Liver Disease NASH/Fibrosis Model

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The Liver Disease Spectrum

- Fatty liver
- Cytokines
- Infection
- Injury
- Scarring
- Fibrosis
- Cirrhosis
- Liver cancer

Connective tissue overtakes normal hepatic function.
Liver Diseases Comprise a Growing Market

NAFLD: 10-20% of Americans may suffer undiagnosed.
NASH: Estimated to affect 2-5% of Americans.
Cirrhosis: Approx. 25% of NASH patients progress.
Liver Cancer: Third highest cancer mortality, globally.

SUMMARY FIGURE
GLOBAL REVENUE FOR DRUGS USED TO TREAT LIVER DISEASES BY REGION, 2009-2016
($ MILLIONS)
Mouse Non Alcoholic Steatohepatitis (NASH) Model:

1. Pancreas β-cell insult (STZ) and subsequent liver injury in pups.
2. High fat diet-induced obesity.
3. Progressive liver disease leading to the induction of liver fibrosis.

*Induction of Macrophage-led Injury to the Liver*
The Role of Macrophages in Fibrogenesis is Well Established

Liver fibrosis and repair: immune regulation of wound healing in a solid organ
Antonella Pellicoro, Prakash Ramachandran, John P. Iredale & Jonathan A. Fallowfield

Obesity is associated with macrophage accumulation in adipose tissue
Stuart P. Weisberg¹, Daniel McCann¹, Manisha Desai², Michael Rosenbaum¹, Rudolph L. Leibel¹,³,⁴ and Anthony W. Ferrante, Jr.³,⁴

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²Department of Biostatistics,
³Department of Medicine, and
⁴Naomi Berrie Diabetes Center, Columbia University, New York, New York, USA
NASH Model Details at Woodland – Status

NASH/Liver fibrosis mouse model
High fat diet induced

- Involves a 2 hit approach
  - 1\textsuperscript{st} hit – chemical intervention leads to metabolic distress
  - 2\textsuperscript{nd} hit – dietary intervention that enhances metabolic distress and induces fibrotic remodeling of the liver

- Animals develop type I diabetes and a fibrotic liver with eventual development of hepatocellular carcinoma (HCC)

NASH/Liver fibrosis rat model
Carbohydrate-driven, \textit{under development}

- Involves a dietary intervention alone where high carbohydrate and cholesterol diet leads to metabolic disease and fibrotic remodeling of the liver
- Animals develop type II diabetes and a fibrotic liver with hyperglycemia, dyslipidemia, liver steatosis, abdominal fat deposition, hypertension and hyperinsulinemia.
- Closer to the majority of human disease as it involves dietary intervention alone
- May progress to HCC
- \textit{Currently in collaborator lab}
NASH model development—Study Plan

**ANALYSES**

**General**
- Body weight
- Liver weight
- Liver to body weight ratio

**Plasma**
- Glucose
- ALT/AST
- Inflammatory markers
- ELF Score
- Triglycerides

**Liver**
- Inflammatory markers
- ELF Score
- Hydroxyproline
- Triglycerides

**Histopathology**
- Liver H & E – NAS Score
- Sirius Red – Collagen/fibrosis
- Oil Red O - Steatosis

**COLLABORATORS**
- Woodland Biosciences
- Histologistics
- Tufts Veterinary School
- Brandeis University

**TIMELINE (Weeks)**

**Female C57 (pregnant)**
- Birth of pups

**Chemical hit #1:** STZ injection in pups

**High FAT DIET (Week 5-17)**
- Hepato-cellular carcinoma
- Hepato-cellular Nodule
- Oil Red O
- Sirius Red
- Liver H & E
- NASH
- Fatty Liver

(Bold are standard)
### NASH model: Data

#### Macroscopic view of the liver

![Control liver](image1.png)

![NASH liver](image2.png)

#### Liver weights

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NASH Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver weight (grams)</td>
<td><img src="graph1.png" alt="Graph" /></td>
<td><img src="graph2.png" alt="Graph" /></td>
</tr>
</tbody>
</table>

#### Liver to bodyweight ratio (%)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NASH Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="graph3.png" alt="Graph" /></td>
<td><img src="graph4.png" alt="Graph" /></td>
<td></td>
</tr>
</tbody>
</table>

#### BLOOD GLUCOSE (mg/dl)

<table>
<thead>
<tr>
<th>Animal Age</th>
<th>Week 5</th>
<th>Week 7</th>
<th>Week 10</th>
<th>Week 12</th>
<th>Week 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>198 ± 14</td>
<td>247 ± 48</td>
<td>149 ± 28</td>
<td>186 ± 1.5</td>
<td>271 ± 34</td>
</tr>
<tr>
<td>NASH</td>
<td>422 ± 173</td>
<td>435 ± 96</td>
<td>467 ± 110</td>
<td>432 ± 147</td>
<td>564 ± 162</td>
</tr>
</tbody>
</table>
NASH model: Histological parameters

- H&E
- Oil Red O
- Picosirius Red (Polarized)
- Picosirius Red

Fibrosis Score

Steatosis Score

NASH Activity Score
NASH model development
Collagen Expression

**Picrosirius Red** stains collagen I and III fibers

Large collagen fibers are orange under polarized light

* - Sirius red positive fibrosis staining
Comparative evaluation of High fat diet (Diet A) versus modified high fat diet (Diet B) supplementation on development of NASH in mice

- Mean body weight (terminal) was higher in the mice fed with diet A as compared to the mice receiving diet B
NASH model development– Diet Optimization

- Comparative evaluation of High fat diet (Diet A) versus modified high fat diet (Diet B) supplementation on development of NASH in mice

• The mean liver weights and the mean % liver to body weight ratios of the animals receiving diet B were greater than the animals receiving diet A
• The livers harvested from the animals receiving diet B appeared lighter (pale appearance) in color than the livers harvested from animals receiving diet A
NASH model development—Diet Optimization

- Comparative evaluation of High fat diet (Diet A) versus modified high fat diet (Diet B) supplementation on development of NASH in mice

The mean blood glucose levels were similar across the 2 study groups indicating hyperglycemia/diabetes (glucose > 300mg/dl)
NASH model development– Diet Optimization

- Comparative evaluation of High fat diet (Diet A) versus modified high fat diet (Diet B) supplementation on development of NASH in mice

<table>
<thead>
<tr>
<th></th>
<th>H&amp;E 10X</th>
<th>ORO 20X</th>
<th>Picrosirius Red 20X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet A</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>Diet B</td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
</tbody>
</table>

- Animals receiving diet-B showed greater fibrosis (picrosirius red staining) and greater steatosis (oil red O Staining) than animals receiving diet-A
- Digitized NAFLD imaging/scoring is currently under investigation
NASH model development– Diet Optimization

- Comparative evaluation of High fat diet (Diet A) versus modified high fat diet (Diet B) supplementation on development of NASH in mice

* (Certified pathology lab-manual scoring-blinded analysis)

- The terminal fibrosis score and NASH activity score was greater for the animals receiving diet-B relative to animals receiving diet-A
Sample study results – histology diet B and drug X

- Treatment with drug X has a marginal effect in reducing fatty deposits and collagen-staining in the liver tissue
- Digitized NAFLD imaging/scoring and statistical evaluation is currently under investigation
Biomarker Analyses

**ANALYTES***

**Plasma**
- Galectin-3 (Gal-3)
- ALT/AST
- Inflammatory markers
- ELF Score
- Triglycerides

**Liver**
- Galectin 3 (Gal-3)
- Inflammatory markers
- ELF Score
- Hydroxyproline
- Triglycerides

*Additional charges incurred*
Other *In-Vitro* Capabilities

- ELISA
- Capillary western blot (CLIA)
- Luminex
- qRT-PCR
- Flow cytometry (available through Worcester partners ATR)

*Fully capable to be your resource for inflammation*

- >30 Cytokines
- >40 Mabs
- >270 human cancer cell lines in 19 indications

- Bench-scale protein production
- Cytokine production and bioassays
- Cell-Based Assays (oncology & Inflammation)
Facilities

**In vitro laboratories**
Principal lab space for in vitro operations at our strategic partner, SBH Sciences in Natick, MA

**In vivo laboratories**
Animal rooms are within the AAALAC-accredited facilities at WPI, located in Gateway Park in Worcester, MA
Woodland Biosciences: A strategic alliance of CRO specialists

In vivo lab
60 Prescott Street, Worcester, MA

In vitro lab
Natick, MA

Plus an extensive network of partners