Tech Spec Review
Hepatitis C Treatment

By Jenny Leanna Samira
Introduction

- What is hepatitis C?
- Why we chose it?
How it works?
Chimp study - why nucleosides

- Study tested in vivo effects of nucleoside in chimpanzees
- Injecting 0.2-2 mg of nucleoside/kg of body weight for 12 days
- Found that there was a >5log10(original IU concentration) decrease to the point where they could no longer detect the presence of IU
- Improvement through cell uptake
Model Structure of HCV
IN VITRO

Inject DNA sequence, coding for nucleocapsid and envelope proteins in nucleus of liver culture cells. Inject RNA that codes for nucleosides in the cytoplasm.

After transcription of DNA, via translation the capsid proteins are formed. RNA replication produces multiple copies of the RNA.

Exocytosed HCV debuggers capsids are collected.

The RNA and the capsids assemble and are ready for exocytosis.
IN VIVO

Collect HCV debuggers from liver cell culture

Inject them into blood stream of human infected with HCV

The HCV debuggers are directed to the liver. The endocytose into the liver cell, injecting the engineered RNA.

The RNA is translated to make the specialized nucleosides, which should prevent viral replication.
Engineering the capsid producing DNA
DNA Plasmid for Debugger

- Doxycycline

DNA Plasmid for Debugger

+ Doxycycline
Engineering nucleoside RNA - 1

RNA for Nucleoside Production

5’ NTR

RNA Polymerase

Ribose production

Adenine production

Proteins for Methylation

3’ NTR

Chemical structure of a nucleoside.
Engineering nucleoside RNA - 2

RNA for nucleoside production

5’ NTR  |  3’ NTR

RNA Polymerase  |  Dephosphorylating Enzyme  |  Methylating Enzyme  |  Regulating Enzyme

[Diagram of RNA structure with specific enzymes and chemical structure of nucleoside]
After injecting debuggers in vivo, we would test for a reduction in viral load in 48 hours.
### Parts list – In Vitro

<table>
<thead>
<tr>
<th>Parts</th>
<th>Sourcing</th>
<th>Description</th>
<th>Annotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver Cells</td>
<td>PromoCell</td>
<td>Human Hepatocyte</td>
<td>Will produce HCV capsids</td>
</tr>
<tr>
<td>DNA (Capsid, E1, E2, GFP, terminator)</td>
<td>Integrated DNA Technologies</td>
<td>Deoxyribonucleic acid</td>
<td>Codes for capsid and proteins</td>
</tr>
<tr>
<td>Tet repressor</td>
<td>sequence from Reg. Standard Biological Parts</td>
<td>Controls expression of our DNA</td>
<td>When Tet is present, gene will not be expressed</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>Next Tag</td>
<td>Antibiotic</td>
<td>Allows DNA to be expressed</td>
</tr>
<tr>
<td>Capsid, E1, E2</td>
<td>Sequence from National Center for Biotechnology Information</td>
<td>The capsid and glycoproteins from HCV</td>
<td>Capsid will form around our synthetic RNA and target it to liver cells</td>
</tr>
<tr>
<td>RNA</td>
<td>Integrated DNA Technologies</td>
<td>Ribonucleic acid</td>
<td>codes for mechanism that will make our nucleosides</td>
</tr>
<tr>
<td>Plasmid Backbone</td>
<td>Sequence from Registry of Standard Biological Parts</td>
<td>Circular DNA</td>
<td>Shuttle for DNA</td>
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<tr>
<td>Parts</td>
<td>Description</td>
<td>Annotation</td>
<td></td>
</tr>
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<td>------------------------------</td>
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</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
<td>Codes for mechanism that will make our nucleosides</td>
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</tr>
<tr>
<td>Sequence for RNA Polymerase</td>
<td>Will code for RNA Polymerase</td>
<td>Copy itself, don’t have to inject every day</td>
<td></td>
</tr>
<tr>
<td>Adenine Production Enzyme</td>
<td>Codes for Enzyme that constructs Adenine</td>
<td>Basic structure of nucleoside</td>
<td></td>
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<tr>
<td>Ribose Sugar Enzyme</td>
<td>Adds Ribose Sugar to Adenine</td>
<td>Adenine becomes basic, common nucleoside: Adenosine</td>
<td></td>
</tr>
<tr>
<td>Proteins for Methylation</td>
<td>Proteins methylate adenosine</td>
<td>Methylation makes the nucleoside specific to viral RNAP</td>
<td></td>
</tr>
<tr>
<td>Sequence for GFP</td>
<td>Codes for Green Fluorescent Protein</td>
<td>Will assist in debugging and validation</td>
<td></td>
</tr>
<tr>
<td>Part</td>
<td>Source</td>
<td>Price</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>Doxycycline</td>
<td>Next Tag</td>
<td>$ 3.50</td>
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<tr>
<td>Liver Cells</td>
<td>Promocells</td>
<td>$ 794 / 3-6* 10^6 cells</td>
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<tr>
<td>DNA</td>
<td>Integrated DNA Technologies</td>
<td>$ 1 / base</td>
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</tr>
<tr>
<td>RNA</td>
<td>Integrated DNA Technologies</td>
<td>$ 1 / base</td>
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</tr>
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</table>
Plan for debugging and validation

- **In vitro:**
  - GFP – to detect capsid formation
  - Validation of nucleosides – infect liver culture cells, and then use debuggers to see if they stop viral replication.

- **In vivo:**
  - Taqman assay on infected human and chimp
Impact of solution

- In the chimps experiment, decrease in Infectious Units per milliliter by $1.0 \log_{10}(\text{original IU/ml})$ when Chimps injected with 0.2 mg/kg of body weight per day.

- If we succeed...by enhancing the uptake of nucleoside by the liver cells, we should see a greater decrease in IU/ml of viral load!
Buildable

- Everything exists!
- Black boxes need to be filled in!
Safety

- HCV debuggers are non-infectious.
- The methylated nucleosides are not toxic in the known dosages.
- In vitro work should be carried out in a biosafety lab 3 environment, with necessary precautions.
Security

- Design should not be available to the general public, due to potential for remodeling HCV into another infectious virus!
- Do not want to disclose of the hook that helps RNA assemble with capsid!
Unknowns

- Detailed mechanism for nucleoside production remains unknown.
- Exact mechanism for methylation of nucleosides due to commercialization of product.
- How nucleoside production would affect the energy of the liver cell.
Open issues and ethics

- Human and chimp test subjects will be needed for final validation of design...

- We are using the outer proteins of HCV ... but just as a vaccine...

- Nucleoside production vs. Liver cell energetics
Go/No Go decision

GO
References

- http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6TF9-4DXC1JJ-L&_user=501045&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000022659&_version=1&_urlVersion=0&_userid=501045&md5=08c13591744cba60a58d591f58fb7a
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