In vivo evaluation of anti-HBV CRISPR/Cas9 therapy in the FRG mouse

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December 5, 2017
Gene editing as an approach to cure HBV

- Approved antiviral drugs only inhibit replication
- cccDNA persists in hepatocyte nucleus throughout its life time
- cccDNA elimination or inactivation could prevent HBV persistence
Gene editing ≠ Cas9

Zinc finger nuclease
- 2 ORFs
- ~1.6 kb coding sequence
- Leaves 5’ overhangs
- Relatively simple retargeting
- Moderately high specificity
- Moderately difficult vectorization/delivery

TAL effector nuclease
- 2 ORFs
- ~2.7 kb coding sequence
- Leaves 5’ overhangs
- Very simple retargeting
- High specificity
- Difficult vectorization/delivery

Homing endonuclease
- 1 ORF
- ~0.8 kb coding sequence
- Leaves 3’ overhangs
- More difficult retargeting
- High specificity
- Easiest vectorization/delivery

CRISPR/Cas
- 1 ORF + guide RNA
- >3.3 kb coding sequence
- Leaves blunt ends
- Trivial retargeting
- Specificity controversial
- Difficult vectorization

modified from Schiffer et al, J Virol 2012
Gene editing as a genetic therapy

If cleavage and mutation were to occur within the coding sequence for an essential viral protein, viral production and pathogenesis would be prevented.
### Potential Viral Targets for Gene Editing

<table>
<thead>
<tr>
<th></th>
<th>HIV</th>
<th>HBV</th>
<th>HSV</th>
<th>HPV</th>
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<tbody>
<tr>
<td><strong>Prevalence</strong></td>
<td>+</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td><strong>Disease severity</strong></td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td><strong>Genetic variability</strong></td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>++</td>
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<tr>
<td><strong>Ease of delivery</strong></td>
<td>---</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
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<tr>
<td><strong>Need for complete eradication</strong></td>
<td>---</td>
<td>+</td>
<td>+</td>
<td>++</td>
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</tbody>
</table>
HBV-specific ZFNs inhibit HBV replication

scAAV-LK03 transduces human hepatocytes in FRG mice

scAAV-LK03-smCBA-eGFP
14 days post intravenous delivery
HBV replication in the FRG mouse
(Genotype C)

HBV Genome Copies (Log 10/mL)

- Vehicle
- Entecavir
- Compound A
- Compound B

Day 3 (predose)  Day 30  Day 37  Day 44  Day 51  Day 58  Day 66  Day 73  Day 80  Day 87
S. aureus CRISPR/Cas9 for HBV

- S. pyogenes Cas9 has shown good activity against HBV in vitro
- spCas9 is too large to be effectively used with AAV vectors
- S. aureus Cas9 is smaller and can be readily used with AAV vectors
HBV genotype C saCas9 sgRNA test

Reporter constructs (4 total)

MND → eGFP → SV40pA

Target 1-Target 2-Target 3

Untreated

Reporter

GFP6-20

GFP7-20

GFP+ve cells (% of untreated)

NTC  GFP-6-20  GFP-7-20  C7  C14  C16

All  Mid-Hi  Hi
Timeline for HBV-C+ FRG mice

IV AAV delivery
5 x 10^{11} vg/mouse

Entecavir

No Entecavir

Weeks

0 4 8

Anti-GFP

Anti-HBV

* scheduled sacrifice
** found dead (No terminal blood sample)
*** sacrificed due to health (Terminal blood sample)
# sacrificed early as part of group B1
In vivo tolerability of anti-HBV gene editing

No weight loss that differed from animals receiving control anti-GFP therapy

No morbidity or behavioral findings that differed from control animals

No histopathologic changes attributable to AAV/Cas9 therapy

Study Day versus Body Weight (Weekly)
in vivo gene editing of HBV

<table>
<thead>
<tr>
<th>Liver samples</th>
<th>Mouse ID#</th>
<th>anti_GFP or anti-HBV</th>
<th>Liver collection day</th>
<th>C14 Mutation</th>
<th>C7 mutation rate</th>
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<tr>
<td>4M006</td>
<td>HBV</td>
<td>28</td>
<td>0</td>
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</tbody>
</table>
Viral loads during CRISPR/Cas9 treatment

AAV day 0

HBV IU/ml

10^10
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2

-24 -17 -10 -3 7 14 21 28 35 42 49 -10 -17 14 56

10^3
10^4
10^5
10^6
10^7
10^8
10^9
10^10

SLU qPCR

UW qPCR

1M001*
1M002*
2M001*
2M002*
3M001
3M002
3M003*
3M004*
4M001
4M002
4M003
4M004*
4M005*
4M006

Anti-GFP

Anti-HBV

Premature death

Planned sacrifice day 28

Entecavir

Days
Viral loads during CRISPR/Cas9 treatment

AAV day 0

HBV IU/ml

SLU qPCR

UW qPCR

Anti-GFP

Anti-HBV

Entecavir
Conclusions from initial in vivo trial

• in vivo therapy with AAV/Cas9 is well tolerated
• Gene editing of HBV in liver can be achieved
• No gene-edited HBV was observed in plasma, consistent with loss of replicative capacity
• Low-level gene editing does not result in decreased plasma viremia

Next steps

• What was the cause of low editing frequency?
  • poor viral suppression with entecavir
  • Is Cas9 the optimal enzyme?
  • confirmation of efficient hepatocyte transduction and Cas9 expression
• quantitation of gene editing in rcDNA vs. cccDNA
Acknowledgments

Daniel Stone
Martine Aubert

Tom Andrus
Chung Dang

Harshana De Silva Feelixge
Meei-Li Huang
Shiu Liang
Michelle Loprieno

Nixon Niyonzima
Harlan Pietz
Ruth Hall Sedlak
Larry Stensland
Nick Weber
Mary Lamery

Alexander Astrakhan
Paula Cannon
Jordan Jarjour
Hans-Peter Kiem
David Rawlings
Pavitra Roychoudhury
Andrew Scharenberg
Joshua Schiffer
Barry Stoddard

7th Wave Labs
Yecuris
Cellectis
Pregenen/Bluebird
Sangamo

UW Medicine
LABORATORY MEDICINE
VIROLOGY

defeatHIV

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UNIVERSITY OF WASHINGTON

Caladan Foundation

Fred Hutchinson Cancer Research Center
Seattle, WA

Northwest Genome Engineering Consortium