cccDNA inactivation using cytosine base editors

Elena Smekalova*¹, Maria Martinez*², Emmanuel Combe², Michael Packer¹, Luis Barrera¹, Selam Dejene¹, Yvonne Aratyn-Schaus¹, Giuseppe Ciaramella¹, Barbara Testoni², Francine Gregoire¹, Fabien Zoulim²⁻⁵

September 27, 2021

2021 International HBV meeting
Session I: Preclinical Targets & Therapies for Chronic Hepatitis B

1. Beam Therapeutics, Cambridge, MA, USA
2. Cancer Research Center of Lyon, INSERM, U1052, Lyon, France
3. Hospices Civils de Lyon (HCL), Lyon, France.
4. University of Lyon, UMR_S1052, UCBL, 69008 Lyon, France.
5. Institut Universitaire de France (IUF), 75005 Paris, France.

* These authors contributed equally to this work
DISCLOSURE

- I am a Beam employee and shareholder
Unmet need in patients with chronic HBV

- Vaccination is 95% effective, and still not universally administered
- 257 million are chronically infected worldwide, 800,000 deaths/year
- Antiviral medications (NUC) manage HBV replication, but do not affect cccDNA – no cure
- 20-30% of adults with chronic infection develop HCC or cirrhosis (WHO)

Zoulim F., “Challenges towards the cure of HBV infection”
Biology of Chronic HBV infection

- HBV genome is maintained in the liver cell as 3.2kb nuclear episomal DNA (cccDNA)
- cccDNA – extremely stable, responsible for the persistence of chronic HBV infection
- HBV DNA integrates into the human genome and serves as a source of Hepatitis B Surface antigen (HBsAg) expression

➢ Failure to prevent HBV infection rebound from cccDNA is the key challenge to cure HBV
Cytosine Base Editors (CBE) convert C-G into T-A without double-stranded breaks

- Cytosine Base Editor (CBE) consists of a partially inactivated CRISPR protein fused to a deaminase enzyme
- Guide RNA (gRNA) directs the CBE to a target genomic DNA sequence and exposes the narrow editing window
- Deaminase chemically modifies target cytosine (C) to uracil (U) via deamination
- Uracil (U) is replaced into thymine (T) during DNA repair and/or replication.

Additional features to improve C>T editing outcome
- CBE is fused to uracil glycosylase inhibitor (UGI) to block base excision repair system
- Cas9 retains nickase activity, which promotes mismatch repair system to resolve U-G into T-A
Base editing strategy: Targeting HBV genome with cytosine base editor (BE4)

- Precise and permanent introduction of stop codons / missense mutations in viral genes without generating double-stranded breaks
  - Viral gene silencing
  - Editing multiple sites simultaneously, without the risk of chromosome translocations

- The goal is to achieve functional HBV cure using base editing:
  - prevent HBV rebound by introducing permanent mutations in cccDNA
  - Irreversibly silence HBsAg expression from the integrated HBV DNA
HBV genotype D chosen as a relevant viral genome sequence

Identifying conserved HBV regions with a focus on genotype D

- Most abundant in US
- Existence of cellular and animal models

Geographic distribution of hepatitis B virus genotypes worldwide (World Gastroenterology Organisation Global Guideline, 2015)
Guide RNA selection and screen in Hek293-Lenti-HBV system

- Targeting conserved HBV regions with a focus on genotype D
- \textit{In silico} gRNA selection based on their potential to silence HBV genes
  - 100 gRNAs introducing Stop codons in viral genes (NGG, NGA, NNNRRT PAMs)
  - 24 conserved gRNAs predicted to introduce missense mutations

- Final selection step: editing in Hek293-Lenti-HBV cell line

Selected gRNAs

<table>
<thead>
<tr>
<th>ID</th>
<th>HBV gene</th>
<th>% Functional Edit</th>
<th>% Conservation across HBV genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>C01</td>
<td>Stop-PreCore</td>
<td>42</td>
<td>95</td>
</tr>
<tr>
<td>C02</td>
<td>Stop-PreCore</td>
<td>70</td>
<td>65</td>
</tr>
<tr>
<td>P01</td>
<td>Stop-Pol</td>
<td>68</td>
<td>22</td>
</tr>
<tr>
<td>P02</td>
<td>Stop-Pol</td>
<td>66</td>
<td>15</td>
</tr>
<tr>
<td>X01</td>
<td>Stop-X</td>
<td>65</td>
<td>76</td>
</tr>
<tr>
<td>X02</td>
<td>Stop-X</td>
<td>40</td>
<td>72</td>
</tr>
<tr>
<td>S01</td>
<td>Stop-S</td>
<td>57</td>
<td>40</td>
</tr>
<tr>
<td>P/S1</td>
<td>Pol/S</td>
<td>47</td>
<td>96</td>
</tr>
<tr>
<td>P/S2</td>
<td>Pol/S</td>
<td>25</td>
<td>92</td>
</tr>
<tr>
<td>P/S3</td>
<td>Pol/S</td>
<td>65</td>
<td>95</td>
</tr>
<tr>
<td>P/S4</td>
<td>Pol/S</td>
<td>49</td>
<td>92</td>
</tr>
</tbody>
</table>
Functional gRNA screen in HBV-infected HepG2-NTCP

Assessment of HBV parameters:
- **Extracellular**
  - HBsAg
  - HBeAg
- **Intracellular**
  - Total HBV DNA
  - 3.5kb viral RNA
Functional gRNA screen in HBV-infected HepG2-NTCP identifies two lead gRNAs targeting HBsAg and Core genes

- **gRNAS01** (Stop-S) reduces HBsAg
- **gRNAC02** (Stop-PreCore) efficiently reduces HBeAg
- Each also lowers the total HBV DNA and 3.5kb RNA levels
Multiplexing gRNAs simultaneously reduces respective HBV viral parameters in HepG2-NTCP

BE4 & gRNAS01(Stop-S) + gRNAC02(Stop-PreCore)
- Target same cccDNA strand; > 1kb distance in between
- Multiplexing gRNAS01+gRNAC02 reduces HBsAg and HBeAg, as well as total HBV DNA and 3.5kb RNA
Base editors function through cccDNA editing, without reducing cccDNA level

- HepG2-NTCP
- cccDNA enriched through ExoI/ExoIII nuclease treatment

- Base editing does not affect cccDNA level

- Robust cccDNA editing
  - ~38% for gRNAs01 (Stop-S)
  - ~60% for gRNAC02 (Stop-PreCore)
Efficacy of base editing + lamivudine in HepG2-NTCP

Objectives:

1) Pretreatment with lamivudine will remove intermediate HBV DNA species, leaving only cccDNA – efficacy in this system would confirm MoA

2) Assessing the efficacy of the combinatorial treatment

Lamivudine – standard of care antiviral, nucleoside reverse transcriptase inhibitor, which blocks HBV viral replication
Pretreatment with lamivudine improves editing, which results in high antiviral efficacy in HepG2-NTCP

- Pretreatment with Lamivudine improves base editing by 20% in HepG2-NTCP
- High cccDNA editing in Lam pretreated conditions suggests that CBE directly targets cccDNA
- Combinatorial treatment leads to robust reduction of HBV viral markers

![Diagram of experimental setup](image)

**Functional cccDNA Editing, C>T, %**

- BE4 & gRNAs(S01+C02)
  - gRNAC02 site
  - gRNAS01 site

- - Lam
- + Lam

**Graphs showing HBsAg, HBeAg, and 3.5kb RNA levels**

- Lamivudine pretreatment for 3 days
- HBV Infection 4 days post infection
- Transfection BE4 mRNA&gRNAs(S01+C02)
Primary human hepatocyte (PHH) co-culture system for longitudinal studies of HBV infection

- Primary hepatocyte co-culture system (PhoenixBio) maintains hepatocyte differentiation and metabolic activity for over 30 days
- Persistent HBV infection, maintains cccDNA level

Assessment of antiviral activity of base editing relative to lamivudine

**Conditions:**
1) HBV, non treated control
2) Lamivudine, 8 days, then discontinued
3) BE4 / control gRNA
4) BE4 / HBV_gRNAs(37+40)

**HBV parameters assessment:**
- Extracellular
  - HBV DNA (time course)
  - HBsAg
  - HBeAg
- Intracellular
  - HBV DNA total
  - 3.5kb RNA
  - cccDNA editing

**Diagram:**
- PHH
- HBV infection
- Lamivudine treatment for 8 days
- Day post infection: 0, 4, 11, 25
- Transfection 1: BE4 mRNA & gRNAs
- Transfection 2: BE4 mRNA & gRNAs
Base editing prevents HBV rebound in primary hepatocyte co-cultures

- HBV rebounds after discontinuation of lamivudine
- No HBV rebound for 2 weeks after the 2nd transfection with the base editing reagents
Base editing prevents HBV rebound in primary hepatocyte co-cultures

- **Reduction of all HBV viral markers by 70-80% at the end of the experiment**
- **HBV rebounds after discontinuation of lamivudine**
- **No HBV rebound for 2 weeks after the 2nd transfection with the base editing reagents**

---

**Graphs:**
- **HBV replication assessed by HBV DNA qPCR in s/n**
- **HBsAg**
- **HBeAg**
- **3.5 kb RNA**
- **HBV DNA total, intracell.**

---

**Table:**

<table>
<thead>
<tr>
<th>Days post infection</th>
<th>Lamivudine</th>
<th>BE4 &amp; control gRNA</th>
<th>BE4 &amp; HBV gRNAs(S01+C02)</th>
<th>Lam 8 day treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Base editing prevents HBV rebound in primary hepatocyte co-cultures

- ~30% Editing S antigen and ~60% Editing PreCore gene sufficient to enable high antiviral efficacy and prevent rebound in PHH
- HBV rebounds after discontinuation of lamivudine
- No HBV rebound for 2 weeks after the 2nd transfection with base editing reagents
Conclusions

• Cytosine base editing results in gRNA-specific reduction of HBV viral markers in relevant in vitro systems

• Multiplexing two gRNAs introducing Stop codons with CBE leads to a simultaneous reduction of HBsAg, HBeAg, HBV DNA, and 3.5kb RNA in HepG2-NTCP and primary hepatocytes

• Reduction in viral markers appear to be driven by base editing of cccDNA, but not a reduction in cccDNA levels

• Combinatorial treatment of the base editing reagents with standard antiviral lamivudine results in higher base editing efficiency

• Base editing prevents HBV rebound in infected primary hepatocytes

➢ Cytosine base editing introduces permanent mutations in cccDNA preventing HBV rebound in relevant in vitro models
Thank You

Beam therapeutics

Liver Therapeutics
- Francine Gregoire
- Michael Packer
- Selam Dejene
- Yvonne Aratyn-Schaus
- Tom Fernandez
- Rosie Chen
- Genesis Lung
- Lo-I Cheng
- Kara Hoar

Computational
- Luis Barrera

Gene Editing Platform
- Nicole Gaudelli
- Yi Yu

mRNA Team
- Valentina McEneany
- Jason St. Laurent

Analytical Development
- Carlo Zambonelli
- Bo Yan
- Jeff Marshall

Cell Biology
- Deborah Wysong
- Jamie Durbin

INSERM/UCBL Team – Lyon
- Fabien Zoulim
- Maria Guadalupe Martinez
- Barbara Testoni
- Emmanuel Combe

Non-viral delivery team
- Robert Dorkin
- Raymond Yang

A-Team/NGS Team
- Bob Gantzer
- Matt Humes
- Jeremy Decker

In vivo Team
- Dominique Leboeuf
- Monique Otero
- Sarah Smith

Leadership / BD
- Giuseppe Ciaramella
- John Evans
- Elbert Chiang
- Charlie Liu
- Stephen Cavnar
- Courtney Wallace
Questions