Anti-HBV potential of PD-1/PD-L1 blockade in chronic HBV infection

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T cells require three signals for complete activation.

Signal 1 – peptide/MHC:TcR
T cells require three signals for complete activation.

Signal 2 – Co-stimulation
T cells require three signals for complete activation.

Signal 3 – Cytokine support
CHB Background

- Viral clearance in acute HBV is associated with a broad, robust CD4+ and CD8+ T cell response and seroconversion.
- An insufficient T-cell response to HBV antigens is characteristic of chronic HBV and limits durable viral control and clearance.
- T-cell exhaustion is a major pathway mediating an impaired *in situ* response.

Co-inhibitory signaling drives progressive T cell exhaustion

• T cell exhaustion is characterised by progressive loss of proliferative capacity and effector cytokine production, and increased susceptibility to apoptosis.

• Exhausted T cells progressively acquire expression of co-inhibitory surface receptors.

• PD-1/PD-L1 is a dominant co-inhibitory axis mediating T cell exhaustion in CHB.
PD-1 Expression and signalling

- IgSF/CD28 transmembrane protein. Binds PD-L1 (B7-H1) and PD-L2 (B7-DC).
- Important for normal tissue homeostasis and T cell memory formation.
- Expressed on activated and memory T cells - PD-1 signalling drives SHP-1/2 recruitment and impairs both TCR/CD3 and CD28 signal transduction.
- Expressed on B cells, NK, macrophages and dendritic cells.
- Expressed on T_{REG} – signalling enhances function and drives proliferation.
- Expressed on MDSC – signalling drives IL-10 production.

Gianchecchi Al Rev 2013; Hui Science 2013; Yulefpskiy JI 2014; Huang JI 2014; Schumacher Immunity 2018
Regulation of PD-1 and PD-L1 expression is multifactorial

- Transcriptional control
  - Upstream binding sites for AP-1, NFAT1, FOXO1
  - Upstream Interferon specific response elements
  - Expression strongly inhibited by T-bet (early) and Blimp-1 (late)

- Epigenetic control
  - Expression silencing by methylation post-activation
  - Locus constitutively ‘open’ in exhausted T cells
PD-L1 expression and regulation

- IgSF/B7 transmembrane protein, expressed by normal stroma and myeloid cells.
- Expression induced by Interferons-α/β/γ.
- JAK – STAT-1 – IRF1 induction pathway.
- Expressed on Liver sinusoidal endothelium, stellate cells, hepatocytes, infiltrating myeloid cells (KC/DC/MDSC) and B cells.
- HBV infection drives PD-L1 expression in hepatocytes.

PD-1 and PD-L1 blockade in Solid Tumours

- Melanoma-specific TIL also exist in an immunosuppressive microenvironment and exhibit T cell exhaustion.

Roberts *NEJM* 2015
Brahmer *NEJM* 2012
Woodchuck Hepatitis Virus Model

- Chronic infection characterized by poor T cell response and high viral load.

- PD-L1 blockade *in vitro* partially restores T cell proliferation and effector function (Zhang *PLoS ONE* 2011)

- NUC therapy + DNA vaccination + α-PD-L1 enhanced T and B cell function, suppressed WHV replication, and led to seroconversion and viral clearance in a subset of infected animals (Liu *PLoS Path* 2014).

- α-PD-L1 safe and well tolerated, pre-treatment serum surface antigen levels predicted response (Balsitis *PLoS ONE* 2018)
T cell exhaustion and PD-1/PD-L1 blockade in CHB

- Intrahepatic HBV-specific T cells uniformly express PD-1, and exhibit low levels of CD127 and CD28 expression.

- HBV-specific T cells heavily enriched (trapped?) in the liver.

References:

T cell exhaustion and PD-1/PD-L1 blockade in CHB

• PD-L1 blockade \textit{ex vivo} restores T cell proliferation, cytotoxicity and effector cytokine production

• Efficacy of \textit{ex vivo} blockade is best predicted by T cell differentiation status (retention of CD27 expression)

• HBV-specific T cells express an hierarchical array of co-inhibitory receptors, but PD-1/L1 blockade has the greatest effect on restoring T cell function.
At 3 mg/kg, evaluation in HBV-infected patients with advanced HCC demonstrated declines in HBsAg of $>1 \log_{10}$ in $3/51$ (6%) of patients

(Sangro et al, The Liver Meeting 2016, Checkmate-040)
Nivolumab +/- GS-4774

- Monoclonal antibody against PD-1
  - Approved for solid organ tumors and lymphomas\(^1\)
- Human IgG4, half-life 12 days (<3mg/kg) to 20 days (10mg/kg)\(^2\).
- Previous studies had demonstrated vaccine GS-4774 to be immunogenic in CHB but to lack therapeutic efficacy\(^3,4\)

Primary efficacy endpoint: Change in HBsAg $\log_{10}$ IU/mL levels 12 weeks post-Nivo

Clinically approved dose 3mg/kg (melanoma, NSCLC, RCC).
PD-1 is primarily expressed on memory and effector T cells

Gane J Hep 2017; Verdon The Liver Meeting 2017.
PD-1 Receptor Occupancy by Nivolumab

- PD-1 occupancy on T cells retained for $\leq 43 - 85$ days following in vivo following a single Nivolumab infusion.
- Differential occupancy observed at day 85.
- Median occupancy days 8-30 post-infusion was similar for 0.1mg/kg and 0.3mg/kg dosing.
- Median RO days 8-43: 75.9%; Median Peak RO (day 16): 79.5%

Gane J Hep 2017; Verdon The Liver Meeting 2017.
## Safety Profile

<table>
<thead>
<tr>
<th></th>
<th>Nivolumab 0.1 mg/kg n=2</th>
<th>Nivolumab 0.3 mg/kg n=12</th>
<th>Nivolumab 0.3 mg/kg + GS-4774 n=10</th>
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<tbody>
<tr>
<td><strong>Overall Safety</strong></td>
<td></td>
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<tr>
<td>AE</td>
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<td>Any Grade 3/4</td>
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<td>Death, n (%)</td>
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<tr>
<td>Any Grade 3–4</td>
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<tr>
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<td>ALT Grade 1</td>
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</tbody>
</table>

*Fatigue (n=1), headache (n=1), cough (n=1); †subject with HBsAg loss. AE, adverse event; SAE, serious AE.

No patient with autoimmune manifestations, including pneumonitis, colitis, rash, or endocrinopathies

Gane J Hep 2017; Verdon The Liver Meeting 2017.
**HBSAg Changes from Baseline**

- 2/22 (9%) at Week 12 and 3/22 (14%) at Week 24 with a \(>0.5 \log_{10}\) reduction in HBsAg
  - Only one patient with \(>1 \log_{10}\) reduction in HBsAg at either clinical timepoint
- No baseline demographic feature associated with \(>0.5 \log_{10}\) reduction in HBsAg
Case Study: Clinical observations

Serum HBsAg (IU/ml) vs. Week post-Nivolumab

- HBsAg IU/ml
- ALT (U/L)
- IFNγ CORE Ag

Week post-Nivolumab: 0, 1, 2, 3, 4, 6, 8, 12, 16, 20, 24

ALT (U/L)
IFNγ spots/1e6 PBMC

Gane J Hep 2017; Verdon The Liver Meeting 2017.
Case Study: Lymphocyte subset redistribution
Summary and Conclusions

• Peripheral PD-1 receptor occupancy is maintained in CHB patients for up to 85 days following a single 0.3mg/kg IV dose of Nivolumab, without evidence of immune-mediated AE.

• 20/22 (90%) of patients treated with 0.3mg/kg Nivolumab exhibited some decline in serum HBsAg at week 12.

• One patient experienced complete and sustained HBsAg loss and anti-HBS seroconversion, and remains disease-free at 29mo.
Future directions

• Anti-PD-L1 monoclonal therapy in CHB

• siRNA inhibition of PD-L1 expression (transient?)

• Small molecule inhibitors of PD-1 and/or PD-L1 (more predictable half-life and clearance?)

• Combination checkpoint blockade therapies – both multiple checkpoints (e.g. Tim-3, CD244) and/or agonistic targets (e.g. CD137).
Future directions

- Adoptive Cell Transfer supported by anti-PD1/L1 – *ex vivo* rescue of HBV-specific T cells.
  - Tetramer/Dextramer or activation marker-based isolation (PepMix™ stimulation).
  - Expansion of HBV-specific T cells under ‘restorative’ conditions that modulate cell phenotype and metabolism before reinfusion.
    - Agonistic microbeads (αCD3/28)
    - Cytokine support (*e.g.* IL-12)  
    - Costimulation (*e.g.* CD137L or agonistic αCD137)

Verdon et al. unpublished data
We extend our thanks to the patients, their families, and participating investigators.

This study was funded by Gilead Sciences, Inc.