Novel Therapies in Alcoholic Hepatitis
UO1 Consortium
DASH - Defeat Alcoholic SteatoHepatitis

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Alcohol affects multiple organs

**BRAIN**
- inflammation
- cognitive impairment
- addiction

**LIVER**
- steatosis
- inflammation
- liver damage

**GUT**
- decreased barrier function
- microbial translocation
- inflammation

Alcohol
Inflammation is the response of the liver to danger signals from pathogens and/or injured self.
Inflammation is the response of the liver to danger signals from pathogens and/or injured self.
The pathogenesis of alcoholic liver disease is multifactorial

- Ethanol
- Adipose
- Adiponectin
- TNF, MCP-1
- Steatosis
- Hepatocyte damage
- Liver injury
- ROS
- Mitochondrial damage
The pathogenesis of alcoholic liver disease is multifactorial.

Key factors include:

- Steatosis
- Hepatocyte damage
- Adipose tissue factors such as Adiponectin, TNF, MCP-1
- Ethanol from the gut and increased bacterial overgrowth
- Mitochondrial damage

The diagram illustrates the interplay between these factors leading to liver injury.
The pathogenesis of alcoholic liver disease is multifactorial.
TLR4 downstream signaling

MyD88-independent signaling pathways play a key role in alcoholic liver disease

Mandrekar & Szabo, J Hepatol. Hritz et al, Hepatology Petrasek et al, Gastroenterology
Dual role of the innate immune signaling pathways and IRF3 in alcoholic liver disease

Petrasek et al, PNAS 2013
Interactions between cell death, TLRs and inflammasomes in ALD

TLR
Pattern recognition receptors

PAMPs (Signal 1)
LPS

Inflammasome
NALP-3
ASC
pro-Caspase1

pro-IL-1β

Nucleus

Endosome

mitophagy

DAMPs (Signal 2)
Uric acid
ATP
K+
HMGB1
ROS

mitochondria

mature IL-1β

apoptosis/
necrosis

Alcohol
Metabolic stress
FFA

Hepatocyte injury

Apoptosis/
Necrosis

Mitochondria

Hepatocyte

• Myeloid cells

• Hepatocyte
Progression of alcoholic liver disease

- Normal liver
- Fatty liver (90-100%)
- Alcoholic hepatitis (steatohepatitis) (10-35%)
- Cirrhosis (8-20%)
- Hepatocellular cancer (Upto 70%)
Progression of ALD

Acute alcoholic hepatitis

Cirrhosis

Fibrosis

Steatohepatitis

Fatty liver

ALD

Years
Knowledge gaps in alcoholic liver disease

1. Predisposing factors for ALD in the alcoholic patient
   - genetic, host, environmental factors, co-morbidities

2. Triggers of severe acute alcoholic hepatitis?

3. Clinical classification of the different clinical stages of ALD

4. Effective therapies

5. Biomarkers of ALD, predictors of clinical outcome and response to therapy
Novel Therapies in Alcoholic Hepatitis

Consortium Administration - UMMS
G Szabo

Cleveland Clinic
A McCullough

UT Southwestern
M Mitchell

Data collection/Statistics
B Barton

DSMB

Clinical Trial

UMass Med
G Szabo

Univ Louisville
C McClain

Translational Project -1
Cleveland Clinic
L Nagy

Translational Project -2
UMMS
G Szabo

Translational Project -3
Univ Louisville
C McClain

Translational Project-4
Mount Sinai
PI: N Nieto
Focus on key elements of the pathogenesis of alcoholic hepatitis

- Inflammatory cascade and innate immune activation
  - a demarcating feature of severe AH compared to mild to moderate alcoholic liver disease

- Gut integrity
  - that is significantly altered in alcoholic hepatitis allowing pathogen-associated molecular patterns (PAMPs) to enter the liver and systemic circulation and induce innate immune activation,

- Cell survival and death pathways
  - that contribute to liver dysfunction and the release of damage-associated molecular patterns (DAMPs) that further fuel inflammation.
- The syndrome of acute alcoholic hepatitis (AAH) results from severe inflammation and dysregulated cytokines.

- Gut derived endotoxins and other bacterial products that trigger inflammation are a consequence of increased permeability and altered gut barrier function.

- Compounds that improve the gut barrier function (both in moderate and severe disease) AND reduce the associated inflammation (severe disease) AND prevent the development of hepatorenal syndrome and other organ failure (severe disease) have utility in the treatment of severe AAH.
## Natural history of Acute Alcoholic Hepatitis: Lab values

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<th>AST</th>
<th>ALT</th>
<th>GGT</th>
<th>T. Bili</th>
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Two Multicenter Pilot Clinical Trials in AH

Identify Potential Subjects with Acute Alcoholic Hepatitis

Stratify for Disease Severity

MILD-MODERATE AH

- MELD <20
  - Novel Treatment

SEVERE AH

- MELD 20-31 + DF>32
- MELD >31 + DF>32
  - Novel Treatment

Observational study
Aim #1: Severe AH

Aim 1: Evaluation of the effects of corticosteroids versus a combination of interleukin-1 receptor antagonist plus pentoxifylline plus zinc supplements in patients with MELD > 20.

IL-1 receptor antagonist attenuates ASH and progression of liver damage in mice

Petrasek... Szabo. JCI 2013
Multicenter randomized double-blind pilot study in severe alcoholic hepatitis

SEVERE AH

- Primary outcome: 6 month mortality
- Secondary outcomes:
  - 30, 90 day mortality
  - changes in MELD at 30, 90, 180 days
  - changes in gut mucosal integrity
  - endotoxin levels & cytokine profiles

* IL-1RA: Interleukin-1 Receptor Antagonist
**Aim #2: Moderate AH**

**Aim 2:** Evaluation of the effects of probiotic supplements versus standard care on improvement in MELD score and gut mucosal integrity in patients with MELD < 21.

**Probiotics modulate intestinal integrity/mucins and liver injury in human AH**

McClain et al (unpublished data)
Multicenter randomized double-blind pilot study in moderate alcoholic hepatitis

- **Primary outcome:**
  - 30 day change in MELD

- **Secondary outcomes**
  - 90, 180 day change in MELD
  - Changes in gut mucosal integrity
  - Endotoxin levels & cytokine profiles

**MILD-MODERATE AH**

- **MELD <20**
  - Placebo (n = 68)
  - Probiotic (n = 68)
DASH - UO1 Clinical Trial

Specific Aims

**Aim 1:** Evaluation of the effects of corticosteroids versus a combination of interleukin-1 inhibitor plus pentoxifylline plus zinc supplements in patients with MELD > 21.

**Aim 2:** Evaluation of the effects of probiotic supplements versus standard care on improvement in MELD score and gut mucosal integrity in patients with MELD < 21.

**Aim 3:** Develop new clinical trials for patients with alcoholic hepatitis using lead compounds identified by the translational science components of the U01 consortium.

**Aim 4:** Create a data and tissue biorepository
Novel therapeutics for alcoholic hepatitis
Translational Component - UMMS
PI: Gyongyi Szabo, MD, PhD

Overall Hypothesis
Inflammation is a driver of acute alcoholic hepatitis and it is an attractive target for biomarker discovery and therapy.

Clinical Component

Specific Aim #1: Novel biomarkers
- MicroRNAs
- TLR4 tolerance
- Unique circulating biomarkers

Specific Aim #2: Therapy with IL-1 inhibition
- Bedside to bench: Inflammatory cascade activation in pt samples
- Bench-to-bedside: Mechanistic studies in the animal model

Specific Aim #3: Exploration of novel therapies
- miRNA-155 inhibition
- FXR agonists
- Uric acid or ATP inhibitors

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- FXR agonists
- Uric acid or ATP inhibitors
Acute alcohol binge drinking increases circulating endotoxin, bacterial DNA and microRNAs in humans
Novel therapeutics for alcoholic hepatitis
Translational Component - Louisville
PI: Craig McClain, MD

Specific Aim 1: Role of Probiotics in modulating mucins/gut barrier function in rodent/human AH
- Rodent intestinal HIF/ITF
- Probiotic supernatant efficacy
- Gut barrier in human AH and effects of probiotics

Specific Aim 2: PDE metabolism in human AH and efficacy of inhibitors
- Human AH in vivo/ex vivo studies
- PDE therapy in animal models of AH

Specific Aim 3: Glucocorticoid resistance
- Biomarkers and mechanisms PDE4:c
- AMP interactions
Novel therapeutics for alcoholic hepatitis

Cleveland Translational Component

PI: Laura E. Nagy, PhD

Specific Aim 1: Biomarkers for severity of AH and sensitivity to treatment in AH

- Exhaled breath
- Urinary biomarkers

Specific Aim 2: Pharmacogenetic analysis

Sensitivity to treatment in AH

Specific Aim 3: Preclinical studies to identify and validate new drug targets

- Complement inhibitors: target inflammation and maintain hepatoprotection
- Tributyrate: provide fuel source to colonocytes to maintain gut integrity
Biomarkers of Alcoholic Hepatitis
PAF and AzPC in urine of patients with ASH
(Cleveland cohort)

Ethanol
\[ \downarrow \]
ROS
\[ \downarrow \]
PAF/PAF-like lipids
\[ \downarrow \]
PAFR
\[ \downarrow \]
NOx2
\[ \downarrow \]
MPO/HOCI
\[ \downarrow \]
Renal Injury

Latchoumycandane et al.,
Hypothesis: Since OPN is protective for the intestinal mucosa, we postulated that enhancing OPN expression in the liver and consequently in the blood and/or in the gut could protect from early alcohol-induced liver injury.

Results:
1. WT, Opn−/− and OpnHEP Tg were chronically fed either the control or the ethanol Lieber-DeCarli diet.
2. Ethanol increased hepatic, plasma, biliary and fecal OPN more in OpnHEP Tg than in WT mice.
3. Steatosis, inflammation, liver injury, LPS, macrophages and TNFα+ cells were lesser in ethanol-treated OpnHEP Tg mice.
4. OPN showed affinity for LPS and the binding prevented macrophage activation, ROS and RNS generation and TNFα production.
5. Treatment with milk OPN blocked LPS translocation in vivo and protected from early alcohol-induced liver injury.

Conclusion: Natural induction plus forced overexpression of OPN in the liver and treatment with m-OPN protect from early alcohol-induced liver injury by blocking the gut-derived LPS and TNFα effects in the liver.
**DASH - UO1 Clinical Trial**

**Specific Aims**

**Aim 1:** Evaluation of the effects of corticosteroids versus a combination of interleukin-1 inhibitor plus pentoxifylline plus zinc supplements in patients with MELD > 21.

**Aim 2:** Evaluation of the effects of probiotic supplements versus standard care on improvement in MELD score and gut mucosal integrity in patients with MELD < 21.

**Aim 3:** Develop new clinical trials for patients with alcoholic hepatitis using lead compounds identified by the translational science components of the U01 consortium.

**Aim 4:** Create a data and tissue biorepository
DASH - UO1 Clinical Trial
Data and Biorepository

Data Coordinating Center
Director: Bruce Barton (UMMS)
Team: Aimee Knoll, Peter Lazare, Dana Anderson

Redcap system:
- Comprehensive data collection
- Patient demographic and clinical information
- Prospective data collection on all patient groups
  - observational study
  - severe alcoholic hepatitis
  - moderate alcoholic hepatitis
- Biosample labeling + tracking

DSMB
- SAE reporting
- Quality control and safety
Biorepository
Located at each site
Central depository - Cleveland Clinic (L Nagy)

Redcap system:
  Biosample labeling + tracking

Biosamples:
  Plasma, serum
  Urine
  Stool
  Liver biopsies

Biosamples collected at multiple timepoints from each patient along with clinical data.
Synergy between the UO1 components
Knowledge gaps in alcoholic liver disease

1. Predisposing factors for ALD in the alcoholic patient
   - genetic, host, environmental factors, co-morbidities

2. Triggers of severe acute alcoholic hepatitis?

3. Clinical classification of the different clinical stages of ALD

4. Effective therapies

5. Biomarkers of ALD, predictors of clinical outcome and response to therapy
1. Established a clinical network
   1. - 4 Clinical sites (7 hospitals)
   2. - 4 clinical Pis and >10 co-investigators

2. Established Data Coordinating Center
   1. Redcap system
   2. DSMB

3. Established Biorepository

4. Started 2 novel clinical trials
   1. Severe alcoholic hepatitis
   2. Moderate alcoholic hepatitis

5. Integrated translational science components
**UO1 - Alcoholic Hepatitis**

**ALD/ASH**

**No specific therapy as of 2014**

- Improve definition of alcoholic liver disease and its clinical stages
- Design new treatment paradigms
- Centralize data collection
- Expand the clinical network
- Capture additional clinical data/samples (liver biopsy)
- Educate patients/public about ALD

**Goal:**

Cure alcoholic liver disease for the benefit of our patients
THANK YOU

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Karen Kodys

Collaborators
Evelyn Kurt-Jones
Kate Fitzgerald
Victor Ambros

Funding from NIAAA
Effects of alcohol withdrawal on gut permeability and inflammatory markers
(U Louisville cohort)

Subjects:
- Otherwise healthy alcohol-dependent subjects (n = 49) were withdrawn from alcohol during a 28-day inpatient protocol at the NIAAA inpatient unit in the National Institutes of Health (NIH) Clinical Center.
- No patient had clinically evident liver disease or bilirubin elevation.
- Subanalysis was performed in patients with modest abnormalities of AST/ALT or totally normal AST/ALT.

Study Design:
- The day after admission (Day 1), blood was obtained for measurement of liver function tests and markers of gut permeability.
- Blood was obtained again on Days 8, 15, and 22 of abstinence.

These patients served as “alcoholic controls” without clinically-significant liver disease. They will be contrasted with our U01 moderate and severe alcoholic hepatitis subjects.
Alcohol withdrawal improves serum inflammatory markers (U Louisville cohort)

**LPS**

- **LPS - all subjects**
  - Plasma LPS, EU/L
  - Day 1, Day 8, Day 15, Day 22
  - ALT < 40, ALT > 40

**IL-8**

- **IL-8 - all subjects**
  - Plasma IL-8, pg/ml
  - Day 1, Day 8, Day 15, Day 22

Graphs showing changes in LPS and IL-8 levels over days with and without ALT markers.