Innovations in Non-invasive Imaging Assessment of Treatment Response in NASH

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• Co-founder: Liponexus Inc.
Outline

• Epidemiology
• Definition
  • NAFLD: NAFL versus NASH
• Natural history of NAFLD
• Advances in imaging assessment
• Pharmacologic treatment
• Novel therapies in NASH
Nonalcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease in the US
  - Afflicts 80-100 million Americans

Ethnic predisposition
  - More common in Asian Indians>Hispanics>Caucasians>African Americans

Risk factors include metabolic syndrome
  - Obesity, hypertension, hypertriglyceridemia, insulin resistance and diabetes
  - PNPLA3 genotype

NAFLD is diagnosed
  - Either on biopsy or imaging evidence of hepatic steatosis (≥ 5% liver fat) in individuals who consume little or no alcohol without any other cause for liver disease or hepatic steatosis
Subtypes of NAFLD

Caveats
- Presence of steatosis in ≥ 5% hepatocytes
- Minimal alcohol use
- Biopsy consistent with NAFLD
- No other etiology for liver disease
- No secondary causes of NAFLD
  - Medications
  - HIV
  - Lipodystrophy

NAFLD

NAFL
- Non-progressive

NASH
- Progressive

Borderline NASH
Nonalcoholic steatohepatitis (NASH)

- steatosis
- lobular inflammation
- ballooning
- with or without zone 3 fibrosis

Third leading indication for liver transplant in the US
Natural history of NASH

20 million Americans

Fibrosis progression rate in NASH: 1 stage per 7 year
20% patients are fast progressors: to cirrhosis in 10 years

Risk of death in NASH
1st CVD
2nd Cancer
3rd Liver

Liver death
Liver transplant
HCC
Cirrhosis
Fibrosis
NASH

Multiple sources: Over 40 studies
Key histologic predictors of mortality in NAFLD

Fibrosis is the single most important predictor of mortality in NASH

Loomba et al. Gastroenterology 2015

Angulo et al. Gastroenterology 2015
Loomba and Chalasani. Gastroenterology 2015
There are no FDA Approved Therapies for NASH
Outline

Quantitative, Imaging biomarker assessment and development program

- Assessment of hepatic steatosis
- Assessment of hepatic fibrosis
- Longitudinal changes in disease severity
  - MRI-PDFF
  - MRE

Traditional paradigm

New paradigm

Improve efficiency
Traditional paradigm for assessment of treatment response

• 2005: NASH CRN Histologic Scoring System was developed
  • NAFLD Activity Score is proposed: A summary score ranging from 0-8
    • Steatosis (0-3)
    • Lobular inflammation (0-3)
    • Ballooning (0-2)

• 2010: PIVENS Trial (Sanyal et al. NEJM 2010)
  • Vitamin E versus pioglitazone versus placebo
  • 96 week duration
  • Paired liver biopsy before and after treatment
  • Primary endpoint: 2-point improvement in NAFLD Activity Score
Problems with traditional approach

• Duration of trials: 96 weeks or 72 weeks

• Liver histologic features have low kappa
  • Ballooning: $K = 0.44$

• Subjective assessment

• Invasive

• High risk of type 2 error in early phase trials
  • Small sample size and small treatment effect size

Solution: Quantitative, non-invasive, accurate, reproducible, precise and have significance in natural history and eventually show improvement in liver-related and overall mortality
NAFLD Activity Score (NAS) = Max Score 8

<table>
<thead>
<tr>
<th>Item</th>
<th>Score</th>
<th>Extent</th>
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<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>&gt;33-66%</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>&gt;66%</td>
</tr>
<tr>
<td><strong>Lobular Inflammation</strong></td>
<td>0</td>
<td>No foci</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>&lt;2foci/200x</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2-4 foci/200x</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>&gt;4 foci/200x</td>
</tr>
<tr>
<td><strong>Hepatocyte Ballooning</strong></td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Few balloon cells</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Many cells/prominent balloon</td>
</tr>
<tr>
<td><strong>Fibrosis</strong></td>
<td>0 - 4</td>
<td></td>
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</tbody>
</table>

Novel MR imaging assessment of liver Fat, NASH and fibrosis
Cohort 1: UCSD NAFLD Cohort

Suspected NAFLD

Confirm NAFLD:
- Quantify alcohol use
- Rule out other causes of liver disease

Pathology
- NAFLD histology
  - NAFL
  - NASH
- Fibrosis stage

MRI
- Quantify fat by Imaging
  - MRI-PDFF/MRS
- Quantify fibrosis
  - MRE/ARFI

Paired samples
- Plasma/DNA/Stools

N = 300 (200 paired stool/plasma samples) NAFLD patients available as Feb 2018
Assessment of liver fat
Fat (TG) has a chemical signature

This chemical signature can be detected directly by magnetic resonance spectroscopy (MRS).

Performed properly, MRS quantifies the proton density fat fraction (PDFF), a standardized measure of liver tissue [TG].

Limitations of MRS
- One 8cm³ voxel
- Not available on routine scanners
- Requires expertise

Imaging method to estimate PDFF would have advantages....

Thomsen MRI 1994
Hamilton JMRI 2009
Hamilton NMR Biomed. 2011
Reeder JMRI 2011
MR Imaging Methods to Estimate PDFF

MRI-PDFF addresses confounding factors, unlike conventional in-phase and opposed-phase
MRI-PDFF *not* affected by
- Scanner field strength
- Patient factors: age, sex, BMI, etiology of liver disease
- Concomitant liver abnormalities: iron overload, necroinflammation

Yu MRM 2008
Bydder MRI 2008
Bydder MRI 2010
Hansen MRI 2012
Kang Invest Radiol 2012
Kuhn Radiology 2012
Tang Radiology 2013
Dulai, Sirlin, Loomba J Hep 2016
Co-localized MRI-PDFF and cross-validated with MRS

- PDFF recorded in regions of interests (ROI)s ~300-400mm²
- The same ROIs in each of the 9 liver segments measured at baseline and post-treatment.
- Each segment fat fraction = 1 ROIs
- Total liver fat fraction = average 9 ROIs

Loomba et al. Hepatology 2015
MR-based fibrosis assessment in NASH: Innovations in fibrosis assessment

Loomba et al. Hepatology 2014
MR Elastography Diagnoses Advanced Fibrosis

“Stiffness” cutoff: 3.63 kPa
Sensitivity 0.86
Specificity 0.91

AUC for diagnosis of advanced fibrosis 0.924

Loomba et al 2014
Innovations in clinical trial design

How will future clinical trials assess NASH?
Fat- and Stiffness-mapping before and after treatment

Why do we need to co-localize?
Heterogeneity in distribution
More comprehensive assessment

Higher precision and accuracy

Enhanced responsiveness

Efficiency in clinical trial

Modified from Loomba et al. Hepatology 2015
Prevalence of NAFLD and advanced fibrosis among patients with Type 2 diabetes in primary care

- Screen for NAFLD MRI-PDFF ≥ 5%
- NAFLD 65%
- Screen for advanced fibrosis MRE ≥ 3.6 kPa
- Advanced fibrosis 7.4%

Doycheva et al. APT 2016
Nonalcoholic fatty liver disease with cirrhosis increases familial risk for advanced fibrosis

Cyrielle Caussy,¹,² Meera Soni,³ Jeffrey Cui,⁷ Ricki Bettencourt,¹,³ Nicholas Schork,⁴ Chi-Hua Chen,⁵ Mahdi Al Ikhwan,¹ Shirin Bassirian,¹ Sandra Cepin,¹ Monica P. Gonzalez,¹ Michel Mendler,⁶ Yuko Kono,⁶ Irine Vodkin,⁶ Kristin Mekeel,⁷ Jeffrey Haldorson,⁷ Alan Hemming,⁷ Barbara Andrews,⁶ Joanie Salotti,¹,⁶ Lisa Richards,¹,⁶ David A. Brenner,⁶ Claude B. Sirlin,⁶ Rohit Loomba,¹,³,⁶ and the Familial NAFLD Cirrhosis Research Consortium⁹

Caussy, Loomba. JCI, 2017
Advanced fibrosis on MRE is highly prevalent in first-degree relatives of NAFLD cirrhotics

The prevalence of advanced fibrosis in relatives

- Relates of non-NAFLD controls: 1.4%
- Relatives of patients with NAFLD without advanced fibrosis: 12.0%
- Relatives of patients with NAFLD cirrhosis: 18.0%

The risk of advanced fibrosis is significantly increased in first-degree relatives with NASH cirrhosis

12 times higher odds of advanced fibrosis among first-degree relatives of probands with NASH cirrhosis

Caussy, Loomba. JCI, 2017
Management of NASH
Intensive lifestyle modification causes weight loss in NASH

A

7% weight loss

Promrat et al. Hepatology 2010
Q1. How much weight loss is needed for improvement in NASH?

- 5% weight loss will start showing improvements in liver fat and liver stiffness

- 5-7% weight loss will start showing improvements in NAFLD Activity Score

- 10% weight loss will lead to resolution of NASH in 90% and 45% will have improvement in fibrosis stage
A Randomized, Placebo-Controlled Trial of Pioglitazone and Vitamin E for Nonalcoholic Steatohepatitis (PIVENS)

The Nonalcoholic Steatohepatitis Clinical Research Network
Primary outcome: PIVENS

Vitamin E improves liver histology in NASH

Vitamin E vs Placebo p-value <0.001
Pioglitazone vs. Placebo p-value <0.04

Sanyal et al. NASH-CRN. NEJM 2010
Summary on Vitamin E

The glass is half full

• Does Vitamin E improve NASH? = **Yes**

• Does Vitamin E reverse NASH? = **Yes**

• Does Vitamin E improve fibrosis? = **No** (based upon RCTs)

• Does Vitamin E improve long-term outcomes? = **No data**
PIVENS: Weight

Pioglitazone causes weight gain

Sanyal et al. NASH-CRN. NEJM 2010
When and how to use pioglitazone

• Biopsy-proven NASH with diabetes or prediabetes
• Monitor-
  • Body weight
    – Lifestyle interventions
      » Exercise and diet
  • ALT and AST response
  • DEXA Scan
Emerging Therapies in NASH
# NASH Therapeutic Targets by Mechanisms and Sites of Activity and Type of Outcomes

## Fatty Acid Synthesis
- PPAR agonist
- Aramchol
- ASK-1 inhibitors
- DGAT inhibitors
- ACC inhibitors
- Anti-CB1
- MetAP2 inhibitors
- Thyroid B agonist

## Insulin Sensitivity
- DPP-4-i
- PPAR agonist
- SGLT2-i
- FGF-19
- FGF-21
- ISIS-ANGPTL3
- others

## Bile Acid Synthesis
- OCA
- FXR agonist
- ASBT-I
- FGF-19
- others

## Anti-Inflammatory
- PPAR agonist
- CVC
- Anti-JNK
- ASK-1 inhibitors
- Anti-CB1
- others

## Anti-Fibrotic
- OCA
- Anti-JNK-1
- ASK-1 inhibitors
- PPAR agonist
- DHA
- Nox inhibitors
- Others

## Anti-Fibrotic
- ASK-1
- Simtuzumab
- Anti-gal 3
- Anti-CTGF
- ACE-R-blockers
- Pentraxin-2
- Anti-IL-17
- Anti-TGF-beta

### Targets by Stages

**Steatosis, ballooning, and inflammation**
- Stage 1-3 fibrosis
- Stage 3-4 fibrosis

**Resolution of NASH**
- Reduce the rate of progression of fibrosis or improvement in fibrosis
- Reversal of advanced fibrosis or improvement in fibrosis
Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial

Brent A Neuschwander-Tetri, Rohit Loomba, Arun J Sanyal, Joel E Lavine, Mark L Van Natta, Manal F Abdelmalek, Naga Chalasani, Srinivasan Dasarathy, Anna Mae Diehl, Bilal Hameed, Kris V Kowdley, Arthur McCullough, Norah Terrault, Jeanne M Clark, James Tonascia, Elizabeth M Brunt, David E Kleiner, Edward Doo, for the NASH Clinical Research Network*

Partial funding for the trial, obeticholic acid, and placebo were provided by Intercept Pharmaceuticals under a Collaborative Research and Development Agreement with the NIDDK.
FLINT primary endpoint

- Improvement in NAFLD activity score* (NAS) ≥ 2 pts
  - * NAS = steatosis grade (0-3) + inflammation grade (0-3) + ballooning grade (0-2)
- No worsening of fibrosis

FLINT Trial Summary

• Obeticholic acid improved histological features of NASH including fibrosis

• Obeticholic acid treatment was associated with pruritus that was severe in 3%

• Elevated total and LDL cholesterol and decreased HDL cholesterol warrant further scrutiny in future trials

• Large phase 3 trials are being planned to assess it’s efficacy in NASH
Elafibranor, an Agonist of the Peroxisome Proliferator—Activated Receptor—\( \alpha \) and \( \delta \), Induces Resolution of Nonalcoholic Steatohepatitis Without Fibrosis Worsening

Vlad Ratziu,\(^1,2\) Stephen A. Harrison,\(^3\) Sven Francque,\(^4\) Pierre Bedossa,\(^5\) Philippe Lehert,\(^6,7\) Lawrence Serfaty,\(^8\) Manuel Romero-Gomez,\(^9\) Jérôme Boursier,\(^10\) Manal Abdelmalek,\(^11\) Steve Caldwell,\(^12\) Joost Drenth,\(^13\) Quentin M. Anstee,\(^14\) Dean Hum,\(^15\) Remy Hanf,\(^15\) Alice Roudot,\(^15\) Sophie Megnien,\(^15\) Bart Staels,\(^16\) and Arun Sanyal,\(^17\) on behalf of the GOLDEN-505 Investigator Study Group

**Randomized**

1) GFT505 80 mg
2) GFT505 120 mg
3) Placebo

**Population**

270 patients with biopsy proven NASH

**Endpoints**

Resolution of NASH
GOLDEN—Primary Results

- Primary endpoint was not met in initial assessment

  - After controlling for baseline heterogeneity of severity and center effect, the primary endpoint was met

Abbreviation: ELF, elafibranor; NAS, NAFLD Activity Score; PBO, placebo.

* Per protocol, # modified criteria

Pilot study shows that GLP-1 agonist leading to improvement in insulin resistance and weight loss led to improvement in liver histology in NASH.
Study Design: Cenicriviroc vs Placebo

NASH stage 1-3 fibrosis

- CVC 150 mg orally daily
- CVC 150 mg orally daily/placebo
- Placebo

1-year results

<table>
<thead>
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<th>Improvement in 1 stage of fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
</tr>
<tr>
<td>10</td>
</tr>
</tbody>
</table>

Year 1

Endpoint
- 2 pt improvement in NAS
- Without any worsening fibrosis

2 years total
GS-4997, an Inhibitor of Apoptosis Signal-Regulating Kinase (ASK1), Alone or in Combination with Simtuzumab for the Treatment of Nonalcoholic Steatohepatitis (NASH): A Randomized, Phase 2 Trial

Rohit Loomba¹, Eric Lawitz², Parvez S. Mantry³, Saumya Jayakumar⁴, Stephen H. Caldwell⁵, Hays Arnold⁶, Anna Mae Diehl⁷, C. Stephen Djedjos⁸, Catherine Jia⁸, Robert P. Myers⁸, G. Mani Subramanian⁸, John G. McHutchison⁸, Zachary D. Goodman⁹, Nezam H. Afdhal¹⁰, Michael R. Charlton¹¹

¹University of California at San Diego, San Diego, CA; ²Texas Liver Institute, San Antonio, TX; ³The Liver Institute at Methodist Dallas, Dallas, TX; ⁴University of Calgary, Calgary, AB, Canada; ⁵University of Virginia, Charlottesville, VA; ⁶Gastroenterology Consultants of San Antonio, San Antonio, TX; ⁷Duke Clinical Research Institute, Durham, NC; ⁸Gilead Sciences, Inc., Foster City, CA; ⁹Inova Fairfax Hospital, Falls Church, VA; ¹⁰Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; ¹¹Intermountain Medical Center, Salt Lake City, UT
Results: Fibrosis Responses

Data for patients with liver biopsies evaluable for fibrosis at baseline and week 24 (N=67).

Loomba et al. Hepatology 2018
Acetyl-CoA Carboxylase Inhibitor GS-0976 Leads to Significant Improvements in MRI-PDFF in a Phase 2, Randomized, Placebo-Controlled Trial of Patients with NASH

Rohit Loomba,¹ Zeid Kayali,² Mazen Noureddin,³ Peter Ruane,⁴ Eric J. Lawitz,⁵ Norman Gitlin,⁶ Michael Bennett,⁷ ElizaJing Harting,⁸ Bryan J. McColgan,⁸ Robert P. Myers,⁸ G. Mani Subramanian,⁸ John G. McHutchison,⁸ Michael S. Middleton,¹ Claude Sirlin,¹ Michelle Lai,⁹ Michael Charlton,¹⁰ Stephen A. Harrison¹¹

1. University of California at San Diego, La Jolla, CA; 2. Inland Empire Liver Foundation, Rialto, CA; 3. Cedars-Sinai Medical Center, Los Angeles, CA; 4. Ruane Medical and Liver Health Institute, Los Angeles, CA; 5. Texas Liver Institute, University of Texas Health San Antonio, San Antonio, TX; 6. Atlanta Gastroenterology Associates, Atlanta, GA; 7. Medical Research Associates Group, San Diego, CA; 8. Gilead Sciences, Inc., Foster City, CA; 9. Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; 10. University of Chicago, Chicago, IL; 11. Pinnacle Clinical Research, San Antonio, TX
Study Design:
Randomized, Placebo-Controlled Trial at 41 U.S. Sites

♦ Key inclusion criteria
  – Clinical diagnosis of NAFLD
  – MRI-PDFF ≥8% and MRE ≥2.5 kPa, or biopsy consistent with NASH and F1-F3
  – Noncirrhotic (FibroTest < 0.75, historical imaging and liver biopsy)
♦ Stratified by presence or absence of diabetes

MRI-PDFF; MRI-proton density fat fraction; MRE, magnetic resonance elastography; NAFLD, nonalcoholic fatty liver disease.
Results: Significant Reduction in MRI-PDFF

GS-0976 20 mg resulted in a clinically significant reduction in MRI-PDFF\(^1,2\)


\(p\)-values for change in MRI-PDFF at Week 12 by Wilcoxon rank-sum test.
\(p\)-values for proportion of subjects with \(\geq30\%\) reduction in MRI-PDFF by Mantel-Haenszel test with adjustment for diabetes status.

Future of NASH: Rationale for combination therapy

- FABAC
- TGF-β1
- CCL2
- Apoptosis CXCL9/10 IL-22 PTX2
- TGF-β1 Int (αv)6 PDGF-BB CCL2 CXCL12 FXR IL-22 Cholangiocyte
- TGF-β1 PDGF-BB IL-4Rα1 IL-13Rα1 IL-13Rα2 CXCR1 PPARγ
- Macrophage Th1 cell Th2 cell Th17 cell
- IL-1β IFN-γ IL-4 IL-13 IL-17A
- FXR agonist
- FARN FABAC
- PPARs
- CVC
- ET-1 PDGF-BB CCL2 NO
- Multi-pronged approach

- Apoptosis signaling kinase-1 (ASK-1) Inhibitors
- ACC i
- CB1 i

- Damaged hepatocyte (excess oxidative stress)

- Inflammatory cells

- Portal fibroblast
- Quiescent hepatic stellate cell

- Activated myofibroblast

- Contractility
- ETAR
- PDGFRβ
- PI3K/mTOR/Akt

- Excess ECM deposition: collagen, elastin
- LOXL2
- Crosslinking
- Scarring, cirrhosis
- Reversal by fibrolisis? stem cell therapy?

- Proliferogenic targets
- Putative fibrolisis-inducing targets
How about longitudinal quantitative changes in fibrosis assessment?
MRE and whole body composition for progression or regression monitoring

MRE showing a fibrosis progression to cirrhosis

MRE showing improvement in stiffness after bariatric surgery

AMRA collaboration: Whole body MRI assessing total visceral fat, total subcutaneous fat, and total muscle mass
Shifting the paradigm

Quantitative, Imaging biomarker assessment and development program
- Assessment of hepatic steatosis
- Assessment of hepatic fibrosis
- Longitudinal changes in disease severity
  - MRI-PDFF
  - MRE

New paradigm
- Shorter trial
- Advanced MRI-PDFF X 30 trials
- MRE X 10 trials
- Greater precision
- Greater efficiency
- Smaller sample size
- Faster to Phase 3
- Liver histology in Phase 2b/3 trials

Improve efficiency
Conclusion

• NASH can lead to cirrhosis and HCC
  • Initial assessment
  • Natural history

• MRI-PDFF is emerging to be the lead candidate for non-invasive steatosis assessment in NAFLD

• MRE is emerging to be the lead candidate for non-invasive fibrosis assessment in NAFLD

• Several exciting molecules are in clinical development for the treatment of NASH
Thank you

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