HBV – an update from AASLD 2016

Sanjay Bhagani
Summary

• HBV re-activation post, peri-DAA therapy for HCV

• New strategies for HBV treatment – towards a cure?
  – Early clinical/pre-clinical data of Host-Targeting agents and Direct Antiviral Agents
  – Sequential NA and PegIFN-alpha
  – Combination of HBs secretion inhibitor/NA/PegIFN

• Tenofovir Alafenomide (TAF)
FDA Adverse Events Database
Bersoff-Matcha, et al., LB-17

• Descriptive analyses of 29 reported cases
• Median time from DAA initiation to HBV reactivation – 46 days (most within 4-6 weeks)
• Significant de-compensation (n=3), death (n=2), transplantation (n=1) and hospitalisation (n=6)
• Pre-DAA HBV status (very heterogenous)
  – HBsAg+ 13
  – HBsAg- 4
  – HBcAb+ 6
  – HBcAb unreported 23
  – HBV DNA+ 9
  – HBV DNA- 16
Take Home messages

• Know your patients’ HBV status pre-DAA therapy
• Cirrhotic HBsAg+ patients should be on HBV therapy, as per guidelines
• Non-cirrhotic HBsAg+ patients – HBV therapy pre-DAA if indicated
• Vigilance re: serum aminotransferases for HBsAg+, HBcAb+ patients whilst on DAA – ?HBV DNA monitoring for F3/F4 patients
The ‘cure’ agenda in HBV

Duantel and Zoulim, J Hepatol 2016; 64: S117
Reversal of T cell exhaustion: the goal of checkpoint inhibitors

Effective T cells control virus

Exhausted T cells lose control of virus

Check point blockade
Excessive co-inhibitory signals drive T cell exhaustion
Harnessing innate and adaptive immunity: TLR agonists e.g. GS-9620

- TLR-7/8 mediate innate immune activation in human liver:
- CD56\textsuperscript{bright} NK cells and MAITs produce antiviral cytokine IFN-\(\gamma\)

Gane EJ, J Hepatol, 2015
Lanford et al. Gastroenterology 2013
TLR-7 agonist GS-9620 can improve HBV-specific T cell and NK cell responses in nucleos(t)ide suppressed patients with chronic hepatitis B

Carolina Boni¹, Andrea Vecchi², Marzia Rossi³, Diletta Laccabue¹, Tiziana Giuberti³, Arianna Alfieri³, Pietro Lampertico⁵, Glenda Grossi³, Floriana Facchetti³, Maurizia R. Brunetto⁴, Barbara Coco⁵, Daniela Cavallone⁶, Alessandra Mangia⁴, Rosanna Santoro⁴, Anuj Gaggar⁵, G Mani Subramanian⁵, Carlo Ferrari¹

1. Laboratory of Viral Immunopathology, Unit of Infectious Diseases and Hepatology, Azienda Ospedaliero-Università di Parma, Parma, Italy
2. Division of Gastroenterology and Hepatology, Fondazione IRCCS Ca’ Granda, Ospedale Maggiore Policlinico, Università degli Studi di Milano, Italy
3. Hepatology Unit and Liver Physiopathology Laboratory, University Hospital of Pisa, Italy
4. Liver Unit, IRCCS, “Casa Sollievo della Sofferenza”, San Giovanni Rotondo, Foggia, Italy
5. Gilead Sciences, Inc. Foster City, CA, United States

PATIENT POPULATION and TREATMENT SCHEDULE

Immunological analysis and clinical monitoring

<table>
<thead>
<tr>
<th>SCR</th>
<th>BASELINE</th>
<th>Wk 8</th>
<th>Wk 12</th>
<th>Wk 24</th>
</tr>
</thead>
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NUC

NUC + GS-9620

Experimental therapy

NUC

Follow-up

NUC + GS-9620 (n=26)

- 1 mg GS-9620 PO weekly (n=9)
- 2 mg GS-9620 PO weekly (n=8)
- 4 mg GS-9620 PO weekly (n=9)

Chronic naive patients (eligible for therapy, n=13)

Viremic, HBeAg neg, Genotype D
**Clinical Efficacy**
HBsAg changes during GS 9620 therapy:

- GS 9620
  - 4 mg
  - 2 mg
  - 1 mg

<table>
<thead>
<tr>
<th>Week</th>
<th>0</th>
<th>6</th>
<th>12</th>
<th>18</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBSAg [U/mL]</td>
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- HBsAg changes were minimal in all cohorts (no patient declines in HBsAg at week 24)
- No patients had HBsAg loss at week 24

**In Vitro HBV-Specific T Cell Analysis**
GS 9620 can induce a transient improvement of IL2 production by HBV-specific T cells

- IFN-γ
- TNF-α
- IL2

<table>
<thead>
<tr>
<th>Week</th>
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<th>12</th>
<th>18</th>
<th>24</th>
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<tr>
<td>CD3 [% total CD3]</td>
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- The GS 9620 effect on IL2 production is detectable with HBV-specific but not with HBV-unrelated control peptides

**In Vitro HBV-Specific T Cell Analysis**

- HBV peptides
- CMV-EBV-FLU control unrelated peptides

<table>
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<th>Week</th>
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<th>6</th>
<th>12</th>
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<tr>
<td>CD3 [% total CD3]</td>
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Summary and conclusions

- The TLR7 agonist GS 9620 can transiently boost the improvement of T cell responses induced by NUC therapy

- CD8 responses are more improved than CD4

- Polymerase and core are always the most powerful T cell immunogens

- TLR7 agonist therapy can induce the acquisition of an activated/inflammatory NK cell phenotype and a modulation of NK cell receptors which are associated with a functional NK cell improvement

- NK cells appear to be poorly inhibitory for T cell during GS 9620 therapy suggesting a positive T cell/NK cell interplay
LUNAR™-HBV, a UNA Oligomer Combination for the Treatment of Chronic Hepatitis B Virus Infection

LUNAR Delivery Technology

Four Components: ATX Lipid, Stealth Lipid, Z Lipid, Cholesterol

RNAi Targeting of HBV Transcripts with UNA Oligomers

<table>
<thead>
<tr>
<th>Feature</th>
<th>Benefit</th>
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<tbody>
<tr>
<td>Proprietary Chemistry</td>
<td>Nuclease resistance</td>
</tr>
<tr>
<td>Potency</td>
<td>Clinical Efficacy</td>
</tr>
<tr>
<td>Longevity of action</td>
<td>Less frequent dosing</td>
</tr>
<tr>
<td>Reduces Off-Target Effects</td>
<td>Safety and Tolerability</td>
</tr>
<tr>
<td>Reduces Immunogenicity</td>
<td>Safety</td>
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</tbody>
</table>
The ability of UNA oligomers to target HBV transcripts was measured using the psiCHECK™-2 system in which HBV genotype A2 sequence was cloned downstream of the Renilla luciferase reporter gene. The UNA oligomers were transfected into Hep3B cells immediately following transfection of the HBV reporter plasmid. Six days later, Dual-Luciferase Reporter Assay kit (Promega) was used to measure Renilla luciferase activity. Activity of a constitutively expressed Firefly luciferase gene was also measured as an internal control for transfection efficiency.

Human hepatocytes isolated from PXB-Mouse were plated overnight and inoculated with HBV genotype Ae, Bj, C or D the next day. 12 days later the cultured cells were transfected with 3 or 15nM LUNAR-HBV combination. HBsAg and hAlbumin levels were measured in cell culture supernatant collected on day 5 post-treatment.
HBV capsid assembly modulator (CAM): Background

- HBV core is a key protein in the HBV life-cycle.
  - Forms the viral capsid.
  - Essential for HBV replication and the formation and maintenance of cccDNA.
  - Reported to possess immunosuppressive activity and to be involved in cccDNA transcription.
- CAMs accelerate capsid assembly kinetics, prevent encapsidation of (pg)RNA and block HBV replication.
- First in class CAM Novira 3-778 demonstrated proof of mechanism in a Phase I clinical trial.
  - Mean -1.72 log HBV DNA change from baseline after 28 days of treatment.
  - Mean -0.82 log HBV RNA change from baseline after 28 days of treatment.
Capsid assembly modulators: Induction of two distinct capsid phenotypes

• CAMs induce the formation of two types of capsids *in vitro*:
  - Empty capsids with normal geometry and size (class I MOA).
    • Phenylpropenamides (e.g. AT130) and sulfamoylbenzamide derivatives.
  - Empty capsids with abnormal geometry and size (class II MOA).
    • Heteroaryldihydropyrimidines (e.g. BAY41-4109).

**Electron microscopy**

Recombinant HBV core dimers + 150mM NaCl +/- 30μM CAM (24h)
JNJ-379: Biochemical mechanism of action studies

Size exclusion chromatography

Electron microscopy

DMSO

JNJ-379 (class I)

BAY41-4109 (class II)

Dose-dependent induction of capsid assembly in presence of JNJ-379, results in the formation of morphologically intact HBV capsids (class I MOA).
JNJ-379: Effect on cccDNA in HBV-infected PHHs

Dose-dependent inhibition of cccDNA formation in presence of JNJ-379. No inhibition of cccDNA formation observed for nucleos(t)ide analogues.
JNJ-379: Time of addition studies in PHHs

preS1 peptide: 2,000nM
ETV; JNJ-379: 5,000nM

0h  4h  8h  24h

D-1   D0   D1   ...
Seeding   Infection   Wash +CPD

D5   ...

D8   ...

D12

Refresh +CPD

JNJ-379 blocked infection when added 0-8 hours after HBV infection.
ETV did not block the infection at any of the time points.
Conclusions

- JNJ-379 is a capsid assembly modulator with class I MOA.
- JNJ-379 is a potent and selective inhibitor of HBV replication across genotype A-H clinical isolates.
- JNJ-379 blocks capsid assembly and cccDNA formation in PHHs, resulting in the reduction of HBV RNA and antigens.
- JNJ-379 is currently being evaluated in Phase 1b trials.
Exploring Combination Therapy for Curing HBV

Preclinical Combo Studies with Capsid Inhibitor AB-423 and siRNA Agent ARB-1740

Amy C.H. Lee
14 November 2016
RNAi & Core Protein/Capsid Inhibitor
Two Novel Agents studied in combination with SoC

**AB-423 (Core/Capsid Inhibitor)**
- Orally administered small molecule
- Sub-micromolar potency
- Misdirects capsid assembly and inhibits pgRNA encapsidation

**ARB-1740 (RNAi)**
- Second generation RNA interference agent
- Three siRNAs encapsulated in a lipid nanoparticle delivery system
- Primarily, targets surface antigen produced by cccDNA & integrated DNA

*Both these investigational agents possess pan-genotypic activity*
Combining Novel Agents with Standard of Care

Triple therapy provides greatest reduction of HBV DNA

Triple drug combinations provided even more reduction of virus in serum
Unlike the other agents, ARB-1740 causes similar reductions in serum HBsAg, HBeAg and HBV DNA.

HBV antigens are produced at high levels and have immune suppressive effect.
Liver Reservoir of cccDNA

Not just “how many copies” but also “is it transcriptionally active?”

Pre-established cccDNA was unchanged after 6 wk treatments;
HBV rcDNA suppression may have reached a maximum with chosen combos;
However, differential control of cccDNA transcriptional activity was observed
Preclinical investigations of drug combinations can provide supportive data to help inform the design of investigative human trials.

- Combination of novel MOA agents AB-423 (capsid inhibitor) and ARB-1740 (RNAi) can enhance control of HBV by current standard drugs.

- These data support the hypothesis that HBV antigen removal will promote immune recognition and viral control.
Increased and sustained HBsAg loss in HBeAg positive CHB patients switched from NUC to Peg-IFN alfa-2a: A randomised open label trial (NEW SWITCH study)

Peng Hu#, Xiaoguang Dou#, Jianning Jiang, Jia Shang, Wenhong Zhang, Guozhong Gong, Yonguo Li, Xinyue Chen, Qing Xie, Yongtao Sun, Yufang Li, Yingxia liu, Guozhen Liu, Dewen Mao, Xiaoling Chi, Hong Tang, Xiao Ou Li, Yao Xie, Xiaoping Chen, Jiaji Jiang, Ping Zhao, Jinlin Hou, Zhiliang Gao, Huimin Fan, Jiguang Ding, Hong Ren*

Nov 13 2016 Boston

# These authors contributed equally
*Corresponding author
Final Analysis

- Randomized, multicenter, open-label study
- Patient population: previous HBeAg pos CHB patients treated with NUC experienced for 1-3 year and with partial response
  - Partial response: HBV DNA<200 IU/mL and HBeAg loss

Randomization 1:1 (stratified by ADV, ETV, LAM)
Extending the duration of Peg-IFN to 96 weeks showed slight improvement compared with 48 weeks (End-of-treatment).

All P value > 0.05
Sustained HBsAg response at End-of-follow up in patients with HBsAg response at End-of-treatment

PegIFN α-2a 48weeks arm (n=153)

PegIFN α-2a 96weeks arm (n=150)

*data missing at EOF considered as non-sustained
Conclusions

• HBsAg loss can be achieved in NUC-treated CHB patients by switching to PegIFN treatment. In addition, quantification of HBsAg level at baseline and week 24 may be a predictor of HBsAg loss at week 48.

• The prolonged treatment duration of PegIFN may improve HBsAg loss rate.

• NEW SWITCH study provide essential data to support the new treatment strategy which might be able to maximize the chance to achieve sustained HBsAg loss which is viewed as “clinical cure”.

Preliminary safety and efficacy of REP 2139-Mg or REP 2165-Mg used in combination with tenofovir disoproxil fumarate and pegylated interferon alpha 2a in treatment naïve Caucasian patients with chronic HBeAg negative HBV infection

(REP 401 protocol)

Nucleic Acid Polymers (NAPs)

Subviral particles (bulk of serum HBsAg)

Infected hepatocyte

Nucleus

cccDNA

Capsids

Virions

NAPs block subviral particle release

Efficient HBsAg clearance from the blood

Vaillant, 2016. Antiviral Res. 133: 32-40
Real et al., 2016 J. Hepatol. 64: S395
Noordeen et al., 2015 PLOS One 10: e0140909
Noordeen et al., 2013 AAC 57: 5299-5306
Nooreen et al., 2013 AAC 57: 5291-5298
Critical effects of HBsAg clearance

NAP mediated HBsAg clearance leads to:

Unmasking pre-existing anti-HBs response
- clearance of virions (HBV DNA and HBV RNA)

Removal of HBsAg mediated immunosuppression
- HBeAg seroconversion (in HBeAg+ patients)
- enhanced immune response in the liver (transaminase flares)
- establishment of functional control off treatment (in some patients)

Improved effect of immunotherapy
- can establish functional control in most patients

Vaillant, 2016. Antiviral Res. 133: 32-40
Al-Mahtab et al., 2016 PLOS One 11: e0156667
M. Bazinet et al., 2016 AASLD Abstract 1848.
Reesink et al., 2016 Hepatol. Int. 10: S2
Noordeen et al., 2015 PLOS One 10: e0140909
Op den Brouw et al., 2009. Immunology, 126: 280-289
Shi et al. 2012 PLOS One 7: e44900
Woltman et al. 2011 PLOS One 6: e15324
Wu et al., 2009. Hepatology, 49: 1132-11
Xu et al., 2009. Molecular immunology, 46: 2640-2646
**REP 401 Design**

**Dosing:**
- **TDF** 300mg PO qD
- **Pegasys** 180ug SC qW
- **NAPs:** REP 2139-Mg or REP 2165-Mg 250mg IV qW
- REP 2165 = REP 2139 variant with improved tissue clearance

**Primary efficacy endpoints:**
- Serum HBsAg reduction
- Appearance of anti-HBs
- Functional control maintained after treatment withdrawal ($\geq 6$ months HBsAg $< 1$ IU/ml, HBV DNA $< 1000$ copies / ml)

29 patients are $> 12$ weeks post-randomization (week 25)
Interim Efficacy data (serum HBsAg)

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

9/9 HBsAg response > 1 log
6/9 HBsAg response > 1 log
Interim Efficacy data (serum anti-HBs)

Elevation in serum anti-HBs correlated with extent of HBsAg reduction

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

REP 2139 and REP 2165 are well tolerated in triple combination with TDF and peg-IFN

NAP therapy is associated with:
  - multilog reduction or clearance of serum HBsAg
  - increases in serum anti-HBs
  - increased incidence and magnitude of serum transaminase flares
    (otherwise asymptomatic and self resolving)

Antiviral effect of REP 2139 is conserved with the more rapidly cleared REP 2165

Reproduces effects of NAPs in previous proof of concept trials leading to functional control
  - HBeAg+ chronic HBV infection (REP 101, REP 102, REP 201 protocols)
  - chronic HBV / HDV co-infection (REP 301 protocol)

Upcoming analyses to be presented:
  - Pre-crossover update in all patients (APASL 2017)
  - Crossover update in control group (EASL 2017)
  - HBcrAg and HBV RNA analysis (EASL 2017)

Vaillant, 2016. Antiviral Res. 133: 32-40
Al-Mahtab et al., 2016 PLOS One 11: e0156667
M. Bazinet et al., 2016 AASLD Absttract 1848
Mechanism of Action

Tenofovir alafenamide (TAF) – A Novel Prodrug of Tenofovir

GI TRACT

RENAL TUBULAR CELL

TFV

OAT 1 & 3

PLASMA

~90% LOWER PLASMA TFV

RENAL TUBULAR CELL

TFV

OAT 1 & 3

HEPATOCYTE

TFV→TFV-DP

HBV

TFV

PLASMA

TFV

OAT 1 & 3

TENOFOVIR DISOPROXIL FUMARATE (TDF) – 300 mg

TENOFUVIR ALAFENAMIDE (TAF) – 25 mg

300 mg

25 mg

short plasma half-life

longer plasma half-life

DIANION

ESTER

AMIDATE

TENOFOVIR (TFV)

DIAMON

ESTER

AMIDATE

TENOFOVIR (TFV)

* T<sub>1/2</sub> based on in vitro plasma data: TDF = 0.4 minutes, TAF = 90 minutes.


Primary endpoint (non-inferiority margin of 10%):
- HBV DNA <29 IU/mL at Week 48

Key secondary endpoints
- ALT normalisation at Week 48
- Renal parameters and bone mineral density at Week 48

95% retention rate through Week 48

Inclusion criteria: HBV DNA ≥20,000 IU/mL; ALT >60 U/L (males), >38 U/L (females), eGFR_{CG} >50 mL/min

*Non-inferiority margin of 10%
Antiviral Efficacy of TAF and TDF at Week 72

Rates of Viral Suppression
HBV DNA <29 IU/mL

- HBV DNA suppression rates were lower in HBeAg+ vs HBeAg− patients
- No significant difference between TAF and TDF
- No resistance was detected through 48 weeks

HBV DNA suppression was comparable between TAF and TDF treatment up to Week 72
Study 108 and 110: Phase 3 CHB Studies: TAF vs TDF

ALT normalisation of TAF and TDF at week 72

- **AASLD Criteria**
  - TAF: 49.1%
  - TDF: 39.0%

- **Central Lab Criteria**
  - TAF: 76.1%
  - TDF: 68.4%

Significantly higher ALT normalisation rate with TAF vs TDF

- **Central Lab Criteria**
  - *p < 0.05
  - †p ≤ 0.001

† <19 and <30 U/L for females and males, respectively
§ ≤34 and ≤43 U/L for females and males, respectively, aged <69 y, and ≤32 and ≤35 U/L, respectively, aged >69 y

Fung, AASLD 2016, Poster 1852
Study 108 and 110: Phase 3 CHB Studies: TAF vs TDF

Changes in Urine Markers of Tubular Dysfunction During Treatment with TAF or TDF

Changes in Quantitative Proteinuria at Week 48

There were smaller changes in protein markers of kidney and proximal tubule function with TAF treatment compared to TDF

* p-values from 2-sided Wilcoxon rank-sum test
Lim, AASLD 2016, Poster 1901
Evaluation of ALT Normalisation with TAF vs. TDF

### Predictors of Week 48 Abnormal ALT by AASLD criteria*

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cirrhosis†</td>
<td>2.64</td>
<td>1.53–4.56</td>
<td>0.0005</td>
</tr>
<tr>
<td>Baseline diabetes†</td>
<td>2.27</td>
<td>1.12–4.63</td>
<td>0.0238</td>
</tr>
<tr>
<td>Female gender</td>
<td>1.79</td>
<td>1.29–2.47</td>
<td>0.0004</td>
</tr>
<tr>
<td>Baseline BMI</td>
<td>1.14</td>
<td>1.09–1.19</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Baseline ALT</td>
<td>1.00</td>
<td>0.99–1.00</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TAF 25-mg (vs TDF 300-mg) treatment</td>
<td>0.60</td>
<td>0.44–0.82</td>
<td>0.0016</td>
</tr>
<tr>
<td>HBV DNA &lt;29 IU/mL at Week 48</td>
<td>0.33</td>
<td>0.22–0.49</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* Multivariate logistic regression using stepwise selection; variables significant in univariate model at 0.15 level were included in multivariate model
† As determined by medical history or concomitant medication.

Higher ALT normalisation rate with TAF vs TDF.
Patients with features of metabolic syndrome were less likely to have normalised ALT levels
**Baseline Factors Associated with Viral Persistence**

### Baseline Factors Associated with HBV DNA ≥2,000 IU/mL at Week 48*

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV DNA ≥8 log_{10} IU/mL</td>
<td>4.98</td>
<td>2.26, 10.99</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HBeAg+</td>
<td>4.79</td>
<td>1.05, 21.77</td>
<td>0.0427</td>
</tr>
<tr>
<td>HBV GT D</td>
<td>2.60</td>
<td>1.31, 5.20</td>
<td>0.0066</td>
</tr>
<tr>
<td>Previous oral antiviral treatment</td>
<td>1.99</td>
<td>1.01, 3.92</td>
<td>0.0456</td>
</tr>
<tr>
<td>CrCl (by CG)</td>
<td>1.01</td>
<td>1.00, 1.02</td>
<td>0.0215</td>
</tr>
<tr>
<td>Adherence rate at Week 48†</td>
<td>0.78</td>
<td>0.66, 0.91</td>
<td>0.0022</td>
</tr>
</tbody>
</table>

- TAF and TDF had similar rates of viral suppression
- 50 patients (4%) had HBV DNA persistence (≥2000 IU/mL) at Week 48
- Several baseline factors were associated with HBV DNA persistence
- HBV DNA ≥8 log_{10} IU/mL, genotype D were highly associated with viral persistence

**A small proportion of patients had HBV DNA persistence at Week 48, but treatment assignment (TAF vs TDF) was not associated with this persistence**

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* Multivariate logistic regression using stepwise selection; variables significant in univariate model at 0.15 level were included in multivariate model
† Adherence rate determined by pill count
Gane, AASLD 2016, Poster 1879
Conclusions

• An new era in HBV management is emerging
• Immediate aim = Functional cure
• Ultimate aim = Sterilising cure
• Lots of molecules targeting host immune system/viral replication pathways
  – Combinations will probably be required
  – Emergence of nanoparticle technology
• Emerging research/consensus in biomarkers/end-points
Acknowledgements

• NATAP
• Keith Alcorn/Liz Highwayman (NAM)
• Malcolm Macartney (J&J)
• Phil Troke (Gilead)