Novel Targets for the Treatment of Chronic Hepatitis B Infection
Hepatitis B viruses

- HBV DNA genome:
  - small (3.2 kb), partial duplex circular DNA
  - RC (relaxed circular) DNA

- Genome replication:
  - Reverse transcription (RT): RNA → DNA

- Antiviral therapy:
  - 1st generation: Lamivudine (LAM), Adefovir
  - 2nd generation: Entecavir (ETV), Tenofovir
- The virus is not cleared, even after long-term therapy: > 50 years therapy needed.
cccDNA & Viral persistence

- A new drug with novel target would block the leakiness of NUC monotherapy.
• HBV Pol-epsilon RNA binding is required.
• HBV Pol inhibits translation via its binding.
• HBV Pol-epsilon RNA interaction represents a novel antiviral target

Cell-based HBV Pol-epsilon RNA Binding Assay

- A small molecule that interferes the Pol-epsilon RNA interaction can be scored.
- HTS-ready cell based assay
Southern blot analysis: HepG2 cell

- We screened > 100,000 compounds via HTS assay.
- Hit compounds inhibit the viral genome replication.

**Future work**
- Hit optimization to get IC$_{50}$ down to double digit nM range: > 100 fold.

**YS 001**  IC$_{50}$ = ~ 2 μM

**YS 002**  IC$_{50}$ = ~ 1.0 μM
HBV infection using HepG2-NTCP cell line
Timelines of Discovery: HBV versus HCV
**Evidence for**
- Pre-S1 peptide binding protein expressed selectively in primary hepatocytes
- Confer the HBV susceptibility to HepG2 cell

**NTCP receptor**
- A key transporter for hepatic uptake of bile acids
- Nine transmembrane domain (TM)
- Taurocholic acids inhibits HBV infection vice versa

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## HBV infection using HepG2-NTCP cells

<table>
<thead>
<tr>
<th></th>
<th>cccDNA</th>
<th>Viral RNA</th>
<th>Viral DNA</th>
<th>Infection efficiency</th>
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<td><strong>Wenhui Li</strong></td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
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<td>Yan et al., 2014</td>
<td>Yan et al., 2013</td>
<td>Zhong et al., 2013</td>
<td>Yan et al., 2012</td>
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<td>Not mentioned</td>
<td>Estimated value: &lt; 30%</td>
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| **Stephan Urban** | ![Image](image4.png) | ![Image](image5.png) | ![Image](image6.png) | ![Image](image7.png) | Determined by immunofluorescence: approximately 70% (at 2.5% DMSO) |
| Ni et al., 2014 | Nkongolo., 2014 | |

| **Takaji Wakita** | ![Image](image8.png) | ![Image](image9.png) | ![Image](image10.png) | ![Image](image11.png) | Determined by immunofluorescence: approximately 50% (at 3% DMSO) |
| Iwamoto et al., 2014 | Watashi et al., 2014 | |

| **W.-S. Ryu**  | ![Image](image12.png) | ![Image](image13.png) | ![Image](image14.png) | ![Image](image15.png) | Determined by immunofluorescence: approximately 70% (at 3% DMSO) |
| (Submitted) | |

References:

- Yan et al., 2014
- Yan et al., 2013
- Zhong et al., 2013
- Yan et al., 2012
- Ni et al., 2014
- Nkongolo., 2014
- Iwamoto et al., 2014
- Watashi et al., 2014

*Estimated values and infection efficiency are indicative of the described experiments.*
HBV infection using HepG2-NTCP cells: Immunostaining by anti-HBc

- A robust HBV infection system established: > 70% cells infection at $10^4$ GEq.
- HBV infection is completely blocked by pre-S1 peptide (MyrCludex B), validating the NTCP-mediated entry.
A precursor of cccDNA, PF-RC DNA, as well as cccDNA was detectable.

Kinetic analysis revealed that precursor-product relationship, as predicted: PF-RC DNA -> cccDNA -> HBV RNAs, -> HBV DNA
Little is known about RC DNA to cccDNA conversion.

- Cellular factors would be attractive antiviral targets for cccDNA control.
1. We established a cell-based assay for HTS screening for HBV packaging inhibitors.

2. A hit from >100,000 chemical library screening was identified: YS001.

3. We established HBV susceptible HepG2-NTCP cell line:
   - Robust, > 70% cells infected.

4. We showed that RC DNA to cccDNA conversion can be now studied.
   - PF-RC DNA, a precursor of cccDNA, detectable
   - Yet-to-be known host factors can be attractive antiviral target for viral clearance.

- Discovery of the NTCP receptor now allowed us to study the full spectrum of the HBV life cycle, including entry and cccDNA conversion.
cccDNA & Viral persistence

- cccDNA
  - Gap-filling
  - Intracellular pathway
    -oretic pathway via Reinfection

- HBsAg
- HBV Pol
- ETV
- RT
- RC DNA
- AN

- NTCP

- It is hoped that multidrug therapy having a novel target will lead to viral clearance
Contributors

- Institut Pasteur Korea / Dr. Windisch Lab
- Chemical library: > 100,000 cpds.