Anti-NASH Effects of Solithromycin in NASH-HCC Mouse Model

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What is Solithromycin?

Currently Approved Macrolide Antibiotics

Erythromycin
Clarithromycin
Azithromycin

Solithromycin – the first fluoroketolide

- More potent than older macrolides
- Activity against resistant strains
- Better stability, better PK
- Oral, intravenous and pediatric suspension
- Well tolerated so far, Phase 1 and Phase 2
- 2 Global Phase 3 trials for pneumonia (CABP) enrolling

Solithromycin
Solithromycin’s Broad Use Potential

**Respiratory Tract Infections (RTI)**
HAP, Simple RTI’s, Pharyngitis, Sinusitis, Bronchitis, Acute Exacerbation of Chronic Bronchitis (AECB)

**Anti-inflammatory**
COPD, Cystic fibrosis, Panbronchiolitis

**Pediatrics and Pregnancy**
Pediatric suspension with broad potential in development
Infections in Pregnancy – neonatal sepsis
Infections in Utero – premature, cerebral palsy, autism

**Multiple Unidentified Pathogens**
Anthrax, Tularemia

**Genital Infections**
Most common reportable infectious diseases.

**Other Infections**
Helicobacter Gastritis, Campylobacteria, Tick and Insect Borne Diseases, Diarrhea, and Ophthalmic Drops

**Special Populations**
BARDA funded
Pediatrics and Pregnancy
Infections in Pregnancy – neonatal sepsis
Infections in Utero – premature, cerebral palsy, autism

**Biodefense**
BARDA funded
Multiple Unidentified Pathogens
Anthrax, Tularemia

**Sexually Transmitted Diseases**
Most common reportable infectious diseases.

**GI & Others**
Ophthalmic
Helicobacter Gastritis, Campylobacteria, Tick and Insect Borne Diseases, Diarrhea, and Ophthalmic Drops
A Novel Macrolide Solithromycin Exerts Superior Anti-inflammatory Effect via NF-κB Inhibition

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Macrolides are used as anti-inflammatories in treating COPD patients

Solithromycin has been shown to have stronger anti-inflammatory properties than the older macrolide antibiotics

Macrolides upregulate HDAC2 promoter – which results in a decrease in cytokine production
Why Test Solithromycin in NASH?

- The strong anti-inflammatory effects of solithromycin will be tested in a Phase 2 study in COPD
- Solithromycin achieves high liver concentrations (as in the lung)
- Since inflammation is known to play a key role in NASH we decided to study the effect of solithromycin in NASH
- Large and growing body of safety data on solithromycin:
  - Well tolerated in approx. 1000 patients and subjects to date – oral capsules, intravenous, pediatric suspension in development
  - Well tolerated in hepatic insufficiency patients – mild, moderate and severe. No dose adjustments needed.
  - No QT effect unlike older macrolides – TQT study complete
  - 90 day toxicology in rat and NHP complete
STAM™: In Vivo Predictive Pharmacology Model for NASH

Healthy Liver → Steatosis → NASH → Fibrosis → HCC

1. **CHEMICAL**
   - 1st Hit: Low dose streptozotocin
   - Pregnant C57BL/6J mice

2. **DIET**
   - Continuous 2nd Hit: High fat diet feeding

Following the initial diabetes model evolves into a model for NASH

- Steatosis evident
- NASH evident
- Fibrosis evident
- Nodule evident
- HCC evident

All mice at 6 weeks have a NAFLD Activity Score (NAS) ≥ 5
Similar to human NASH

Steatosis (+)
ALT↑
NAFLD activity score↑
### NAFLD Activity Score (NAS)

<table>
<thead>
<tr>
<th>Histological feature</th>
<th>Score</th>
<th>Category definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Steatosis</strong></td>
<td>0</td>
<td>&lt;5%</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>5–33%</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>34–66%</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>&gt;66%</td>
</tr>
</tbody>
</table>

**Plus**

| Hepatocyte ballooning | 0     | None                |
|                      | 1     | Few                 |
|                      | 2     | Many                |

**Plus**

| Inflammation         | 0     | None                |
|                      | 1     | 1–2 foci per ×20 field |
|                      | 2     | 2–4 foci per ×20 field |
|                      | 3     | >4 foci per ×20 field |

**NAS total 0–8**

<table>
<thead>
<tr>
<th>Histological feature</th>
<th>Score</th>
<th>Category definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fibrosis</strong></td>
<td>0</td>
<td>No fibrosis</td>
</tr>
<tr>
<td></td>
<td>1a</td>
<td>Zone 3 mild perisinusoidal fibrosis</td>
</tr>
<tr>
<td></td>
<td>1b</td>
<td>Zone 3 moderate perisinusoidal fibrosis</td>
</tr>
<tr>
<td></td>
<td>1c</td>
<td>Periportal/portal fibrosis only</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Zone 3+periportal/portal fibrosis</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Bridging fibrosis</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Cirrhosis</td>
</tr>
</tbody>
</table>

**Fibrosis score 0–4**

**A score of ≥5 with steatosis and hepatocyte ballooning is generally considered diagnostic of NASH**

Study Plan for Assessing the Anti-NASH/fibrosis Effects of Solithromycin in STAM™ Model of NASH

 Analyses

1. General
   - Body weight
   - Liver weight
   - Liver-to-body weight ratio

2. Biochemistry
   - Whole blood glucose
   - Plasma ALT
   - Liver TG

3. Histopathological assay
   - HE staining (NAFLD Activity score)
   - Sirius red staining (Fibrosis area)
   - Immunohistochemistry for F4/80 (Inflammation area)

4. Gene expression assay
   - TNF-α
   - MCP-1
   - MMP-9

- 50 mg/kg mouse dose = 240 mg adult human equivalent dose (Conversion of animal doses to HEDs based on body surface area FDA guidance 2012)
- 800 mg LD/400mg MD is being used in our current pneumonia clinical trials
Mouse Weight and Liver Weight on Week 9

Steatosis seen in week 9 in the model

Student’s T-test
Liver Morphology and Function

Macroscopic Appearance

**Blood Glucose**

Mean ± SD
Student’s T-test

P < 0.01
**Additional Chemistry**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vehicle (n=8)</th>
<th>Solithromycin (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma ALT (U/L)</td>
<td>48 ± 11</td>
<td>50 ± 9</td>
</tr>
<tr>
<td>Liver triglyceride (mg/g liver)</td>
<td>43.3 ± 7.0</td>
<td>36.8 ± 13.7</td>
</tr>
</tbody>
</table>

- ALT not decreased
- No effect on liver triglyceride concentration

Mean ± SD
Student’s T-test
H & E Stained Liver Sections

All of the untreated mice had a NAFLD score of ≥5 and none of the solithromycin treated mice had this score.

NAFLD Activity Score

NAFLD Activity score (NAS) calculated according to Kleiner DE et al. Hepatology. 2005;41:1313-1321.
Components of NAFLD Activity Score

Hepatocyte Ballooning Score

- Mean ± SD
- Student's T-test
- P<0.0001

Lobular Inflammation Score

- Mean ± SD
- Student's T-test
- P<0.01

Steatosis Score

- n.s.

Control untreated

Treated
Short duration of treatment is not expected to improve fibrosis. Fibrosis is not massive even in vehicle treated controls in this study.

Continued treatment, with continued improvement in inflammation could provide a reduction in fibrosis.
Photomicrographs of F4/80 Immunostained Liver Sections

F4/80 antigen is a macrophage-restricted cell surface glycoprotein and the stain is very specific for macrophages.
Future experiments will look at other inflammatory markers will be examined:
- Collagen Type 1, α-SMA, TIMP-1 and TGF-β.
- CK-18 biomarker

Insulin resistance markers
Possible Mechanism of Action
Solithromycin Has No Effect On FXR Signal Pathway

- FXR agonist and antagonist activity measured in hybrid human FXR receptor expressing reporter cell assays
- Solithromycin up to 30 μM

Unlike Obeticholic acid from Intercept, Solithromycin (CEM-101) does not show agonistic activity nor significant antagonistic activity in the human FXR assays

Solithromycin does not show evidence of cytotoxicity in the antagonist assays
Does Solithromycin Act Through It’s Antibacterial Effects in NASH?

- Endotoxin is not likely to be released by solithromycin
  - It does not effect aerobic or anaerobic Gram-negative intestinal bacteria
  - Minimal effect on bowel flora
- Solithromycin is well absorbed after oral administration (78%) and has very little active solithromycin is in the intestinal tract
  - <15% of unchanged solithromycin is found in the feces

Susceptibility of Anaerobic Intestinal Bacteria

<table>
<thead>
<tr>
<th>Organism (No.)</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt; (mcg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroides spp. including B. fragilis (22)</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Prevotella spp. (10)</td>
<td>4</td>
</tr>
<tr>
<td>Porphyromonas spp. (10)</td>
<td>0.06</td>
</tr>
<tr>
<td>Peptostreptococcus spp. (10)</td>
<td>0.25</td>
</tr>
<tr>
<td>Clostridium spp. (10)</td>
<td>0.06</td>
</tr>
<tr>
<td>C. difficile (10)</td>
<td>&gt;64</td>
</tr>
</tbody>
</table>

Antibacterial Effect on Intestinal microflora – Unlikely

- Very high liver concentrations of solithromycin
  - Approximately 70% of the oral dose is metabolized and excreted by the liver
- It is possible that solithromycin is exerting its anti-inflammatory properties in the liver as it does in the lung in COPD

Anti-inflammatory Mechanism

- It does not effect aerobic or anaerobic Gram-negative intestinal bacteria
- Minimal effect on bowel flora
Anti-inflammatory Effect of Solithromycin in a mouse COPD Model

Neutrophil and pro-MMP9 production are inhibited by solithromycin in cigarette-smoke exposed murine lung

SM = Cigarette smoke
EM = Erythromycin
SOL = Solithromycin
Solithromycin
Clinical Development Status to Date
Solithromycin: Clinical Development Status

- Two global Phase 3 studies for community acquired bacterial pneumonia (CABP) enrolling ~800 patients each
- Phase 1 SD, MD, Food effect, DDI, TQT, hepatic insufficiency complete
  - No food effect and no dose adjustment needed in hepatic insufficiency patients. No QT effect.
- Phase 2 CABP – Effective and well tolerated. 5 days QD 800mg LD / 400mg MD
- Approx. 1000 patients or subjects have had up to 5-7 days treatment
- All toxicology studies complete for NDA for CABP
  - 3 month toxicology in rat and NHP complete – 125 mg/kg/day

**Mean Cmax:** 0.02 to 1.96 μg/mL
**Mean AUC\text{inf}:** 0.06 μg•h/mL to 28.9 μg•h/mL
**Mean t_{\text{max}}:** 1.5 to 6.0 hours
**Mean T1/2:** 3.2 to 7.4 hours

Safe and well tolerated – Tested up to 1600 mg SD
Next Steps

- **STAM™** Mouse model is being repeated with dose titration
  - Determination of lowest optimal dose and duration of treatment
  - Determination of duration of effect

- Characterization of additional inflammatory markers

- **MOA in NASH studies**

- We are planning a Phase 2 dose ranging study in NASH patients
  - 3 month treatment period – possible with current toxicology data

- Chemical synthesis program to modify solithromycin to find a new compound without antibacterial properties
  - Next generation product
Key Take Away Messages

- Solithromycin, a novel fluoroketolide, has shown statistically significant effects in decreasing the NAFLD score, including drastically reducing ballooning degeneration and inflammation in the STAM™ mouse model.

- Data indicates that the mechanism is mediated by the anti-inflammatory effects of solithromycin.

- Solithromycin is well tolerated in clinical trials in bacterial pneumonia – approx. 1000 patients/subjects have been exposed for multiple days.

- We expect to follow up these exciting mouse data by starting a Phase 2 study in NASH.