NASH Globally- *Pathways to combination therapies, biomarkers and outcomes*

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Conflicts of Interest

• President, Sanyal Biotechnologies
• **Stock options**: Genfit, Akarna, Tiziana, Indalo, Durect, Exhalenz, Hemoshear
• **Advisor with compensation**: Lilly, Pfizer, Novartis, Ardelyx, Salix, Hemoshear
• **Advisor without compensation**: Galectin, Intercept, Merck, Bristol Myers, Immuron, Gilead, Chemomab, Affimmune, Protalix, Nitto Denko, Novo Nordisk, Cirius, Boehringer Ingelhiem
• **Grants to institution**: Gilead, Tobira, Allergan, Merck, Bristol Myers, Astra Zeneca, Immuron, Intercept, Novo Nordisk, Shire, Boehringer Ingelhiem, Cirius
NAFLD: a silent killer in our midst

- Obesity
- Heart Disease
- Nonalcoholic Fatty Liver Disease
- Type 2 Diabetes

Image: Map of the United States with states color-coded to represent the percentage of the population affected by obesity, with arrows pointing to the related conditions.
NAFLD is driving the national increase in liver cancer.

Liver cancer rate related to obesity is increasing at 3% annually.

Steele et al, MMWR, October 2017
The consequences of inaction will be serious:

- number of those with cirrhosis will triple
- over 300,000 people will have end-stage liver disease
- many of these will be “todays” children

Estes et al, Epub Hepatology, 2017
Making the case for combination therapies
NASH is a disease of metabolic substrate poisoning
Pathogenesis of NASH and targets of therapy

**Dietary fat**
- Chylomicron triglyceride
- Adipose tissue
  - Adipose insulin resistance
  - Fatty acids released from adipose tissue into the blood

**Dietary sugars, Amino acid**
- Fructose, glucose
- Acetyl CoA
- SREBP1c
  - “DNL”
    - ACC
    - FAS
    - SCDs
- Fatty acids made in the liver (de novo lipogenesis)

**Minor sources:**
- Autophagy
- Lipoprotein remnant uptake

**Oxidative disposal:**
- Mitochondria
- Peroxisomes
- CYPs

**Lipotoxic lipids**
- DAGs?
- Ceramides?
- LPCs?
- Others?

**Hepatocellular free fatty acids**
- PNPLA3?

**Triglyceride**
- Lipid droplets

**Oxidative disposal:**
- Mitochondria
- Peroxisomes
- CYPs

**Hepatocellular injury**
- Inflammation/repair

**Modifying factors:**
- OSA/hypoxia
- Uric acid
- Gut microbiome products
- Cholesterol/oxysterols
- Circadian control of metabolism

**NASH**
**Fibrosis**
**HCC**

**Hypertriglyceridemia**

Coutesy Brent Tetri
Disease activity versus disease stage

- **Disease Onset**
  - Steatohepatitis
  - NAFLD activity score
  - Fibrosis

**Disease Activity**
- Metabolic overload
- Cell stress
- Inflammation
- Activity drives Stage
- Stage is a marker of disease progression

**Stage**

**cirrhosis**
- Generally accepted surrogate

Clinically meaningful outcome
- Liver-related outcome
- Death
The progression of NASH is affected by many pathways:

- **Behavior**
  - Gut/Microbiome
  - Metabolic
  - Inflammation
  - Apoptotic
  - Fibrotic

- **Stem cell activation**
  - Regeneration
  - Cell-matrix cross talk
  - Microcirculation
  - Metabolic reprogramming

**Predisposition**

**Disease initiator**

**Phenotype**

- **Perpetuating mechanisms**
- **Restorative mechanisms**

**Progression < healing** (disease resolution)

**Progression = healing** (non-progressive NAFLD)

**Progression > healing** (disease progression)

---

The exclusivity probability is the probability that the pathway is significant in one contrast and not in the other:

\[ P_{EA} = P_A \times (1 - P_B) \]

Sanyal lab, unpublished data
K. Human NASH (NAS 5)

Cazanave et al, Scientific Reports, Epub Dec 2017

TLR signaling - 24 wks

<table>
<thead>
<tr>
<th>Time</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 wks</td>
<td>CD NW</td>
</tr>
<tr>
<td>8 wks</td>
<td>WD SW</td>
</tr>
<tr>
<td>24 wks</td>
<td>CD NW</td>
</tr>
<tr>
<td>24 wks</td>
<td>WD SW</td>
</tr>
<tr>
<td>52 wks</td>
<td>CD NW</td>
</tr>
<tr>
<td>52 wks</td>
<td>WD SW</td>
</tr>
<tr>
<td>52 wks</td>
<td>WD SW tumor</td>
</tr>
</tbody>
</table>

-0.2 0 0.2 0.4
Pathways with super-additive effects on disease activity and fibrosis stage

Sanyal Lab (unpublished data)
DISEASE BIOLOGY PROVIDES TARGETS FOR THERAPEUTICS

- Insulin resistance modifiers
- Cell stress modifiers
- Anti-inflammatory agents
- Anti-fibrotics

Metabolism (insulin resistance) → Cell stress apoptosis → Inflammation → Fibrogenic remodeling → CIRRHOSIS
If everyone took the drug, why did only some individuals improve?

Disease activity burns out with progression into cirrhosis.

Siddiqui et al, Clin Gastro Hep 2015
Tracking the molecular evolution of NASH provides a comprehensive list of potential targets for therapy

Cazanave et al, Scientific Reports, Dec 2017
Lipidomic signature of NASH

Proposed Model of NAFLD

LOX Activation
Oxidation of lipids
DNL
Peroxisomal Dysfunction

Normal  NAFL  NASH

Puri et al, Hepatology 2009
Matching the right patient to the right drug/s
Rational approach to therapeutics for NASH

Mainly anti-fibrotic

Targets:
- Metabolic
- Inflammation
- Fibrosis

Lifestyle
Metabolic targets

Mainly metabolic + Inflammatory
Hypothetical patient stratification

Pathway category:

- Impaired satiety mechanisms
- Impaired thermogenesis
- Periph adipogenesis/lipolysis
- Adipose inflammation
- Augmented DNL
- Impaired TG formation
- PNPLA3? → Inappropriate TG lipolysis
- Active lipotoxic lipid synthesis
- Liver inflammatory pathways
- Impaired wound response
- Augmented fibrogenesis

Likelihood of contributing to NASH phenotype:

- Low
- High

“Lean NASH”
Network analysis reveal vitamin E specific pathways that are relevant for its effects on NASH

Sookoian and Pirola, Clin Liv Dis, 2012
Baseline metabolites predict response to future treatment with vitamin E

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>gamma-CEHC</td>
<td>0.11</td>
<td>0.01-0.995</td>
</tr>
<tr>
<td>2-palmitoylglycerolphosphoethanolamine</td>
<td>0.08</td>
<td>0.01 - 0.56</td>
</tr>
<tr>
<td>myristoleate (14:1n5)</td>
<td>0.04</td>
<td>0.002-0.64</td>
</tr>
<tr>
<td>3-phenylpropioninate</td>
<td>29.4</td>
<td>1.23-707.0</td>
</tr>
<tr>
<td>Asparagines</td>
<td>20.2</td>
<td>1.2-338.6</td>
</tr>
<tr>
<td>indolepropionate</td>
<td>16.2</td>
<td>1.45-180.7</td>
</tr>
</tbody>
</table>

Only those that were significant are listed

Cheng et al, PlosONE, 2012
Stratification → targeted treatment
“Personalized medicine”

Pathway category:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pt A</th>
<th>Pt B</th>
<th>Pt C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impaired satiety mechanisms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impaired thermogenesis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Periph adipogenesis/lipolysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adipose inflammation</td>
<td></td>
<td></td>
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<td>Impaired TG formation</td>
<td></td>
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<tr>
<td>PNPLA3? → Inappropriate TG lipolysis</td>
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<td></td>
</tr>
<tr>
<td>Active lipotoxic lipid synthesis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver inflammatory pathways</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impaired wound response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Augmented fibrogenesis</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

“lean NASH”

Central modulation
PGC1α activators
PPARγ ligands
JNK inhib?
ACC1, SCD inhib.
DGAT2 activators
CYP4 inhibitors
iPLA₂ inhibitors
Hedgehog inhib
Galectin inhib?
Combination therapy: targeting multiple organs simultaneously
Pathogenesis of beta cell failure in type 2 diabetes

Janikiewicz, et al; Biochemical and Biophysical Research Communications, Volume 460, Issue 3, 2015, 491–496

http://dx.doi.org/10.1016/j.bbrc.2015.03.153
The Liver-Heart connection

- Ectopic fat
- Injury/inflammation
- Fibrosis
- Increased CVS risk
- Subclinical disease
  - Acute MI
- Heart failure
- NAFL
- NASH
- CIRRHOSIS

Time
Disease Severity in NAFLD Drives Atherogenic Dyslipidemia

Small Dense LDL-Cholesterol

- Normal
- Increased Liver Fat

A

- Low-Nml ALT
- High-Nml ALT
- Elevated ALT

B

- LEAN
- OBESE
- NAFL
- NASH

* for the effect of NAFLD: p=0.03
(for obesity: p=0.15)

References:
Siddiqui et al. Gastroenterology. 2013
The more advanced the NASH, the greater the risk of cardiac events

<table>
<thead>
<tr>
<th></th>
<th>Age, Sex-adjusted</th>
<th>Multivariable-adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Hazard ratio (95% CI)</td>
</tr>
<tr>
<td>All cause</td>
<td>778</td>
<td></td>
</tr>
<tr>
<td>Minimal</td>
<td>251</td>
<td>1</td>
</tr>
<tr>
<td>Intermediate</td>
<td>404</td>
<td>1.50 (1.20-1.88)</td>
</tr>
<tr>
<td>Advanced</td>
<td>123</td>
<td>2.26 (1.59-3.21)</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>296</td>
<td></td>
</tr>
<tr>
<td>Minimal</td>
<td>81</td>
<td>1</td>
</tr>
<tr>
<td>Intermediate</td>
<td>167</td>
<td>2.43 (1.69-3.50)</td>
</tr>
<tr>
<td>Advanced</td>
<td>48</td>
<td><strong>3.34 (2.00-5.60)</strong></td>
</tr>
</tbody>
</table>

Kim et al, Hepatology, 2013
Fibrosis Stage is Linked To Diastolic Dysfunction and Exercise Capacity

Exercise Time

Peak VO$_2$

Exercise E/E’

Siddiqui et al. AASLD 2017
Finding the patients- an urgent need to develop noninvasive methods for assessment
Targeting the population at risk

Healthy

NAFLD
NAFLD + inflammation

NASH

High-risk NASH
NASH and fibrosis type 2 diabetes, high BMI

Cirrhosis HCC
Liver Failure Death

86 – 108m in USA\textsuperscript{2,3}

9 – 15m in USA\textsuperscript{4}

6 – 10m affected\textsuperscript{5}
At greatest risk of progression to cirrhosis or other serious liver conditions\textsuperscript{1}
Liver biopsy is an inadequate tool for routine assessment

- Invasive, painful
- Risks- morbidity and mortality
- Sampling variability
- Observer variability
- Limited workforce capacity

With a mortality risk of 1:1000 and population at risk of 60 million, the total number of Diagnostics-associated mortality would be 60000
Biomarker development process

- Drug Approval Process
- Scientific Community Consensus
- Biomarker Qualification Program

- Data Driven
- Subject to regulatory scrutiny
- More than one process can go on
- **Liver Forum** integrates biomarker development process across FDA and EMA

*Facilitating Biomarker Development: Strategies for Scientific Communication, Pathway Prioritization, Data-Sharing, and Stakeholder Collaboration; Published June 2016, Duke-Margolis Center for Health Policy*
Disease activity versus disease stage

Disease Onset

Steatohepatitis
NAFLD activity score

Disease Activity

Fibrosis

Metabolic perturbation
Microbiome products
Systemic inflammation
Cell stress
Hepatic inflammation

Stage

cirrhosis

Generally accepted surrogate

Clinically meaningful outcome

Liver-related outcome
Death
“Fit for Purpose” biomarkers

Adapted from Chris Leptak...Liver Forum Biomarker Workshop 2017
Trans-atlantic initiatives for NASH biomarker development

Liver Forum

NIMBLE
FNIH-BC

LITMUS
IMI

FDA
The path to approval
Evidence burden to have therapy approved for NASH

Pre-cirrhotic stages

Subpart H

Resolution of steatohepatitis
Improvement/no worsening in NAS and/or
Improvement in fibrosis
With at least no worsening of activity

Post-subpart H
Progression to cirrhosis

Cirrhosis

Subpart H

Improvement in stage
Reduction in MELD progression

Post-subpart H
Improved outcomes

Sanyal et al, Hepatology 2015
CIRRHOSIS

Metabolism (steatosis)

PPARs, FXR, GLP-1, FABAC, FGF21, Thyroxine analog

Mean 42% fat reduction in 75% of subjects

PPAR α/γ, PPAR α/δ, mTOT

Cell stress apoptosis

Vitamin E, ASK1

CCR2-CCR5 (Cencriviroc blocks this target)

Fibrogenic remodeling

Anti-fibrotics

CIRRHOSIS
Endpoints: disease activity vs stage

- Disease Onset
  - Steatohepatitis (NAFLD activity score)
  - Fibrosis

- Disease Activity
  - Activity drives Stage
  - Stage is a marker of disease progression

- Stage
  - Clinically meaningful outcome
    - Liver-related outcome
    - Death

- Cirrhosis
  - Generally accepted surrogate
In pre-cirrhotic stages, fibrosis is relevant mainly as a marker of *disease progression* towards cirrhosis.

Progression to cirrhosis is a generally accepted surrogate endpoint for approval.

*Disease progression includes metabolic reprogramming, cell death, stem cell recruitment, regenerative activity, cell differentiation, changes in microcirculation, matrix, bile flow. Fibrosis is an easily visible and quantifiable surrogate for this process.*
Implications of decreased fibrosis in pre-cirrhotic stages of NASH is linked to drug mechanism of action

NASH

- Decreased NASH activity (high biological plausibility) → ↓ FIBROSIS
- Decreased disease activity with some direct anti-fibrotic effects (depends on which effect predominates) → ↓ FIBROSIS
- Primary anti-fibrotic effect (uncertainty over impact of unrestrained upstream drivers) (may position drug for combination therapy) → ↓ FIBROSIS
# Impact of Fibrosis on Clinical Events

Increased risk of clinical events with:

- Higher baseline hepatic collagen content and ELF
- Worsening of fibrosis (by Ishak stage, collagen content, ELF)

<table>
<thead>
<tr>
<th>Metric</th>
<th>Hazard Ratio *</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ishak stage 5 vs 6 (baseline)</td>
<td>1.25</td>
<td>0.68, 2.29</td>
<td>0.48</td>
</tr>
<tr>
<td>No improvement vs improvement</td>
<td>9.63</td>
<td>1.33, 69.81</td>
<td>0.025</td>
</tr>
<tr>
<td>Hepatic collagen (baseline), per 5%</td>
<td>1.39</td>
<td>1.15, 1.69</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Change from baseline, per 5%</td>
<td>1.20</td>
<td>1.03, 1.39</td>
<td>0.017</td>
</tr>
<tr>
<td>ELF (baseline)</td>
<td>2.37</td>
<td>1.69, 3.31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Change from baseline</td>
<td>1.54</td>
<td>1.10, 2.15</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Sanyal et al, EASL 2017

* Separate multivariate models run with baseline and change from baseline for each variable.
How cirrhosis leads to clinically meaningful outcomes

- CIRRHOSIS
  - Reversal

- CLINICALLY SIGNIFICANT PORTAL HYPERTENSION
  - VARICEAL BLEED
  - ASCITES/SBP/HRS ENCEPHALOPATHY
    - Need to show for a specific MOA decrease in HVPG
      - Reduces clinical outcomes

- DECLINE IN FUNCTION ➤ LIVER FAILURE
  - Decreased progression to MELD ≥ 15
  - Composite clinical outcomes
MELD as an endpoint

<table>
<thead>
<tr>
<th>Pros</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Relates to mortality</td>
</tr>
<tr>
<td>• Well known to clinicians</td>
</tr>
<tr>
<td>• Widely available</td>
</tr>
<tr>
<td>• Easy to measure</td>
</tr>
<tr>
<td>• Threshold value of 10 or 14 identifies a important stage in clinical course</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Inter-lab variability</td>
</tr>
<tr>
<td>• Related to 3 month mortality</td>
</tr>
<tr>
<td>• Rate of progression of MELD score not linear</td>
</tr>
<tr>
<td>• Most patients with compensated cirrhosis have a MELD &lt; 10</td>
</tr>
</tbody>
</table>

*Increase in MELD to ≥ 15 represents a point in course of disease where Transplant should be considered*
Take home messages

• NASH is a clinical syndrome driven my metabolic substrate overload to the liver.

• The biology of NASH has significant collinearity with the biology of HFPEF and type 2 diabetes

• Integrated approaches to noninvasive assessments that provide a read out of disease activity and stage in key end organs is needed.

• Therapeutics should go after nodal targets that are key for disease development and progression. Combinations should be rational and based on proper step wise clinical development.

• Trial design innovations are under way to allow accelerated assessment of combination therapies to improve clinical outcomes
Huang Dee: Nai-Ching (2600 BC, First Medical Text)

Translation:
Superior doctors prevent the disease
Mediocre doctors treat the disease before evident
Inferior doctors treat the full-blown disease
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