The prospects of curing chronic HBV infection using gene therapy

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## Disclosure

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<th>Relations that could be relevant for the meeting</th>
<th>Company names</th>
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<tr>
<td>Sponsorship or refund funds</td>
<td>• Johnson &amp; Johnson</td>
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<td>Payment or other financial remuneration</td>
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Current treatment of HBV infection

- Licensed drugs have modest cure rate
  - Nucleoside & nucleotide analogues
  - Interferon-α
- Identifying suitable new targets has been difficult
- Eliminating/disabling cccDNA is vital
- Lines of investigation for new drug development include advancing
  - Immunoregulatory molecules,
  - Entry inhibitors,
  - Inhibitors of viral particle formation and
  - Approaches employing gene therapy to disable HBV replication
Anti-HBV nucleic acids

- Gene silencers
  - Expressed and
  - Synthetic RNAi activators
- Gene editors
  - TALENs
  - CRISPR/Cas
  - ZFNs

- Systemic administration (not ex vivo)
- Durable effect
- Specificity
HBV replication and targets for gene therapy
HBV replication and targets for gene therapy
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Considerations for advancing therapeutic anti-HBV Gene Editing

- Not all HBV replication models produce cccDNA
- Difficult replication intermediate to assay
- Fate of mutated cccDNA unclear
- Off target mutagenesis and effect on integrated HBV DNA a concern
- Immunity to effectors
- Delivery & scalability challenges
CRISPR/Cas

- First article in 2014

The CRISPR/Cas9 System Facilitates Clearance of the Intrahepatic HBV Templates *In Vivo*

Su-Ru Lin¹, Hung-Chih Yang¹,²,³, Yi-Ting Kuo¹, Chun-Jen Liu²,³,⁴, Ta-Yu Yang¹, Ku-Chun Sung¹, You-Yu Lin²,⁶, Hurng-Yi Wang², Chih-Chiang Wang², Yueh-Chi Shen¹, Fang-Yi Wu¹, Jia-Horng Kao²,³,⁴,⁵, Ding-Shinn Chen²,³,⁴,⁵ and Pei-Jer Chen²,³,⁴,⁵

- Now about 20 publications on the topic
- Interest in smaller *Staph aureus* CRISPR/Cas
  - Smaller size can be accommodated in recombinant AAVs
HBV Targets of CRISPR/Cas from *Staph. aureus*
Anti-HBV efficacy following transfection of Huh7 cells
Anti-HBV efficacy following transfection of Huh7 cells
ssAAVs encoding SaCas9 and HBV-targeting guides

AAV2 for cell culture and AAV8 for in vivo

ssAAV

- ITR
- CMV
- SaCas9
- pA
- sgRNA
- ITR

- U6 sgRNA 8 (HBV)
- U6 sgRNA HIV
- U6 sgRNA scrambled
Inhibition of HBV replication in HepG2.2.15 cells

- Similar effect on VPEs
- Confirmed in hNTCP cells

Short deletions the predominant type of mutation

T7E1

Hirt extraction of cccDNA

- Day 15
- Day 21

**Indel %**

- No guide: <1%
- HBV-sgRNA-8: 28%
- HIV-sgRNA: <1%
- No guide: <1%
- HBV-sgRNA-8: 33%
- HIV-sgRNA: <1%
Efficacy in HBV transgenic mice

- Poor efficacy in vivo and minimal mutagenesis
- No evidence of toxicity (transaminases and histology)
- Pre-existing adaptive immunity to Sa Cas9?
Pre-existing adaptive immunity to Sa Cas9?

Identification of preexisting adaptive immunity to Cas9 proteins in humans

Carsten T. Charlesworth¹, Priyanka S. Deshpande¹, Daniel P. Dever¹, Joab Camarena¹, Viktor T. Lemgart¹, M. Kyle Cromer¹, Christopher A. Vakulskas², Michael A. Collingwood³, Liyang Zhang¹, Nicole M. Bode⁴, Mark A. Behlke⁵, Beruh Dejene¹, Brandon Cieniewicz⁶, Rosa Romano⁷, Benjamin J. Lesch⁸, Natalia Gomez-Ospina⁹, Sruthi Mantri¹, Mara Pavel-Dinu¹, Kenneth I. Weinberg¹¹ and Matthew H. Porteus¹³

¹Department of Pediatrics, Stanford University, Stanford, CA, USA. ²Integrated DNA Technologies, Inc., Coralville, IA, USA. ³e-mail: kwl@stanford.edu

High prevalence of *Streptococcus pyogenes* Cas9-reactive T cells within the adult human population

Dimitrios L. Wagner¹²,³, Leila Amini¹²,³, Desiree J. Wendering¹²,³, Lisa-Marie Burkhardt¹, Levent Akyüz¹, Petra Reinke¹²,³, Hans-Dieter Volk¹,²,⁴,⁵ and Michael Schmuck-Henneresse¹²,⁴,⁵

¹Institute for Medical Immunology, Charité - Universitätsmedizin Berlin, Berlin, Germany. ²Berlin-Brandenburg Center for Regenerative Therapies (BCRT), Charité - Universitätsmedizin Berlin, Berlin, Germany. ³Berlin Institute of Health (BHI), Berlin, Germany. ⁴Berlin Center for Advanced Therapies (beCAT), Charité - Universitätsmedizin Berlin, Berlin, Germany. ⁵These authors jointly directed this study. ⁶Hans-Dieter Volk, Michael Schmuck-Henneresse.

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TALEN targets
Targeted mutation of HBV sequences in the liver

Bloom et al. (2013) Inactivation of hepatitis B virus replication in cultured cells and in vivo with engineered Transcription Activator-Like Effector Nucleases, Molecular Therapy, 21, 1889-1897.
Mutation of HBV DNA in transgenic mice

![Diagram with bands and disruption percentages]
Little (no) evidence of toxicity

Pro-inflammatory cytokine release

- Liver histology normal
Improving TALEN specificity

- Obligate heterodimeric TALENs
  - Site-specific mutation at the FokI dimerisation interface
  - Increased specificity of target site cleavage
  - Lowered dimerisation energy
  - Diminished toxicity

Target cleavage

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<th>Wild type homodimer</th>
<th>Fok I wt</th>
<th>Fok I wt</th>
<th>+</th>
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<tbody>
<tr>
<td>Mutant homodimer</td>
<td>Fok I</td>
<td>Fok I</td>
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<tr>
<td>Mutant homodimer</td>
<td>Fok I</td>
<td>Fok I</td>
<td>-</td>
</tr>
<tr>
<td>Mutant heterodimer</td>
<td>Fok I</td>
<td>Fok I</td>
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Nature Biotech. 25, 786-793
Obligate heterodimeric variant TALENs in vivo

Similar effects on VPEs
NGS: mutation in 50-65% of targets for WT and Var
No evidence of toxicity
HBV mutations predominantly deletions and similar types in WT and variant TALENs
Repressor TALEs’ efficacy in vivo

Gets around problem of cleaving integrated HBV DNA
Durability of effect needs to be established
Conclusions

• Gene editing offers the means to achieve cure from HBV infection
  – However important challenges remain: delivery, specificity, unintended effects etc.
• Synthetic components essential for scalability (e.g. mRNA & NVVs)
• Developments with other anti-HBV therapies will influence whether gene therapy is the preferred treatment modality
• Advances in gene therapy for HBV infection will be informed by progress in other fields, e.g. cccDNA biology & NA vectorology
• Pairing with other treatment strategies may be more effective
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