fold) in comparison to the pre-treatment profile

ASSAY

HBsAg Epitope Mapping

19 mAbs





[Adapted from Torresi et al., Virology. 2002. 293:305-313]

1. Magnetic bead
2. Capture Ab: 19plex mouse anti-HBs mAbs to HBsAg 'a' determinant
3. Patient HBsAg sample
4. Reporter Ab: PE conjugated polyclonal anti-HBs

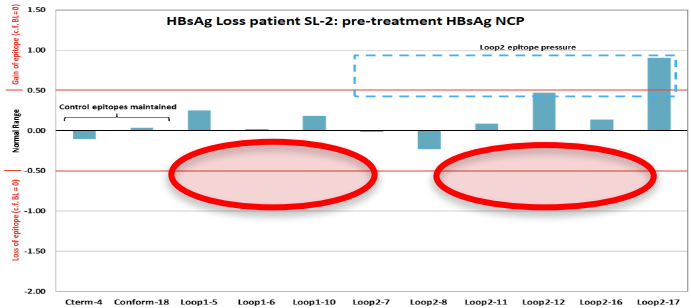
HBsAg clearance profile (CP)

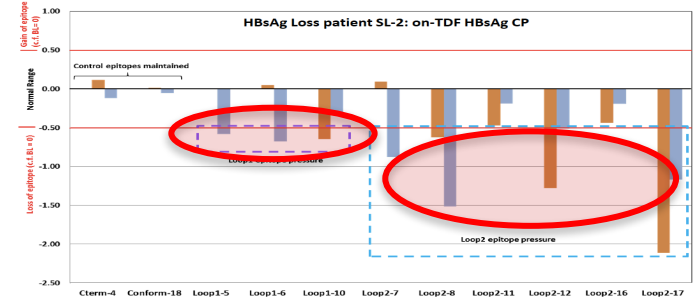
- HBsAg epitope pressure (reduced recognition) at *both* loop 1 **AND** loop 2 epitopes

HBsAg non-clearance profile (NCP)

- No change in HBsAg epitope profile, OR reduced epitope binding at *only* one loop

HBsAg CP development





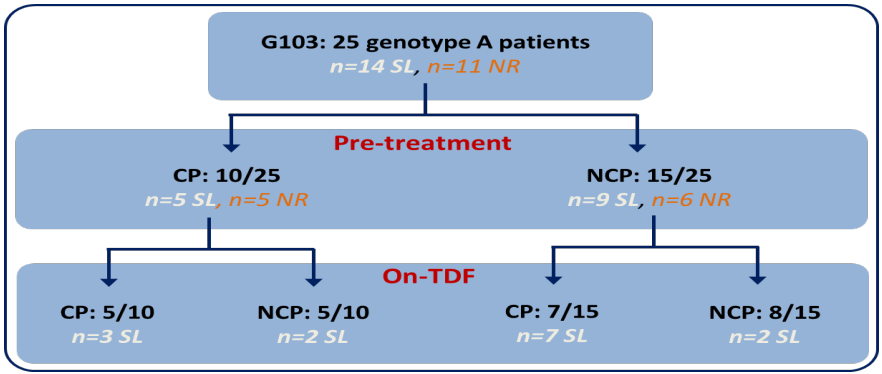
HBsAg NCP pre-treatment / study

During study patient develops

HBsAg CP prior to HBsAg loss/seroconversion

HBsAg CP: HBsAg epitope pressure (reduced recognition) at *both* loop 1 **AND** loop 2 epitopes

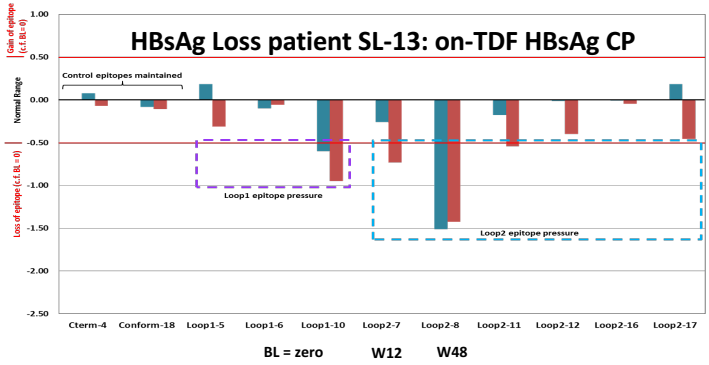
Results (to W48)

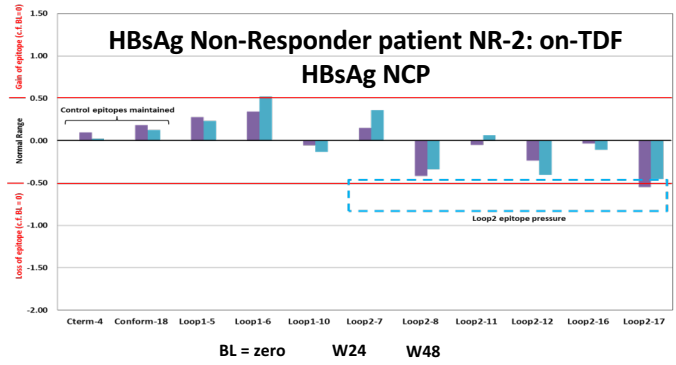


- 7/15 patients with an NCP pre-treatment developed a CP on-TDF, all 7 then achieved SL
- 8/15 patients with a pre-treatment NCP maintained an NCP, only 2 attained SL
- 5/10 patients with a pre-treatment CP maintained a CP on-TDF, and 3 developed SL
- 5/10 patients with a pre-treatment CP switched to an NCP by week 48, only 2 had SL

Results (to W48)

- During 48 weeks of TDF therapy, analysis of HBsAg response and HBsAg profile identified:
  - 12/25 patients with an HBsAg CP patients, of which 10 achieved HBsAg SL
  - 13/25 patients had an NCP, and only 4 achieved HBsAg SL
- There is a significant association (p-value 0.02) between the development, maintenance or enhancement of a HBsAg CP and an outcome of HBsAg loss/seroconversion (PPV 83%).

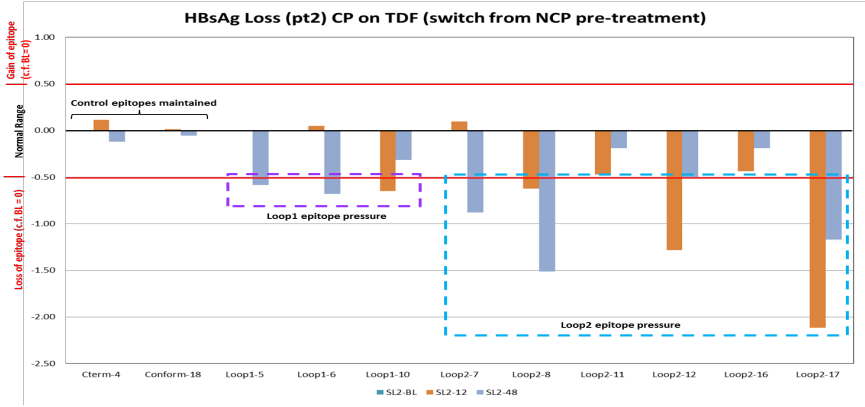


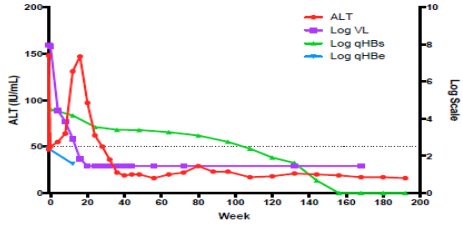


On-TDF HBsAg CP in SL patient

Typical on-treatment CP (CP developed from pre-existing NCP at BL)for HBsAg loss/seroconversion

[normalised to patient's baseline profile]





Pre-TDF baseline

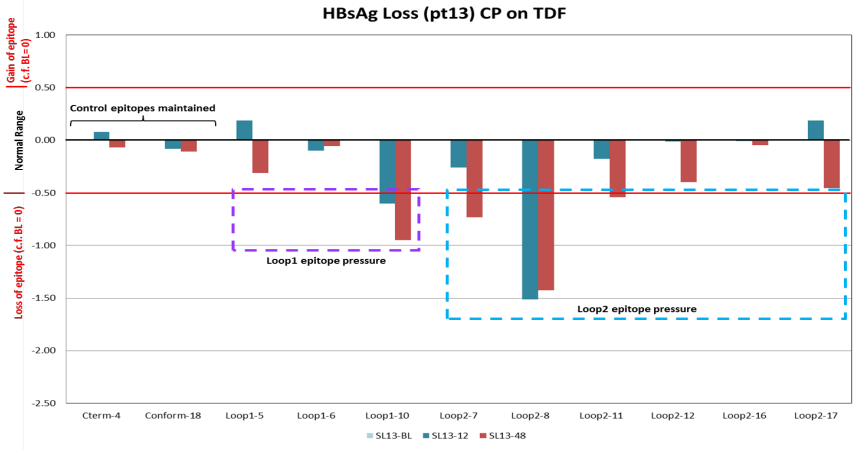
HBsAg loss/serconversion patient with HBsAg CP on-TDF:

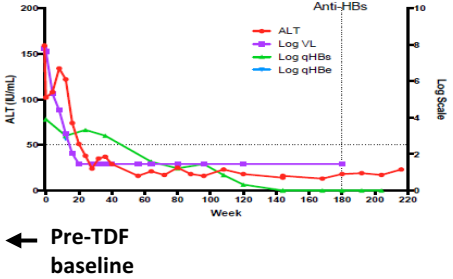
- Switched to CP from pre-treatment NCP
- CP by wk12 [blue] &maintained at wk48 [orange]
- HBsAg loss by wk180

On-TDF HBsAg CP in SL patient

Typical on-treatment CP (CP maintained from pre-existing CP at BL)for HBsAg loss/seroconversion

[normalised to patient's baseline profile]





← **Pre-TDF baseline**

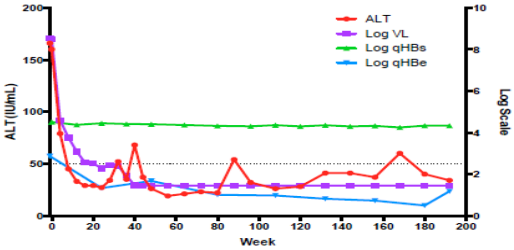
HBsAg loss/serconversion patient with HBsAg CP on-TDF:

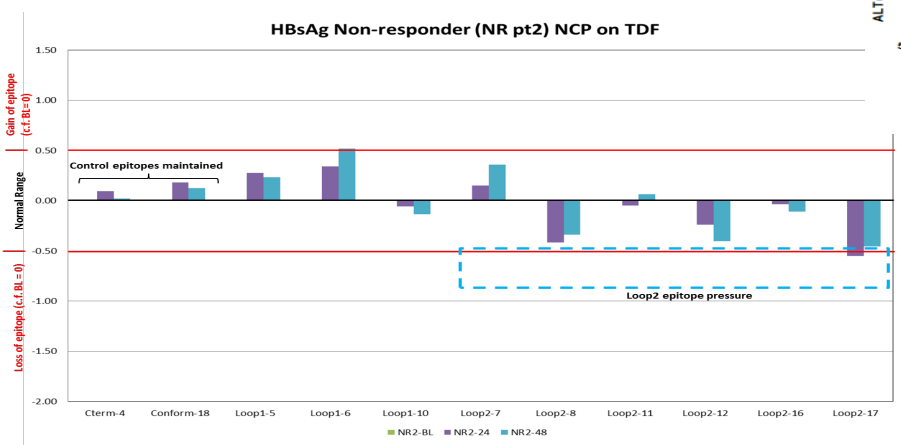
- CP maintained from pre-treatment at wk12 [blue] & wk48 [red]
- HBsAg loss by wk168

On-TDF HBsAg NCP in NR patient

Typical on-treatment NCP (NCP maintained from pre-existing NCP at BL)for HBsAg loss/seroconversion

[normalised to patient's baseline profile]





Pre-TDF baseline

HBsAg non-responder patient with HBsAg NCP on-TDF:

- NCP maintained from pre-treatment at wk24 [purple] & wk48 [blue]

Summary (to W48)

- HBsAg clearance is likely driven by the selective pressure of an effective anti-HBs response.
- HBsAg epitope occupancy influences the HBsAg profile, and mapping on-treatment changes of the HBsAg profile revealed that recovery of the anti-HBs response was promoted on-TDF therapy.
- There is a significant association between an HBsAg CP and HBsAg SL in GT A patients on-TDF therapy in the G103 cohort
- The HBsAg CP may be a predictive biomarker for HBsAg response/SL , potentially associated with an

p-value 0.02 **Positive predictive value 83%** **Negative predictive value 69%**

- HBsAg NCP was associated with a NPV of 69% for HBsAg SL
- HBsAg CP is associated with reduced epitope recognition/availability across both loop 1 AND 2 epitopes

Results (to EOS or SL)

Strong predictive association between HBsAg CP and SL is enhanced (p-value 0.001) by the end of study (EOS) or the last time point before SL, with a PPV 92% (NPV 77%)

Development of HBsAg CP	At Baseline	By Week 48 or prior to SL	By EOS or prior to SL
HBsAg Loss (SL), n=14	5	10 (2/10 maintained from BL)	11
Non-Responder (NR), n=11	5	2 (maintained from BL)	1
p-value	*0.697*	***0.015***	***0.001***
PPV	n.a.	**83%**	**92%**
NPV	n.a.	**69%**	**77%**

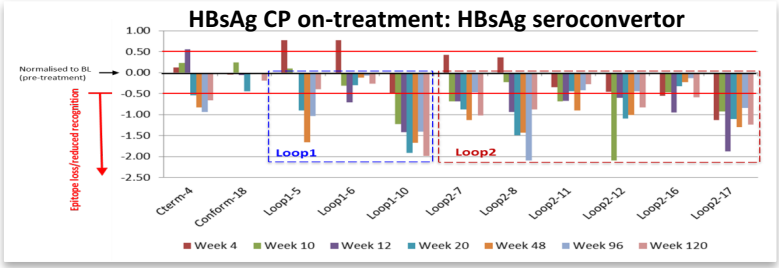
Development of an HBsAg CP with an outcome of SL could also be significantly correlated to:

- The level of HBsAg decline (p-value 0.003 to 0.0005)
- An ALT flare / immune response (p-value 0.017), preceding HBsAg response and SL

HBsAg CP coinciding with HBsAg decline or ALT flare	0.5 $\log_{10}$ HBsAg decline	1 $\log_{10}$ HBsAg decline	2 $\log_{10}$ HBsAg decline	Coinciding with ALT flare
HBsAg Loss (SL), n=14	6	8	10	12
Non-Responder (NR), n=11	2	0	0	4
p-value	*0.234*	***0.003***	***0.0005***	***0.017***

HBsAg profile to EOS: SL vs NR

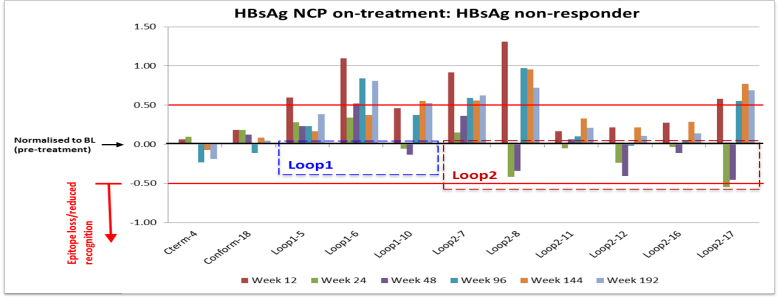
CHB patient who achieved HBsAg clearance and seroconversion = Functional cure



HBsAg CP detected from W10

- coinciding with HBsAg decline from W8
- preceding HBsAg clearance at W144

CHB non-responder patient



HBsAg NCP detected

- No HBsAg response/decline

Conclusions (to EOS or SL)

HBsAg clearance to achieve functional cure is presumably driven by an effective anti-HBs response. HBsAg epitope profile, influenced by epitope occupancy, and the detection of an HBsAg CP, may be a predictive biomarker, reflective of an emerging anti-HBs response

- **HBsAg CP was significantly associated with an outcome of HBsAg clearance** at Week48 (p-value 0.015), and enhanced preceding HBsAg loss/seroconversion (p-value 0.001), with strong positive predictive value

p-value 0.001 **Positive predictive value 92%** **Negative predictive value 77%**

- **Complexed anti-HBs when correlated with an HBsAg CP, was strongly associated with HBsAg clearance/seroconversion** (p-value 0.004; PPV91%; NPV 71%), and is indicative of a developing immune response
- The serological and biomarker profile of CHB patients who achieve SL is indicative of effective and functional immune recovery

Questions

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Discussion

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