Leveraging Clinical Pharmacology to Optimize Drug Development for Nonalcoholic Steatohepatitis (NASH) and Cholestatic Liver Diseases

Sheraton Silver Spring Hotel | Magnolia Ballroom | Silver Spring, MD
December 9, 2019
Welcome & Overview

Mark McClellan, MD, PhD
Director, Duke-Margolis Center for Health Policy
Opening Remarks

Patrizia Cavazzoni, MD
Deputy Center Director for Operations, Center for Drug Evaluation and Research (CDER)
Leveraging Clinical Pharmacology to Optimize Drug Development for Nonalcoholic Steatohepatitis (NASH) and Cholestatic Liver Diseases

Opening Remarks from FDA

Patrizia Cavazzoni, M.D.
Deputy Center Director for Operations
CDER/FDA
December 9, 2019
NASH—A Public Health Concern

- The incidence of NASH is on the rise in the U.S.
- Given the ever-increasing patient population for NASH, the time is now to collectively derive solutions for this public health issue.
Challenges in NASH

• Timely identification of patients: there is an absence of clear clinical, biochemical, or histological criteria that can identify patients with NAFL who are at risk for progression to NASH
• Development of noninvasive methods for diagnosis and staging
• Inclusion of appropriate study population
• Translation of early phase results to late phase success
CDER Initiatives

- 21st Century Review Initiative
- Computational Science Center
- Critical Path Initiative
- Equal Voice Initiative
- Modernizing FDA's New Drugs Regulatory Program
- Pharmaceutical Quality for the 21st Century
- Safety First Initiative
- Safe Use Initiative
- Scientific Public Private Partnerships and Consortia
- Sentinel Initiative
- Transparency Initiative
- Unapproved Drugs Initiative

OCP actively contributes to CDER Initiatives aimed at facilitating drug development through our regulatory review, policy development, research programs, and strategic engagement.
OCP’s Innovative Solutions to Support CDER Initiatives

MODEL-INFORMED DRUG DEVELOPMENT (MIDD)
MIDD Pilot Program ongoing to advance the use and potential of model-based approaches to accelerate drug development and patient access to safe and effective drugs

THE INTERSECTION OF NEW TECHNOLOGIES AND REGULATORY SCIENCE
Keeping up with the latest advances at the intersection of technology/approaches and regulatory science, such as machine learning/artificial intelligence and harnessing the power of RWD/RWE

LEVERAGING COLLECTIVE ACTION
Helping to advance clinical trial design and strategies by leveraging collective action (C-PATH, etc.) to address contemporary regulatory challenges

OCP SUPPORTING CDER THROUGH INNOVATION

www.fda.gov
Driving Innovation Through Translational Medicine: FDA/OCP Workshops

Workshops provide an unparalleled opportunity to engage thought-leaders and create dialogue on the latest in scientific methods and modern challenges in drug development.

Select FDA/OCP Workshops in 2019:
- Development of Best Practices in Physiologically Based Pharmacokinetic Modeling to Support Clinical Pharmacology Regulatory Decision-Making
- Enhancing the Accessibility and Utility of Drug Interaction Information Labeling Workshop
- Leveraging Clinical Pharmacology to Optimize Drug Development for Nonalcoholic Steatohepatitis (NASH) and Cholestatic Liver Diseases
- Precision Dosing: Defining the Need and Approach to Deliver Individualized Drug Dosing in the Real World Setting
- Topical Drug Development-Evolution of Science and Regulatory Policy

www.fda.gov
Driving Innovation Through Engagement: Vision for Today’s Workshop

**BRAINSTORM** on creative solutions to some of the most pressing challenges in NASH

**ENGAGE** in two-way scientific dialogue to share, educate, and inform

**DRIVE** expeditious drug development in this area
Leveraging Clinical Pharmacology to Optimize Drug Development for Nonalcoholic Steatohepatitis (NASH) and Cholestatic Liver Diseases

Clinical Development of Drugs for the Treatment of NASH: General Considerations, Challenges, and the Role of Clinical Pharmacology

Shirley K. Seo, Ph.D.
Director, Division of Cardiometabolic and Endocrine Pharmacology
Office of Clinical Pharmacology
OTS/CDER/FDA
Disclaimer

• The opinions contained in this presentation are my own and do not necessarily represent the views of the FDA.
The Urgency

- Non-Alcoholic Fatty Liver Disease (NAFLD) is the most common chronic liver condition in the U.S.¹
- Because most patients are asymptomatic in the initial stages, NAFL can progress to NASH and other serious complications without identification and treatment
- Up to 25% of all adults in the U.S. have NAFLD. And 20% of those adults have NASH ¹
- NASH is the leading cause of liver transplant in women and the 2nd leading cause in men ²
- Yet, no approved treatment currently exists in the U.S.

¹American Liver Foundation: https://liverfoundation.org, accessed 12/4/19
Complexities of the Liver as Both the Pharmacologic Target and the Clearing Organ

- Pharmacologic Target
- Drug Metabolism and Transport
- Liver Blood Flow and Drug Distribution
- Synthetic Function
- Efficacy—Healed Liver
Building Blocks of Clinical Pharmacology and Confidence in Proof of Concept

Adapted from Vicini & van der Graaf, Clinical Pharmacology & Therapeutics (2013); 93:5, 379-381.
General Considerations and Questions for Today

• NASH can be a “silent disease”
  – Need better early diagnostic tools
  – Ideally, should have predictive biomarkers to better forecast the benefit of pharmacologic therapy

• Pharmacologic target(s)
  – Identification of the appropriate target
  – Should combination therapy be the mainstay?

• Dose of selected therapeutic agents
  – Is proper dose selection performed in all cases?
  – Is the right dose being given to the right patient population?
Using Clinical Pharmacology to Poise Development for the Best Chance of Success

- **Clinical efficacy trials**: Ensuring right population, right dose, and/or drug combination are studied
- **Early phase studies**: Ensure well-designed and timely studies are properly informing Phase 3. Translatability of Phase 2 results to Phase 3
- **Preclinical**: Need to consider species translatability and appropriate characterization of target engagement
Using Clinical Pharmacology to Poise Development for the Best Chance of Success

Data on hepatic impairment is critical

- Given all the complexities of the liver, it is vital to study individuals with hepatic impairment
- Represent a non-trivial subset of the NASH patient population
- Hepatic dysfunction can impact either safety or efficacy, depending on the underlying mechanism for the change in drug disposition → differential risk/benefit calculus
Potential Challenges and Areas of Opportunity

• Beginning with nonclinical: no single ideal preclinical model exists so it is imperative to find relevance/translatability of animal model data to humans.

• Target identification and engagement: is the right pharmacologic target being engaged at the right stage of disease? (PPAR agonist, FXR agonist, Apoptosis signal-regulating kinase 1 (ASK1), etc.)

• Biomarkers at all stages are needed: diagnostic, prognostic, and surrogate endpoints that are predictive of long-term benefit
Potential Challenges: Good News/Bad News

**Good news:** The liver is healing. Recovery of enzyme activity, transporter function, protein synthesis, increased blood flow and better distribution of drug molecule.

**Semi-bad news:** Dynamic process occurring during treatment, presenting challenges for predictability of drug disposition and continued dosing. Need to consider these complexities and implications on intra-hepatic concs and DDIs.
Can We Leverage Experiences from Another Liver Disease?

<table>
<thead>
<tr>
<th></th>
<th>HCV</th>
<th>NASH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex pathophysiology</td>
<td>![Checkmark] Complex pathophysiology</td>
<td>![Checkmark] Complex pathophysiology</td>
</tr>
<tr>
<td>Hepatotoxicity of liver-targeted drug is a concern</td>
<td>![Checkmark] Hepatotoxicity of liver-targeted drug is a concern</td>
<td>![Checkmark] Hepatotoxicity of liver-targeted drug is a concern</td>
</tr>
<tr>
<td>Targeting multiple pharmacologic mechanisms led to success</td>
<td>![Checkmark] Need for targeting multiple mechanisms...?</td>
<td>![Checkmark] Need for targeting multiple mechanisms...?</td>
</tr>
<tr>
<td>Healing liver did not impact dose because of shorter-term treatment</td>
<td>![Checkmark] Will the healing liver cause the dose to evolve over longer term treatment?</td>
<td>![Checkmark] Could changes in DDIs occur over long-term treatment?</td>
</tr>
<tr>
<td>The extent and types of DDIs have been impacted by the healing liver</td>
<td>![Checkmark]</td>
<td>![Checkmark]</td>
</tr>
</tbody>
</table>
Acknowledgements

• The entire workshop organizing committee at the FDA:
  – Anand Balakrishnan
  – Jenny Cheng
  – Dilara Jappar
  – Insook Kim (steering member)
  – Tracey Lee
  – Steven Li
  – Martina Sahre
  – Elizabeth Shang
  – Donny Tran (steering member)
  – Jack Wang (steering member)
  – Yao-Yao Zhu

• All speakers and panelists who have contributed to the program
• The entire Duke-Margolis Workshop Organizing Team and C-PATH partners
• Issam Zineh (Office Director, OCP)
• Patrizia Cavazzoni (Deputy Center Director for Operations, CDER)
Session 1: Liver Disease Pathophysiology and the Impact of Liver Dysfunction on Pharmacokinetics, Pharmacodynamics, Drug Safety and Efficacy

9:35 am – 10:35 am
### DISCLOSURE(S)

#### Research Support
- NIH / NIDDK (NASH CRN)
- Gilead Sciences
- Genfit Pharmaceuticals
- Conatus Pharma
- TaiwanJ
- Bristol Meyers Squibb
- Poxel
- Allergan
- Gilead Sciences
- Galmed
- Galmed
- Blade
- Intercept
- NGM Biopharma
- Madrigal
- Novo Nordisk
- Prometheus
- Celgene
- Durect

#### Consultant /Scientific Advisory Board
- Bristol Meyers Squibb
- Allergan
- Novo Nordisk
- TaiwanJ
- NGM Pharma
- Blade
- Madrigal
- Medimmune
- Prometic

#### Speaker’s Bureau
- Alexion
- Clinical Care Options
- Medscape
- NASH Education Program

No conflict of interest or financial disclosure for today’s presentation.
My presentation....

Leveraging Clinical Pharmacology to Optimize Drug Development

Diagnostic Approach
Cohorts at Risk
Epidemiology
Spectrum of Disease

Overview for Discussion NAFLD and Cholestatic Liver Disease in 15 min
Spectrum of NAFLD

Dynamic process

Variations in Disease
Natural History

Epigenetics
Environmental Modifiers
Genetic
Outcome
NAFLD Is Endemic—A Global Epidemic

Progression of NAFLD

Prognosis of NAFLD and NASH

Overall Survival in NAFLD vs. the General Population

HR 1.34 (95% CI 1.003-1.76)
P=0.03

Follow-up (years)

N=420, Mean f/u 7.6 yrs

Overall Survival in NAFLD by Fibrosis Stage

N=619, Median f/u 12.6 yrs

*Adams et al, Gastro. 2005; Angulo et al, Gastro. 2015*
Risk Stratification / High Risk Populations

- Sedentary life style / Western diet (high fructose consumption)
- Overweight / obese
- Metabolic syndrome (3 or more features)
- Type 2 diabetes (T2DM)
- Ethnicity (Hispanic/ Asian)
- Dyslipidemia
- Polycystic ovary syndrome
- Endocrinopathies (Panhypopituitarism)
- Obstructive sleep apnea
- First degree relative with NASH cirrhosis

*Age > 50 years and T2DM and/or 1st degree relative with NASH cirrhosis* are the strongest clinical predictors for NASH & fibrosis.
NAFLD and Hepatic Fibrosis in Patients With vs Without T2DM By Diagnostic Approach

Prevalence of NAFLD/NASH

Prevalence of Fibrosis

Diagnosing and Staging NAFLD/ NASH Noninvasively

- Metabolic syndrome (3 or more features)
- Vague right upper quadrant pain
- Hepatomegaly on exam
- Little (< 20 gm/day) to no alcohol use
- “Bright” liver on ultrasound
- “Seronegative” chronic hepatitis (ALT> AST)
  - Viral serologies (negative HBsAg, HCV Ab)
  - Iron profile
  - Autoimmune markers (ANA, ASMA, AMA)
  - Ceruloplasmin
  - Alpha-1 antitrypsin
- Elevated liver enzymes (normal F< 20 U/L; M< 30 U/L)
- *Concern for advanced liver disease or decreased function*: Platelet count < 150K, albumin < 3.5 /dl, increased bilirubin, MELD > 12, Fibroscan > 12 kPA, Fib-4 > 3.25, NFS > 0.67, MRE stiffness > 3.63

Clark JM, Am J. Gastro. 2003
Cholestatic Liver Diseases

Intrahepatic
- Drugs
- Alcohol
- Infiltrative liver disease
- Pregnancy
- Heart failure
- Primary biliary cholangitis
- Infection
- Immunodeficiency
- Sarcoidosis
- Ischemia

Extrahepatic
- Primary sclerosing cholangitis
- Pancreatitis
- Tumors
- IgG4 cholangitis
- Congenital biliary anomalies
- Cystic fibrosis
Biologic Spectrum of Cholestatic Liver Disease

FAMILIAL
CFTR, ABCB4
OLIGOGENIC
PSC & PBC

ENVIRONMENT

GENETICS

SPORADIC
INFECTIONS, DRUGS, DIET
POLYGENIC
Primary Biliary Cirrhosis Cholangitis

Male : Female 9:1
Age Typically > 45 years
Laboratory Increased ALP, GGT, AST, ALT
Exposures Bacterial mimics/ xenobiotic chemicals
Serologies AMA + ~ 95%, ANA + 35-50% , +/- ASMA
Liver Histology Lymphocytic infiltrate, inflammatory “florid” duct lesion, granulaoms may be present
MRCP / ERCP Normal appearing biliary tree
Co-existing IBD Not typically present

Younossi Z et al. Am J Gastro. 2019
Carey EJ. et al. Lancet. 2015
PouponR. J of Hepatol. 2010
### Elevated ALP is typical of PBC

<table>
<thead>
<tr>
<th>Test</th>
<th>Finding</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP</td>
<td>↑</td>
<td>Values associated with disease progression</td>
</tr>
<tr>
<td>AST/ALT</td>
<td>↑</td>
<td>May be suggestive of PBC with features of AIH</td>
</tr>
<tr>
<td>GGT</td>
<td>↑</td>
<td>Reflects cholestatic liver injury</td>
</tr>
<tr>
<td>IgM</td>
<td>↑</td>
<td>Elevated values associated with disease</td>
</tr>
<tr>
<td>AMA (&gt;1/40)</td>
<td>+</td>
<td>Diagnostic in &gt;90% of cases in correct clinical context</td>
</tr>
<tr>
<td>Specific ANA</td>
<td>+</td>
<td>Specific immunofluorescence patterns* present in 30%</td>
</tr>
<tr>
<td>Anti-gp210</td>
<td>+</td>
<td>Specific immunoassays available</td>
</tr>
<tr>
<td>Anti-sp100</td>
<td>+</td>
<td>Specific immunoassays available</td>
</tr>
<tr>
<td>Anti-centromere</td>
<td>+</td>
<td>Associated with portal hypertensive phenotype</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>↑</td>
<td>Elevation at late stages frequently indicative of cirrhosis†</td>
</tr>
<tr>
<td>Platelets</td>
<td>↓</td>
<td>Indicative of cirrhosis</td>
</tr>
<tr>
<td>INR</td>
<td>↑</td>
<td>Indicative of cirrhosis</td>
</tr>
<tr>
<td>Albumin</td>
<td>↓</td>
<td>Indicative of cirrhosis</td>
</tr>
</tbody>
</table>

† Except in patients with ductopenic non-cirrhotic variant

EASL CPG PBC. J Hepatol 2017;67:145–72
Pathogenesis of Primary Sclerosing Cholangitis

- Immunological Factors
  - Adaptive response (strong association with HLA loci)
  - Gut-Liver Axis
  - Innate response
  - T-cell homing

- Environmental Factors
  - Explain about 50% of risk

- Genetic Predisposition
  - Known risk loci (>20) explain <10% of risk

- Microbiome

- Toxic Bile

- Activated / inflammatory / senescent biliary phenotype

- Cancer
- Inflammatory bowel disease
- Autoimmunity

Karsen et al. J Hepatol 2017, 67 1258-1323
Primary sclerosing cholangitis - Overview

Male : Female 2:1 (overt cases)
silent disease in F

Age at presentation: 25-45 years

Median OS: 12-21 years
w/o dominant stricture: 23 years
w dominant stricture: 14 years

Laboratory abnormalities: ALP, GGT (but also AST, ALT)

Autoantibodies: pANCA (26-94%)
(atyp. nuclear staining)
IgG4 < 4x ULN

Associated IBD: 60-80 % Northern Europe,
USA
20-37% Asia

Malignancies: premalignant disease, esp. CCA (11-20%) GB, CRC

Recurrence after OLT: 25% (less after colectomy)
Diagnostic Approach to Cholestasis

Elevated serum ALP/GGT and/or conjugated bilirubin (HBsAg and anti-HCV negative)

History, physical examination, abdominal US
- No abnormalities

AMA, ANA (anti-sp100, anti-gp210)
- Negative and no specific drug history

Extended imaging: MRCP (± EUS)
- No abnormalities

Liver biopsy
- No abnormalities

Genetics

Observation/re-evaluation

Suspicion of DILI
- Focal lesions; dilated bile ducts

Serum antibodies

Stenoses (sclerosing cholangitis)

Parenchymal damage
- Biliary lesions

Gene mutations

Hurdles for NASH and Cholestatic Liver Disease

Lack of diagnostic / prognostic biomarkers

Limited pharmacologic therapies

Lack of tailored approaches

Promising Future Ahead......
Altered Hepatic Transporter Function in Patients with NASH: Implications for Drug Disposition, Efficacy and Safety

Kim L. R. Brouwer, PharmD, PhD

William R. Kenan Jr., Distinguished Professor
Associate Dean for Research & Graduate Education

The University of North Carolina at Chapel Hill
Conflict of Interest Disclosure

• **Commercial Financial Interest:** Co-inventor of the sandwich-cultured hepatocyte technology for quantification of biliary excretion (B-CLEAR®) and related technologies, which have been licensed exclusively to Qualyst Transporter Solutions, LLC, recently acquired by BioIVT

• **Scientific Consultant:** Merck Research Laboratories, Indalo Therapeutics, Inc.

• **Grants/Research Support:** National Institute of General Medical Sciences, National Institutes of Health (R35 GM122576); Intercept Pharmaceuticals; Otsuka Pharmaceutical Development & Commercialization, Inc.; Gilead Sciences, Inc.
Outline

• Hepatic Transport Proteins in Health and Disease

• Altered Hepatic Transport in Patients with Nonalcoholic Steatohepatitis (NASH) and Pharmacokinetic Implications
  ➢ Clinical Probes to Assess Transporter Function
    − $^{99m}$Tc-Mebrofenin (MRP2 probe)
    − Morphine/Morphine Glucuronides (MRP3 probe)

• Increased Bile Acid Concentrations and Impact on Hepatic Transporters
  ➢ OSTα/β Induction

• Implications for Drug Disposition, Efficacy and Safety, and Knowledge Gaps
Hepatic Uptake and Efflux Transporters

BSEP (Bile Salt Export Pump);
NTCP (Sodium-Taurocholate Cotransporting Polypeptide);
MRP (Multidrug Resistance–Associated Protein);
OST (Organic Solute Transporter)

(Adapted from Ho and Kim, Clin Pharmacol Ther, 78:260, 2005)
Altered Hepatic Transport Protein Levels in Liver Tissue from Patients with NASH

**Hepatic Uptake Transporters**

- OATP1B1
- OATP1B3
- OATP2B1

**Hepatic Efflux Transporters**

- MRP1
- MRP3
- MRP4
- P-gp
- BCRP

**Human Liver Tissue**

Clarke et al., *J Hepatol*, 61:139, 2014

Hardwick et al., *Drug Metab Dispos*, 39:2395, 2011
Altered MRP2 Expression and Localization in Liver Tissue from Patients with NASH

Clarke et al., Liver Int, 37:1074, 2017

Hardwick et al., Drug Metab Dispos, 39:2395, 2011
$^{99m}$Tc-Mebrofenin (Choletec®):
Probe for Transporter-Mediated Hepatobiliary Excretion

- Used clinically as a hepatobiliary imaging agent
- Liver uptake ~98%; negligible metabolism
- Urinary excretion <2% of dose
- Transporter-mediated hepatobiliary disposition
  - Hepatic uptake via OATP1B1 and OATP1B3
  - Biliary excretion via MRP2
  - Basolateral excretion via MRP3

Simulations Predict Increased Hepatic Exposure to MRP2 Substrates in Patients with NASH

Clinical Study Design: $^{99m}$Tc-Mebrofenin

- Healthy subjects (n=14) and biopsy-confirmed NASH patients [n=7; NAFLD activity score (NAS)≥4] admitted on morning of study after overnight fast
- Attenuation correction obtained with a cobalt-57 flood source
- Subjects positioned supine under gamma camera

- Subjects discharged following exit exam

- Screen/Informed Consent
- $^{99m}$Tc-mebrofenin PK
- Attenuation correction
- Urine collection
- Continuous γ-scintigraphy
- Urine collection
- Safety questionnaire & discharge
Hepatic $^{99m}$Tc-Mebrofenin Exposure was Increased in Patients with NASH

Blood

- 1.9-fold ↑ in $C_{\text{max}}$
- 1.7-fold ↑ in exposure

Liver

- 2-fold ↑ in exposure
- $t_{1/2} = 50$ min
- $t_{1/2} = 38$ min

Mean ± SD
- Control (n = 14)
- NASH (n = 7)

Ali...Brouwer, *Clin Pharmacol Ther*, 104:749, 2018
Model Scheme Describing $^{99m}$Tc-Mebrofenin Disposition and Parameter Estimates

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy (n=14)</th>
<th>NASH (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$CL_{uptake}$ (L/min)</td>
<td>1.14 (0.73-2.27)</td>
<td>0.731 ** (0.382-1.04)</td>
</tr>
<tr>
<td>$CL_{efflux}$ (L/min)</td>
<td>0.00800 (0.00481-0.0139)</td>
<td>0.00579 (0.00475-0.00903)</td>
</tr>
<tr>
<td>$CL_{bile}$ (L/min)</td>
<td>0.0354 (0.0157-0.0728)</td>
<td>0.0171 ** (0.0110-0.0207)</td>
</tr>
<tr>
<td>$V_{central}$ (L)</td>
<td>11.1 (9.55-12.5)</td>
<td>6.32 ** (5.69-9.69)</td>
</tr>
<tr>
<td>$V_{liver}$ (L)</td>
<td>0.958 (0.527-1.39)</td>
<td>0.891 (0.648-1.43)</td>
</tr>
</tbody>
</table>

Median (range); **p<0.001

Ali...Brouwer, *Clin Pharmacol Ther*, 104:749, 2018
**99mTc-Mebrofenin Hepatic Uptake Clearance was Lower in NASH Patients even with “Normal Function” SLCO1B1 Genotype**

<table>
<thead>
<tr>
<th>SLCO1B1 Genotype</th>
<th>Healthy (# of subjects)</th>
<th>NASH (# of subjects)</th>
<th>Function</th>
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</thead>
<tbody>
<tr>
<td>*15/*15</td>
<td>1</td>
<td></td>
<td>Low (LF)</td>
</tr>
<tr>
<td>*1A/*15</td>
<td>3</td>
<td></td>
<td>Intermediate (IF)</td>
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<tr>
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<td>Normal (NF)</td>
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<td>*1A/*1A</td>
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<td>3</td>
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</tr>
<tr>
<td>*1B/*1B</td>
<td>2</td>
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</tr>
<tr>
<td>*1B/*14</td>
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<td></td>
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<tr>
<td>*1A/*1B</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Ali…Brouwer, *Clin Pharmacol Ther*, **104**:749, 2018
Increased MRP3 Protein Levels in Liver Tissue from Patients with NASH

Normal | Steatosis | NASH (fatty infiltration)
--------|----------|-----------------------
(>5%)    | (<5%)    |

MRP3

~3-fold increase

Relative Protein Expression

Hardwick et al., Drug Metab Dispos, 39:2395, 2011
Hepatic Disposition of Morphine and Metabolites

Morphine

- OCT1 (SLC22A1)
- MRP2 (ABCC2)
- MRP3 (ABCC3)
- ATP
- ATP

blood flow

Morphine

- Morphine-3- (M3G) and Morphine-6- (M6G) Glucuronide

UGT2B7

bile

MorpKine

lucuronide

MorpKine-3- (M3*) and MorpKine-- (M*)

bile

blood flow
Clinical Study Design: Morphine / Glucuronides

- Healthy subjects without insulin resistance: n=14
- Biopsy confirmed NASH patients [NAFLD activity score (NAS)≥4]: n=7

Ferslew...Brouwer, Clin Pharmacol Ther, 97:419, 2015
Increased Morphine-3-Glucuronide (M3G) and Morphine-6-Glucuronide (M6G) Serum Concentrations in Patients with NASH

<table>
<thead>
<tr>
<th>MG Parameters</th>
<th>Healthy (n=14)</th>
<th>NASH (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (nM)</td>
<td>225 (194-261)</td>
<td>343** (284-413)</td>
</tr>
<tr>
<td>$AUC_{0-\text{last}}$ (µM*min)</td>
<td>37 (32-44)</td>
<td>59 ** (42-83)</td>
</tr>
<tr>
<td>Half-life (min)</td>
<td>187 (153-229)</td>
<td>146 (104-205)</td>
</tr>
</tbody>
</table>

Geometric mean (95% CI); ** p<0.01 t-test on log transformed data

Simulations Predict Reduced Enterohepatic Recycling (EHR) of Morphine 3-Glucuronide (M3G) in Patients with NASH

Obese subjects

Obese subjects with NASH-related transporter changes

Without EHR, 21% decrease in M3G AUC

Without EHR, 11% decrease in M3G AUC

Serum concentrations were simulated after 3.76 mg morphine i.v. in 10 x 10 obese virtual subjects (age 33 – 63, 50% female)

Sjöstedt...Brouwer, in preparation, 2020
Increased Conjugated Bile Acids in Serum of NASH Patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total Bile Acids</th>
<th>Glycocholate</th>
<th>Taurocholate</th>
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<tbody>
<tr>
<td>β</td>
<td>β</td>
<td>β</td>
<td>β</td>
</tr>
<tr>
<td>P-value</td>
<td>(0.10)</td>
<td>(0.01)</td>
<td>(0.01)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.004</td>
<td>0.03</td>
<td>0.001</td>
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<tr>
<td>P-value</td>
<td>0.02</td>
<td>0.006</td>
<td></td>
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</table>

Mean ± SEM; *p<0.05

β: regression parameter estimate (SE)

Serum and Urine Bile Acids at Screening

Serum

Urine

Mean ± SEM, Healthy: n=15 (13 for urine), NASH: n=7

CDCA = Chenodeoxycholic Acid
CA = Cholic Acid
DCA = Deoxycholic Acid
LCA = Lithocholic Acid
UDCA = Ursodeoxycholic Acid

Ferslew et al., *Dig Dis Sci*, **60**:3318-3328, 2015
Organic Solute Transporter (OSTα/β) SLC51A/B is Upregulated in Patients with Liver Disease

Is OSTα/β an Overlooked “Safety Valve” for Hepatocellular Efflux of Bile Acids?

Patients with Nonalcoholic Steatohepatitis (NASH) and Primary Biliary Cirrhosis (PBC)

Altered Hepatic Transporter Function in Patients with NASH may Increase Hepatic and/or Systemic Exposure to Drugs and Susceptibility to Drug-Induced Liver Injury

(Adapted from Ho and Kim, Clin Pharmacol Ther, 78:260, 2005)
Altered Hepatic Transporter Function in Patients with NASH: Implications for Drug Disposition, Efficacy and Safety

- Altered hepatic transporters (↓OATP1B1 and OATP1B3, ↓MRP2, ↑MRP3, ↑OSTα/β) in patients with NASH may **impact the systemic and/or hepatic exposure** to substrates [drugs, metabolites, and endogenous compounds (e.g., bile acids)]

- Systemic concentrations may **not** accurately reflect hepatic exposure

- Patients with NASH may be **predisposed to bile acid-mediated drug-induced liver injury** by medications that inhibit hepatic efflux transporters
Knowledge Gaps in Predicting Drug Disposition, Efficacy and Safety in Patients with NASH

- Impact of disease progression (NAFLD → NASH → Cirrhosis) on hepatic transporter function
- Pathophysiologic changes in input parameters for physiologically-based pharmacokinetic (PBPK) models during disease progression
- Altered transporter function in other key organs of drug absorption/elimination (e.g., intestine, kidney)
- Relationship between systemic concentrations and tissue exposure
Graduate Students/ Postdoctoral Fellows/ Visiting Scholars
- Izna Ali
- James Beaudoin
- Jacqueline Bezençon
- Brian Ferslew
- Giulia Ghibellini
- Curtis Johnston
- Josh Kaullen
- Kathleen Köck
- Melina Malinen
- Noora Sjöstedt
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Collaborators
- Sid Barritt
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- Arlene Bridges
- Wei Jai
- Mary Paine
- Paul Watkins

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- Clinical & Translational Research Center

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- National Institutes of Health Grants
  R01 GM41935, R35 GM122576, T32 GM86330 (NIGMS); UL1 TR001111 (NCATS)
- Finnish Cultural Foundation, Orion Research Foundation, Sigrid Jusélius Foundation
- Erasmus+ Programme
- IQVIA Clinical PK/PD Fellowship
Bile Acid Synthesis in Human NASH

Lake et al., Toxicol Appl Pharmacol, 268:132, 2013
Orthogonal partial least squares discriminant analysis (OPLS-DA) ($R^2_{X_{\text{cum}}} = 0.380$, $R^2_{Y_{\text{cum}}} = 0.077$, $Q^2_{Y_{\text{cum}}} = 0.0419$) scores plot discriminating the serum bile acid profiles over time.
Clinical Pharmacology Questions for Drug Development for NASH and Cholestatic Liver Diseases

Insook Kim, Ph.D.
Team Leader, Gastroenterology and Hepatology Products
Division of Inflammation and Immune Pharmacology
Office of Clinical Pharmacology
OTS/CDER/FDA
Disclaimer

The views and opinions expressed here are my own and do not represent official guidance from the FDA.
Outline

• Current trends of clinical trials for liver diseases
• Draft NASH guidances
• Major Clinical Pharmacology Questions at the IND Stage
• Summary
Unmet needs

• Limited number of approved drug products for non-viral, non-oncology liver diseases
  – Ursodeoxycholic acid for primary biliary cirrhosis (1997)
  – Rifaximin for reduction in risk of overt hepatic encephalopathy recurrence (2010)
  – Obeticholic acid for primary biliary cholangitis (2016; Subpart H based on ALP reduction)
• No approved drugs for NASH or PSC

ALP: Alkaline Phosphatase
PSC: Primary sclerosing cholangitis
Number of IND Submission

NASH/Guidances

YEAR SUBMITTED

Courtesy of Dr. Yao-Yao Zhu
Submission Trends

• Development Program
  – Commercial IND #
    • 132 (73 active) for NASH/NAFLD, 32 (14 active) for PBC/PSC
  – Mostly in phase 1 and 2; a few completed phase 3
  – Many phase 2 study protocols to open an IND

• Investigational Treatment
  – NME (small molecules >>> biologics)
  – Repurposing of previously approved/studied drugs
    • e.g. T2DM agents, anti-hyperlipidemia, weight loss
  – Combination therapy
  – One drug for both NASH and cholestatic liver diseases in some cases

• Recent late phase attrition

www.fda.gov
Draft Guidances for NASH

• Noncirrhotic NASH with Liver Fibrosis (2018)
  – Accelerated approval pathway based on liver histology
    • Improvement in fibrosis stage > 1 and no worsening of steatohepatitis OR
    • Resolution of steatohepatitis and no worsening of liver fibrosis OR
    • Both resolution of steatohepatitis and improvement in fibrosis

• NASH with Compensated Cirrhosis (2019)
  – Efficacy: Time to clinical outcome events
  – Compensated cirrhosis by histology (e.g., F4 fibrosis);
  – Exclusion of MELD score > 12
Biomarkers

Noninvasive, disease-specific biomarkers; standard measures of liver injury (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)); and imaging modalities that assess liver stiffness or hepatic fat content are acceptable as POC study endpoints as long as the sponsor can scientifically justify them.

Once proof of pharmacological activity has been demonstrated in a NASH population of interest, the phase 2 program should explore the treatment effect on histological endpoints.

The sponsor should provide information supporting the proposed biomarker strategy for the late phase 2 program. This can include, but is not limited to, the following:

- Inclusion of biomarkers that can reliably predict the histopathological evidence of NASH with or without liver fibrosis and, as such, can increase the likelihood of a confirmatory liver biopsy, reduce the number of screening failures, and expedite the screening of eligible patients

- Inclusion of diagnostic biomarkers that may provide evidence of progression to cirrhosis

- Inclusion of prognostic biomarkers that may robustly predict liver-related complications

Early Clinical Trials for Dose Selection

• Proof of mechanism
  – Identification of target engagement biomarkers
  – PK/PD for biomarkers

• Proof of concept in patient population
  – Biomarkers that correlate with liver histology
    • Histology for NASH (reasonably, likely predict clinical outcome)
  – POC for each component for a combination product
  – Exposure-response relationship

Relationship among MOA, disease-specific biomarkers, imaging biomarkers, and histology should be evaluated and established throughout the program.

MOA: mechanism of action
Major Clinical Pharmacology Questions at the IND Stage

• Dose selection
• Drug-drug interactions with concomitant medications for patients in clinical trials
  Patients with liver diseases often have comorbidities that require medications

• Inclusion/exclusion criteria based on renal and hepatic function
  – Drug ADME, and impact of hepatic and renal impairment on PK
    Patients with liver diseases can have concurrent HI and RI

ADME: absorption, distribution, metabolism, excretion
Early evaluation of the effects of hepatic impairment on PK to support dosing across the spectrum of NASH liver disease

Until a sponsor can characterize a drug’s initial tolerability, preliminary safety, and pharmacokinetics, patients with evidence of abnormal liver synthetic function should be excluded from early phase trials (i.e., phase 1 and early proof-of-concept (POC) clinical trials). In addition, the sponsor should study the effects of hepatic impairment on the drug’s pharmacokinetics early during the drug development program in a dedicated hepatic study to support appropriate dosing and dose adjustment across the spectrum of NASH liver disease.
Importance of Right Dosage for Patients with Hepatic Impairment

<table>
<thead>
<tr>
<th>Staging/Classification</th>
<th>Non-Cirrhotic or Compensated Child-Pugh Class A</th>
<th>Child-Pugh Class B or C or Patients with a Prior Decompensation Eventa</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Starting OCALIVA Dosage for first 3 months</strong></td>
<td>5 mg once daily</td>
<td>5 mg once weekly</td>
</tr>
<tr>
<td><strong>OCALIVA Dosage Titration after first 3 months, for patients who have not achieved an adequate reduction in ALP and/or total bilirubin and who are tolerating OCALIVAb</strong></td>
<td>10 mg once daily</td>
<td>5 mg twice weekly (at least 3 days apart)</td>
</tr>
<tr>
<td><strong>Maximum OCALIVA Dosage</strong></td>
<td>10 mg once daily</td>
<td>10 mg twice weekly (at least 3 days apart)</td>
</tr>
</tbody>
</table>

---

**WARNING: HEPATIC DECOMPENSATION AND FAILURE IN INCORRECTLY DOSED PBC PATIENTS WITH CHILD-PUGH CLASS B OR C OR DECOMPENSATED CIRRHOSIS**

See full prescribing information for complete boxed warning.

- In postmarketing reports, hepatic decompensation and failure, in some cases fatal, have been reported in patients with primary biliary cholangitis (PBC) with decompensated cirrhosis or Child-Pugh Class B or C hepatic impairment when OCALIVA was dosed more frequently than recommended. (5.1)
- The recommended starting dosage of OCALIVA is 5 mg once weekly for patients with Child-Pugh Class B or C hepatic impairment or a prior decompensation event. (2.2)

---

Ocaliva Label: https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/207999s003lbl.pdf
Hepatic impairment study

- Single or multiple dose PK study
- Initial tolerability in subjects with hepatic impairment
- Cirrhosis patients
- Degree of HI based on Child-Pugh score
  - Mild HI: Child-Pugh Class A (score 5-6)
  - Moderate HI: Child-Pugh Class B (score 7-9)
  - Severe HI: Child-Pugh Class C (score 10-15)
- Other classification may be used with collection of Child-Pugh score

To support the dosing for patients with cirrhosis in clinical trials

To support clinical trials for patients with non-cirrhotic NASH and cholestatic liver diseases
- Patients may asymptotically progress to compensated cirrhosis during the trials

PK in patients with non-cirrhotic NASH or cholestatic liver disease need to be collected in the clinical trials

Case: Repurposing of Drug A

• Approved for a non-liver disease
• Proposed for non-cirrhotic NASH with fibrosis (stage F2/F3)
  – Patients with cirrhosis on biopsy excluded from efficacy trials
• To study the same dose as the approved dose for the non-liver disease
• In a legacy HI study, 5-fold higher AUC in subjects with compensated cirrhosis than in subjects with normal liver function

Risk mitigation strategy for patients who may asymptotically progress to cirrhosis during the trial is needed

Biomarkers for progression to cirrhosis
Biomarkers correlated with the PK change
Summary

• Unmet medical needs in the area of NASH and cholestatic liver diseases

• Early characterization of ADME of the drug and impact of hepatic impairment on PK
  – To support dosing in patients with liver diseases
  – To mitigate safety concerns if the liver disease progresses during the trials
Opportunities

• Better understanding of relationship between drug concentrations in plasma and liver

• Better characterization of physiologic parameters that reflect the pathophysiological changes due to liver dysfunction and that may impact PK

• Simultaneous assessment of PK and biomarkers in target patient population to understand and establish the relationship between PK, PD, and disease biomarkers
Acknowledgements

• Clinical pharmacology review team for GI/Liver products
  • Anand Balakrishnan
  • Jenny Cheng
  • Dilara Jappar
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  • Sojeong Yi
• Former members
  • Jack Wang
  • Xiaohui Li
  • Christine Hon

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  • Clinical review team
  • Pharm/Tox review team

• OCP senior leadership
  • Shirley Seo (DCEP)
  • Donny Tran (DCEP)
  • Chandrahas Sahajwalla (DIIP)
  • Suresh Doddapaneni (DIIP)
  • Issam Zineh (Office Director)
Shortfall on Primary Endpoint Shifts Focus for Emricasan to Ongoing NASH Cirrhosis Trials

SAN DIEGO, March 21, 2019 (GLOBE NEWSWIRE) -- Conatus Pharmaceuticals Inc. (Nasdaq:CNAT) today announced top-line results from the company’s Phase 2b ENCORE clinical trial in patients with biopsy-confirmed nonalcoholic steatohepatitis (NASH) and liver fibrosis. The primary endpoint was a ≥1 CRN fibrosis stage improvement with no worsening of liver inflammation.

CymaBay Therapeutics Halts Clinical Development of Seladelpar

NEWARK, Calif., Nov. 25, 2019 (GLOBE NEWSWIRE) -- CymaBay Therapeutics, Inc. (Nasdaq: CBAI), a clinical-stage biopharmaceutical company focused on developing therapies for liver and other chronic diseases with high unmet need, today announced that the company was terminating its Phase 2b study of seladelpar in subjects with non-alcoholic steatohepatitis (NASH) and its recently initiated Phase 2 study of seladelpar in subjects with primary sclerosing cholangitis (PSC). In addition, the company is putting on hold all studies of seladelpar in subjects with primary biliary cholangitis (PBC).

The decision to halt development of seladelpar was based on initial histological findings observed in the Phase 2b study of seladelpar in NASH. Planned, blinded histological assessments of the first tranche of liver biopsies in the trial revealed atypical histological findings, including histology characterized as an interface hepatitis presentation, with or without biliary injury. The company has initiated a series of investigative actions to better understand these findings.

Session 2: Early Discovery and Development—Treatment Mechanisms, Molecular Targets, and Biomarkers in Early Development of Therapies for NASH and Cholestatic Liver Diseases

10:50 am – 12:15 pm
An Overview of Treatment Mechanisms, Molecular Targets, and Biomarkers in Early Development of Therapies in NASH and Cholestatic Liver Diseases

Naga Chalasani, MD
Indiana University School of Medicine
COI Disclosure (12/5/19)

- Ongoing paid consulting activities (or had in preceding 12 months) with NuSirt, Abbvie, Allergan, Madrigal, Siemens, La Jolla, Foresite labs, Axcella, Zydus, Galectin, and Genentech.

- Research grant support from Exact Sciences, DSM, and Intercept where his institution receives the funding.

- No speaking fees since 1999
<table>
<thead>
<tr>
<th>NAFL</th>
<th>NASH w/ F0 &amp; F1</th>
<th>NASH with F2 &amp; F3</th>
<th>NASH Cirrhosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular</td>
<td>Cardiovascular</td>
<td>Liver disease</td>
<td>Liver disease</td>
</tr>
<tr>
<td>Non-hepatic malignancies</td>
<td>Non-hepatic malignancies</td>
<td>Cardiovascular disease</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>?Renal disease</td>
<td>Liver disease</td>
<td>Hepatic &amp; non-hepatic malignancies</td>
<td>Hepatic &amp; non-hepatic malignancies</td>
</tr>
</tbody>
</table>
Liver directed pharmacotherapy

<table>
<thead>
<tr>
<th>Condition</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAFL</td>
<td>No</td>
</tr>
<tr>
<td>NASH w/F0 &amp; F1</td>
<td>Probably Not</td>
</tr>
<tr>
<td>NASH w/F2&amp;F3</td>
<td>Yes</td>
</tr>
<tr>
<td>NASH Cirrhosis</td>
<td>Yes</td>
</tr>
</tbody>
</table>
## NASH w/ F2 and F3

<table>
<thead>
<tr>
<th>Major causes of morbidity and mortality</th>
<th>Desired Outcomes</th>
<th>Valid surrogate(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver failure</td>
<td>Prevention of progression to cirrhosis</td>
<td>Resolution of NASH with no worsening of fibrosis OR Significant improvement in fibrosis with stable steatohepatitis</td>
</tr>
</tbody>
</table>
NASH Cirrhosis: Early vs Late Stage

**Early cirrhosis**
- Thin septa
- No CSPH
- Preserved synthetic function
- Active SH

Reversal of cirrhosis (≤ 2point drop in Fibrosis score)
- Study duration ≥ 2 yrs
- Combination Rx

Prevention of development of complications of cirrhosis
- Longer study duration
- Combination Rx

**Late Stage cirrhosis**
- Thick septa
- CSPH (Plts <100k, splenomegaly, LSM >18 kPa)
- MELD < 13
- ± Active SH

Prevention of development of complications of cirrhosis
- Longer study duration
  - Combination Rx
## Currently Available Drugs for Treatment of NASH

### Targeting insulin resistance

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mechanism of action</th>
<th>Trials</th>
<th>Primary endpoint(s)</th>
<th>AASLD recommendation as NASH treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin</td>
<td>Multiple</td>
<td>Multiple studies</td>
<td>Various</td>
<td>Not recommended</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>PPARγ agonist</td>
<td>PIVENS*</td>
<td>Improvement in NAS ≥ 2 without fibrosis worsening</td>
<td>May be used in patients with biopsy-proven NASH</td>
</tr>
<tr>
<td>Liraglutide</td>
<td>GLP-1 receptor agonist</td>
<td>LEAN*</td>
<td>Resolution of NASH without fibrosis worsening</td>
<td>Premature to consider GLP-1 receptor agonists</td>
</tr>
</tbody>
</table>

### Targeting Oxidative stress

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mechanism of action</th>
<th>Trial</th>
<th>Primary endpoint(s)</th>
<th>AASLD recommendation as NASH treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin E</td>
<td>Antioxidant</td>
<td>PIVENS*</td>
<td>Improvement in NAS ≥ 2 without fibrosis worsening</td>
<td>May be used in non-diabetic adults with biopsy-proven NASH</td>
</tr>
</tbody>
</table>

*PIVENS: Prospective Investigation of Nondiabetic NASH Study  
LEAN: Long-term Evaluation of Nondiabetic NASH Study  
TONIC: Treatment of NASH with GLP-1 Receptor Agonists  
AASLD: American Association for the Study of Liver Diseases
Global Pipeline for NASH

Slide Courtesy from Michael Charlton, MD
Categorization of NASH Development Compounds

**Targeted Metabolic**
- Insulin sensitizers (GLP-1)
- FGF21/19
- SGLT-1 inhibitors
- KHK inhibitor
- PUFAs
- IBAT inhibitors
- DGAT-2 inhibitors

**ROS stress reduction**
- Vitamin E
- mTOT inhibitors

**Anti-Steatosis**
- ACC inhibitors
- SCD1 inhibitors
- FASN inhibitors
- Omega-3 fatty acid

**Multifactorial Metabolic**
- PPAR agonists
- FXR agonists
- THRβ agonists
- LXR agonists
- Mito pyruvate carrier modulators

**Intestinal permeability**
- Larazotide
- Lubiprostone

**Anti-Inflammatory/Fibrotic**
- CCR2/5 inhibitor
- ASK-1 inhibitors
- Caspase inhibitors
- Galectin-3 inhibitors
- 5-lipogenase inhibitors

**Nuclear Hormone Receptors**
- CB1 inhibitors
- A3AR antagonists
- LOXL2 inhibitors
- NOX1/4 inhibitors
- ROCK2 inhibitors
- αvβ6/1 integrin inhibitors

Slide Courtesy from Peter Traber, MD - Alacrita
## Advanced Phase Monotherapy Programs in Pre-Cirrhotic NASH

<table>
<thead>
<tr>
<th>Phase 3 initiated/completed/posted</th>
<th>Phase 2 initiated/completed/posted</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Obeticholic Acid (FXR agonist)</td>
<td>- Namodenoson (A3 adenosine receptor agonist)</td>
</tr>
<tr>
<td>- Elafibranor (PPAR α/δ)</td>
<td>- IMM-124-E (bovine colostrum)</td>
</tr>
<tr>
<td>- Resmetrion (THRβ agonist)</td>
<td>- CORT118335 (Glucocorticoid Receptor Modulators)</td>
</tr>
<tr>
<td>- Cenicriviroc (CCR2/5 inhibitor)</td>
<td>- Licoglitifozin (SGLT2 inhibitor)</td>
</tr>
<tr>
<td>- Aramchol (SCD-1 inhibitor)</td>
<td>- Icosabutate (SCFA)</td>
</tr>
<tr>
<td>- MSDC-0602K (mito pyruvate carrier modulator)</td>
<td>- AZD4017 (11-βhydroxysteroid dehydrogenase inh)</td>
</tr>
<tr>
<td>- Selonsertib (ASK-1 inhibitor)**</td>
<td>- CC-90001 (JNK inhibitor)</td>
</tr>
</tbody>
</table>

* Failed primary endpoint in phase 2 trial
** Failed primary endpoint in phase 3 trial

Slide Courtesy from Dr. Peter Traber
<table>
<thead>
<tr>
<th>Drug (Company)</th>
<th>MOA</th>
<th>Phase</th>
<th>Study Description</th>
<th>Data (estimate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obeticholic acid ( Intercept)</td>
<td>FXR Agonist</td>
<td>3</td>
<td>REGENERATE: NASH with F2/F3 fib Endpoint: Fibrosis OR Resolution; Composite outcomes</td>
<td>Met primary endpoint</td>
</tr>
<tr>
<td>Elafibranor (Genfit)</td>
<td>PPAR α/δ agonist</td>
<td>3</td>
<td>RESOLVE-IT: NASH with F1-3 fibrosis (n=2000) Endpoints: Resolution [72 wks]; Composite outcomes</td>
<td>Recruit completed</td>
</tr>
<tr>
<td>Resmetriom (Madrigal)</td>
<td>THR β agonist</td>
<td>3</td>
<td>MAESTRO-NASH: NASH with F2-3 fibrosis (n=2000) Endpoint: Resolution [52 wks]; Composite outcomes</td>
<td>Recruiting</td>
</tr>
<tr>
<td>Cenicriviroc (Allergan)</td>
<td>CCR2/5 inhibitor</td>
<td>3</td>
<td>AURORA: NASH with F2-3 fibrosis (n=2000) Endpoints: Fibrosis [12 months]; Composite outcomes</td>
<td>Recruiting</td>
</tr>
<tr>
<td>Aramchol (Galmed)</td>
<td>SCD1 inhibitor</td>
<td>3</td>
<td>ARMOR: NASH with F2/F3 fib (n=~2000) Endpoint: Fibrosis OR Resolution [52 wks]; Composite outcomes</td>
<td>Recruiting</td>
</tr>
<tr>
<td>MSDC-0602K (Cirius)</td>
<td>Mitochondrial pyruvate carrier modulator</td>
<td>3</td>
<td>NASH with fibrosis (n=3600) Endpoint: HbA1c [6 mo] and Resolution [12 mo] Composite hepatic and cardiac outcomes [31 mo]</td>
<td>Not yet recruiting</td>
</tr>
<tr>
<td>Selonsertib (Gilead)</td>
<td>ASK-1 inhibitor</td>
<td>3</td>
<td>STELLAR-3: NASH with F3 fibrosis Endpoints: Fibrosis; Composite outcomes</td>
<td>Failed primary Terminated**</td>
</tr>
</tbody>
</table>

* Includes trials that have been initiated and have information on trial posted on clinicaltrials.gov
** Continuing evaluation in combination clinical trial (ATLAS; NCT03449446)
<table>
<thead>
<tr>
<th>Drug (Company)</th>
<th>MOA</th>
<th>Phase</th>
<th>Study Description</th>
<th>Data</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selonsertib (GILD)</td>
<td>ASK-1 inhibitor</td>
<td>3</td>
<td>STELLAR-4: Comp NASH cirrhosis EP: Fibrosis; composite outcomes</td>
<td>Failed</td>
<td>Also failed STELLAR3 (stage 3 NASH); ATLAS P2 combination trial ongoing</td>
</tr>
<tr>
<td>Obeticholic acid (ICPT)</td>
<td>FXR Agonist</td>
<td>3</td>
<td>REVERSE: Comp NASH cirrhosis EP: Fibrosis; composite outcomes</td>
<td>JUN 2021</td>
<td>Trial ongoing; In Aug 2019 increased patients from 540 to 900 and extended Rx 12 to 18 mo</td>
</tr>
<tr>
<td>Simtuzumab (GILD)</td>
<td>LOXL2 inhibitor</td>
<td>2</td>
<td>Comp NASH cirrhosis EP: Change in HVPG</td>
<td>Failed</td>
<td>Also failed in pre-cirrhotic NASH to improve fibrosis. Program discontinued</td>
</tr>
<tr>
<td>Belapectin (GALT)</td>
<td>Galectin-3 inhibitor</td>
<td>2</td>
<td>NASH-CX: Comp NASH cirrhosis EP: Change in HVPG</td>
<td>Failed</td>
<td>Post-hoc difference in HVPG without varices and reduced development of varices; no effect on fibrosis; P3 trial planned**</td>
</tr>
<tr>
<td>Emricasan (CNAT/Novartis)</td>
<td>Pan-caspase inhibitor</td>
<td>2</td>
<td>ENCORE-PH Change in HVPG ENCORE-LF Complications</td>
<td>Failed</td>
<td>Post-hoc analysis showed some effect in high HVPG sub-group; Currently not progressing</td>
</tr>
<tr>
<td>Pegbelfermin (BMS)</td>
<td>PEG-FGF21</td>
<td>2</td>
<td>Comp NASH cirrhosis EP: Fibrosis</td>
<td>JAN 2020</td>
<td>Completed trial enrollment</td>
</tr>
</tbody>
</table>
# Selected Biomarkers – NAFLD and NASH

<table>
<thead>
<tr>
<th>Soluble biomarkers</th>
<th>Imaging</th>
<th>Function tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>VCTE (Fibroscan)</td>
<td>Hepaquant</td>
</tr>
<tr>
<td>K18 fragments</td>
<td>MRI PDFF</td>
<td>C13 Methacetin breath test</td>
</tr>
<tr>
<td>Owl NASH test</td>
<td>MR spectroscopy</td>
<td></td>
</tr>
<tr>
<td>ELF</td>
<td>MR elastography</td>
<td></td>
</tr>
<tr>
<td>ProC3</td>
<td>Multiparametric MRI</td>
<td></td>
</tr>
</tbody>
</table>
LITMUS (Liver Investigation: Testing Marker Utility in Steatohepatitis)

FACTS & FIGURES
Start Date: 01/11/2017
End Date: 31/10/2022
Call: IMI2 - Call 9
Grant agreement number: 777377

Type of Action:
RIA (Research and Innovation Action)

Contributions: €
IMI Funding: 15 797 881
EFPIA in kind: 24 180 663
Other: 6 483 232
Total Cost: 46 461 776

PROJECT LINKS
Project website: www.litmus-project.eu
Twitter: @LITMUS_IMI
Coordinator: Prof Quentin M. Anstee

EU FP7
€6 million (2010-2013)

Expanded Partner Network

Clinical Application

EU IMI2
€46.5 million (2017-2022)

EU H2020
€6 million (2015-2019)

THE FORUM
For Collaborative Research

THE U.S. FOOD & DRUG ADMINISTRATION

NEWCASTLE UNIVERSITY
NIMBLE Program Team Structure

NIMBLE Program Leadership
Project Co-chairs: Arun Sanyal, Sudha Shankar, Roberto Calle
Members: Claude Sirlin, Anthony Samir, Rohit Loomba, Sarah Sherlock
Scientific Program Manager: Tania Kamphaus

Pathology Expert Team
Cynthia Guy (DCRI)
Melissa Contos (VCU)
Others TBD

Data Analysis & Modeling Expert Team
Nancy Obuchowski (Cleveland Clinic)
Santos Carvajal-Gonzalez (Pfizer)
Cytel

Imaging Markers Work Stream
Claude Sirlin (UCSD) – Co-chair
Anthony Samir (Harvard/MGH) – Co-chair
Sarah Sherlock (Pfizer) – Co-Chair
Academic collaborators

Circulating & Functional Markers Work Stream
Rohit Loomba (UCSD) – Co-chair
Sudha Shankar (Astra Zeneca) – Co-chair
Roberto Calle (Pfizer)
Academic collaborators
Molecular targets and ongoing studies in PBC

- UDCA is the first line agent approved for PBC. Serum AlkP response to UDCA is predictive of long-term outcomes of patients with PBC.
- Obeticholic acid is approved for treating PBC unresponsive, or partially responsive, or intolerant to UDCA.
- Ongoing trials: FXR agonist, PPAR agonists, and FGF19 for Alk P and disease course. IBAT inhibitor (Linerixibat) and kappa opioid receptor agonist (Korsuva) for itching.

<table>
<thead>
<tr>
<th></th>
<th>PPAR-α</th>
<th>PPAR-γ</th>
<th>PPAR-δ</th>
</tr>
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<tbody>
<tr>
<td>Bezafibrate</td>
<td>++++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Seledelpar</td>
<td>Negligible</td>
<td>++++</td>
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<tr>
<td>Saroglitazar</td>
<td>++++</td>
<td>+</td>
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<td>Elafibranor</td>
<td>+++</td>
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</table>
Molecular targets and ongoing studies in PSC

• No approved therapy. UDCA and endoscopic therapies are used clinically.
• norUDCA and Cilofexor (FXR) are being tested in phase3 clinical trials
• Other ongoing trials: PPAR agonists (fenofibrate), FGF19, IBAT inhibitor, HTD 1801, Vancomycin, FMT etc.
Clinical Pharmacology of OCA in Health and Liver Disease

Jeffrey E. Edwards Ph.D.
Executive Director of Clinical Pharmacology
Intercept Pharmaceuticals, Inc.
Obeticholic Acid: Background

• Obeticholic acid (OCA) is a farnesoid X receptor (FXR) agonist

• OCA is a modified bile acid derived from the primary bile acid chenodeoxycholic acid (CDCA), the natural human FXR ligand

• OCA has received marketing authorization in the United States, Europe, Canada, and several other countries for the treatment of primary biliary cholangitis (PBC)

• New Drug Application to the US FDA for OCA in patients with fibrosis due to NASH has been submitted
Pharmacokinetics and Pharmacodynamics of OCA

- Absorption in the gut followed by conjugation to glycine and taurine in the liver (highly extracted by the liver)

- Extensive enterohepatic recirculation with the drug primarily residing within the liver-gut axis (primary location of FXR)

- Approximate 4-day half-life

- Bile acid like toxicity observed at high daily doses of OCA (250 mg) in healthy subjects
PK of OCA in Healthy Subjects versus Cirrhosis

• Higher systemic exposure of endogenous bile acids with moderate and severe hepatic impairment

• Similar proportional increase in systemic exposure of OCA

![Graph showing the effect of liver function on OCA and bile acid exposure](image-url)
Systemic and Liver Exposure of Bile Acids in End-Stage Liver Disease

- Higher systemic concentrations of bile acids
- Modestly higher liver exposure with hepatic impairment

Figure: Fischer, et al. Clinica Chimica Acta. 1996;251:173
Effects of Cholestasis on Systemic and Liver Bile Acid Exposure

Higher liver exposure of bile acids with cholestasis

**Dose/Exposure-Response of OCA in PBC**

- Clear dose- and plasma exposure-response for OCA in PBC patients for reductions in alkaline phosphatase (ALP) and total bilirubin (BILI)

**ALP**

<table>
<thead>
<tr>
<th>Trough Total OCA (ng/mL)</th>
<th>% Δ in ALP from Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-40</td>
</tr>
<tr>
<td>10</td>
<td>-30</td>
</tr>
<tr>
<td>100</td>
<td>-20</td>
</tr>
<tr>
<td>1000</td>
<td>-10</td>
</tr>
<tr>
<td>10 mg QD</td>
<td></td>
</tr>
<tr>
<td>5 mg QD</td>
<td></td>
</tr>
</tbody>
</table>

**Total Bilirubin**

<table>
<thead>
<tr>
<th>Trough Total OCA (ng/mL)</th>
<th>% Δ in BILI from Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>10%</td>
</tr>
<tr>
<td>1000</td>
<td></td>
</tr>
<tr>
<td>10 mg QD</td>
<td></td>
</tr>
<tr>
<td>5 mg QD</td>
<td></td>
</tr>
</tbody>
</table>

Dosing of OCA in PBC Patients with Moderate and Severe Hepatic Impairment

- Alternative dosing in PBC patients with moderate or severe hepatic impairment
  - 5 mg once weekly → 5 mg twice weekly → 10 mg twice weekly

Effects of Fibrosis/Cirrhosis due to NASH on the Hepatic Uptake of OCA/Bile Acids

• Liver extraction of OCA and bile acids are decreased with NASH fibrosis
• Systemic exposure of OCA/bile acids are expected to increase in fibrosis; however, liver is not expected to change significantly
Plasma Exposure of OCA and Bile Acids in Subjects with Fibrosis or Cirrhosis due to NASH

- Increase in plasma exposure but no clear differences in liver exposure with NASH fibrosis (Stage F1-F3) and minimal increase in cirrhosis (Child-Pugh A [F4])

Dose Response of OCA for Markers of FXR Activation in NASH

- OCA plasma exposure: Healthy < NASH F1-F3 < NASH F4
- Results consistent with liver exposure being similar or minimal higher in subjects with fibrosis or cirrhosis due to NASH, respectively
- Similar dose responses for FXR activation between healthy and NASH subjects
Dose Response of OCA for Markers of Liver Injury and Function

- Similar dose response between OCA 10 mg and OCA 25 mg, less for OCA 5 mg
Dose Response of OCA for Markers of Liver Injury and Function

• Increase in plasma exposure but no clear differences in liver exposure with NASH fibrosis (stage F1-F3) and minimal increase with cirrhosis (Child-Pugh A [stage F4])
Dose Response of OCA for Livers Function Based on HepQuant

- Clear dose response for improvement in liver function (HepQuant)
- Results appear more consistent with the improvement in fibrosis from REGENERATE

Potential Pitfall of Using Liver Biomarkers

• Increases in ALP with OCA and other non-steroidal FXR agonist have been observed

• OCA does not directly transcriptionally regulate ALP

• OCA does regulate gene(s) involved in ALP homeostasis (ie, PLD)
Potential Indirect Effect of OCA on Alkaline Phosphatase
Conclusions

• Liver disease, cholestatic and non-cholestatic, may have significant effects on the PK and disposition of drugs used in NASH

• OCA shows significant improvement in biomarkers associated with NASH
  • Dose-response not always matching Phase 3 results
  • Non-invasive technologies may help in aiding dose selection

• Important to determine if changes in biomarkers of liver disease are only due to disease modification
Circulating Biomarkers in Early Drug Development in NASH

Session 2: Early Discovery and Development: Treatment Mechanisms, Molecular Targets, and Biomarkers in Early Development of Therapies for NASH and Cholestatic Liver Diseases

Saurabh Gupta, PhD
Takeda Pharmaceuticals International Co.
Disclosure

Working at Takeda Pharmaceuticals International Co. at Department of Translational Biomarker Research at Gastrointestinal Drug Discover Unit.
Contents

– Background & need for non-invasive biomarkers in drug development in NASH

– Pro-C3 and other extracellular matrix biomarkers in early drug development in NASH:
  • Diagnosis, disease Severity
  • Fast and slow progressing patients
  • Responder and non-responder
  • Prognostic biomarkers for liver related Complications
  • Translatability

– Conclusion & future perspective
Rationale for use of Non-invasive Biomarkers in NASH Drug Development

- Liver biopsy is still a reference standard for staging fibrosis and steatohepatitis
  - Biopsy invasive and painful, may lead to severe complications bleeding, injury to other organs & death
  - Costly, lack of specialists to cater huge patient numbers
  - Not suitable for frequent or longitudinal assessment
  - May not adequately represent disease phenotype as fibrosis in NASH is heterogenous
  - High inter-observer variability in histological scoring even highly skilled & trained pathologists

- Noninvasive, cost effective, readily available, accurate & reproduceable biomarker/s will be desirable for diagnosis, patient stratification, response and prognosis

1. (Anstee et al., Hepatology 2019; Rockey et al., Hepatology 2009, Ratziu et al., Gastroenterology 2005)

www.niddk.nih.gov/health-information/liver-disease/nafld-nash/diagnosis
Current Landscape of Clinical Endpoints in NASH

Early Phase 2 studies:
- In proof of concept studies biomarkers associated with steatosis, steatohepatitis, and fibrosis are used as endpoints
  - e.g.: MRI-PDFF, CK-18, ALT, MRE, ELF, Pro-C3
  - Histological endpoints can be used, provided trial is run for sufficient duration
  - Opportunity to characterize non-invasive biomarkers

Late Phase 2 studies:
- Evidence of efficacy on a histological endpoint (i.e., reduction of inflammatory changes, improvement in fibrosis, or both)
- Scope of Biomarkers
  - Biomarkers that can increase likelihood of confirmatory biopsy
  - Biomarkers that can provide evidence of disease progression
  - Prognostic biomarkers which may robustly predict liver related complications

Phase III studies:
- Resolution of steatohepatitis on overall histopathological reading & no worsening of liver fibrosis on NASH CRN fibrosis score. OR
- Improvement in liver fibrosis greater than or equal to one stage (NASH CRN fibrosis score) & no worsening of steatohepatitis (defined as no increase in NAS for ballooning, inflammation, or steatosis);
- Or Both

Additional Clinical Endpoints used in NASH studies
- Composite long-term outcome composed of all-cause mortality, cirrhosis & liver-related clinical outcomes

(Adapted from Noncirrhotic Nonalcoholic Steatohepatitis With Liver Fibrosis: Developing Drugs for Treatment Guidance for Industry Dec 2018; NASH trials at ClinicalTrial.gov )
NASH Biomarkers: Pro-C3 and Other ECM Turn Over Markers

- NASH patients with hepatic inflammation and hepatocellular injury are at increased risk of progressive fibrosis.
- Hepatic fibrosis is the only predictor of clinical disease progression.
- Fibrosis is abnormal connective tissue turnover, damages structure and function of organ/tissue.
- Core protein of fibrosis is collagen and other structural proteins like laminins and elastin.
- Formation and degradation of collagen and other extracellular matrix (ECM) proteins leads to release of peptide fragments which can be detected in circulation as fibrogenesis and fibrolysis biomarkers (BMs) respectively.

Soluble peptide fragments can be detected in serum/plasma via ELISA based assay

(Schuppan et al., J Hepatol 2017)

(Karsdal et al., Biochemistry of Collagens Laminins and Elastins 2018; Anstee et al., Hepatology 2019)
Learnings from HCV: ECM Remodeling Markers for Diagnosis of Fibrosis

• In a cross sectional study in 403 chronic hepatitis (HCV) patients with biopsy were included in analysis

• Along with ECM markers other biochemical measures including AST and ALT were measured

• Multiple ordered logistic regression models for the detection of fibrosis stratified according to Metavir F stages:
  A) Model 1 combining Pro-C3, C4M, AST, and ALT
  B) Model 2 combining Pro-C3, C4M, age, BMI, and gender

(Nielsen et al., PloS One 2015)
Pro-C3 Correlation with Fibrosis Score and NAS

• Pro-C3 is an ADAMTS (a disintegrin and metalloproteinase with a thrombospondin type 1 motif) generated neo-epitope marker of type III collagen formation
• Not a liver specific marker of fibrogenesis

• Pro-C3 is increased in NAFLD subjects as compared to healthy
• Increase in Pro-C3 according to severity of fibrosis as well as NAS observed in number of studies

(Gupta et al., Hepatology 2017; Sanyal et al., EASL 2019 Poster # LBP-30; Yi et al., Sci Rep. 2018)
Fibrogenesis biomarkers in NASH, Simple Steatosis and NHV

• Besides Pro-C3 other fibrogenesis markers may offer additional information:
  o Pro-C4 ↑ indicates increase in basement fibrosis whereas Pro-C3 ↑ indicates interstitial fibrosis

• Type VI collagen pro-peptide, endotrophin, stimulates fibroblasts and ↑ Pro-C3 in scar in a jar model
  o Collagen fragments also have signaling capabilities

(Gupta et al., Hepatology 2017; Morten et al., Hepatology 2019)
Fibrolysis biomarkers in NASH, Simple Steatosis and NHV

- Fibrolysis biomarkers markers may have limited value in discrimination b/w NASH and SS
- However fibrolysis biomarker are also elevated in NASH as compared to simple steatosis and NHV;
- A direct quantification of the tissue turnover balance (i.e. the ratio between fibrogenesis and fibrolysis), may provide more comprehensive information on a specific collagen
- Ratio of Pro-C3/C3M was significantly ↑(p<0.03) in NAFLD (1.81±0.21) patients vs. NHV (0.86±0.05)
- For collagen IV also formation/degradation ratio was significantly ↑ (p<0.01) in NAFLD patients (12.05±0.25) vs. NHV (10.61±0.37)
- Fibrosis is a high turn over component of NASH pathogenesis

(Gupta et al., Hepatology 2017; Unpublished Data, 2019)
Ethnic Translatability of ECM Turnover Biomarkers in Caucasians and Japanese subjects

Inhouse Data on Pro-C3

<table>
<thead>
<tr>
<th>Fibrosis Score</th>
<th>Yohkohoma collaboration (ng/mL±SD)</th>
<th>Univ. Birmingham collaboration (ng/mL±SD)</th>
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<tbody>
<tr>
<td>F0</td>
<td>16.9 ± 9.8 (N=39)</td>
<td>21.2 ± 22.6 (N=8)</td>
</tr>
<tr>
<td>F3</td>
<td>25.8 ± 16.2 (N=9)</td>
<td>26.2 ± 17.3 (N=20)</td>
</tr>
<tr>
<td>F4</td>
<td>55.4 ± 38.7 (N=3)</td>
<td>27.3 ± 12.4 (N=6)</td>
</tr>
</tbody>
</table>

• While designing a global trial it is important to establish the levels of biomarkers in different ethnicities

(Gupta et al., Hepatology 2017; Unpublished Data, 2019)
Effect of Pharmacological intervention on Pro-C3 levels: Potential for Patient Enrichment?

- In phase –II study, 16 wks. treatment with BMS-986036, a FGF21 analogue F1-F3 NASH patients led to beneficial effects on steatosis (MRI-PDFF), markers of liver injury (ALT, AST) & fibrosis (Pro-C3, MRE).

- Higher ↓ in Pro-C3 was observed in patients with higher >20 ng/mL.

- In phase 2 study NGM-282, a FGF 19 analogue, was treated in biopsy confirmed NASH patients for 12 wks.

- In 3 mg cohort 63 % patients had >2 ↓ in NAS score and 42 ↓ their fibrosis score by >1.

- In responder group Pro-C3 ↓ 56% & Pro-C3 correlated with histological changes.

- NGM313, novel activator of beta-klotho/FGFR1c single dose significantly ↓ Pro-C3 by day 28.

(Abdelmalek M. et al., 2017; AASLD #195650; Sanyal et al., Journal of Hepatology 2017 S89–S90; DePaoli, EASL 2019, PS-108; Sanyal et al., EASL 2019 Poster # LBP-30)
Pro-C3: Clinical Biomarker for Identification of Responders to Anti-fibrotic Treatment

- Farglitazar, PPARγ agonist, 52 wk treatment failed to improve overall histological fibrosis stage and morphometrical collagen in a Phase II CHC; Patients with baseline levels of Pro-C3 > 20.2 ng/mL showed efficacy
- Balaglitazone, PPAR γ agonist, was evaluated in type 2 diabetics with 26 wk. treatment in a phase III study; subjects with the highest tertile of Pro-C3 levels responded to balaglitazone with ↓ in levels of alanine aminotransferase and Pro-C3

Disease specific threshold need to be defined for potential patient enrichment/patient stratification

(Morten et al., AJP-Gastrointest Liver Physiol 2016)
Time course of Change in Pro-C3

• NGM282 at 1 mg and 3 mg doses significantly reduced plasma Pro-C3 at all the time points assessed
• After 12 wks. of treatment Pro-C3 levels ↓ by 21 % and 27% with NGM282 1mg and 3mg dose respectively

PSC study

<table>
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<tr>
<th>Week 12</th>
<th>Pro-C3</th>
<th>R Value</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Conjugated primary bile acids</td>
<td>GCA</td>
<td>0.62</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>TCA</td>
<td>0.52</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>GCDC</td>
<td>0.55</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>TCDC</td>
<td>0.46</td>
<td>0.003</td>
</tr>
<tr>
<td>Conjugated secondary bile acids</td>
<td>GDCA</td>
<td>0.31</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>TDCA</td>
<td>0.28</td>
<td>0.038</td>
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<tr>
<td>Unconjugated primary bile acids</td>
<td>CA</td>
<td>-0.18</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>CDCA</td>
<td>-0.21</td>
<td>0.12</td>
</tr>
<tr>
<td>Unconjugated secondary bile acids</td>
<td>DCA</td>
<td>-0.06</td>
<td>0.65</td>
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</table>

NASH Study

• 43 biopsy confirmed NASH SC treatment with NGM-282, significant decrease observed at both 1 and 3 mg

• Serum Levels of Pro-C3 were significantly reduced at 2-12 wks both in PSC and NASH Patients wks post treatment
• It will be important to understand how long the levels of Pro-C3 need to be lowered to be reliably observe improvement in histological fibrosis scores

1: (Hirschfield et al., EASL 2019 Poster # FRI-027; Harrison et al., Hepatology 2019; Hirshfield et al., Journal of Hepatology, 2019)
Potential of Pro-C3 to Predict Clinical Outcomes

- 47 Liver transplant (LT) patients divided into fast, intermediate or non-progressors towards recurrent fibrosis (RC), RC within 1 yr, RC within 3-5 yrs and no fibrosis 5yrs post LT, respectively Pro-C3 measured at 3, 6, 12 months along with biopsy
- In 137 CHC patients Pro-C3 and C3M were measured at base line and 52 wks, plasma Pro-C3 predicts fibrosis progression

1 (Nielsen et al., EASL 2019 Poster # Thu-088; Nielsen et al., Liver Int. 2015)
Reverse Translatability of ECM Biomarker: Preclinical Data in MCR4KO

- Most ECM biomarkers are translatable across species
- In MCR4KO mice there is significant increase in rPro-C3, Pro-C5, C3M and C4M
- ECM biomarkers are good tools to employ in preclinical settings and may be of value for PK-PD modelling in an more efficient manner

(Unpublished Data, 2019)
Conclusion and Future Perspective

- Extracellular matrix turnover biomarkers especially Pro-C3 has shown promise for:
  - NASH diagnosis, especially F3 and F4
  - Identifying NASH patients potentially in active fibrogenesis phase
  - Stratification biomarker
  - Response biomarker for fibro-static activity
  - Demonstrates quick turnover
  - Preclinical translation

- Early phase NASH clinical trials should try to incorporate multiple non-invasive biomarkers as feasible, to provide further evidentiary strength to biomarkers and their association with endpoints

- Non-invasive biomarker/s are key for expedited NASH drug development
  - Various National and International NASH biomarkers consortia, like NIMBLE and LITMUS are working towards the same objective
Acknowledgements

Gastrointestinal Discovery Unit Takeda

• Shinji Ogawa, Hiroaki Yashiro, et al.

Statistics: Iwona Dobler, Jacob Zhang

University of Birmingham: Gideon Hirschfield et al.

Nordic Bioscience Team
Thank You For Your Attention

Picture From: https://jpninfo.com/wp-content/uploads/2017/06/Climb-Mt-Fuji-featured.jpg
Session 2: Panel Discussion

Early Discovery and Development—Treatment Mechanisms, Molecular Targets, and Biomarkers in Early Development of Therapies for NASH and Cholestatic Liver Diseases
Biomarkers for NASH in Early Clinical Development

Roberto Calle, MD
Clinical Research
Internal Medicine Research Unit
Pfizer, Inc.

FDA Public Workshop: Clinical Pharmacology in Drug Development for Liver Diseases

December 9, 2019
Definition of Non-Alcoholic Steatohepatitis

Histology has helped to characterize and define the disease

- Steatosis (macro-vesicular)
- Ballooning
- Lobular inflammation
- Zone 3 distribution

Kleiner et al, Hepatology 2005

Fibrosis Staging

Stage 1 | Stage 2 | Stage 3 | Stage 4

Histology courtesy of Pierre Bedossa and Arun Sanyal
Implications of Biopsy Based Decision-making

HISTOPATHOLOGY-BASED ASSESSMENT IS NOT AN EFFICIENT STRATEGY FOR DRUG DEVELOPMENT

■ Patient perspective:
  o Invasive
  o Painful
  o Morbidity / Mortality

■ Technical drawbacks:
  o Sampling variability
  o Intra- & Inter-observer variability
  o Limited opportunity for repeat assessment

■ Operational feasibility challenges:
  o Resource intense; needs hepatologist or radiologist and pathologist

Sampling variability: Same biopsy, Two different grades of liver fibrosis
Consortium approach is encouraged by FDA

Updated FDA Draft Guidance published January 2014

**Unmet Need for Drug Development**

A major unmet need to advance drug development for NAFLD is the development of **non-invasive** biomarker or biomarker panels that

1. Identification of individuals with NASH (Diagnostic marker)
2. Identification of individuals at risk of progression to cirrhosis and in need of pharmacological or non-pharmacologic intervention (Prognostic marker)
3. Permits evaluation of effects of one or more pharmacologic interventions on disease progression in subjects at various stages of NASH (Response marker)

“Because of the substantial work needed to achieve qualification, CDER [Center for Drug Evaluation and Research] encourages the formation of collaborative groups to undertake these tool-development programs... A variety of projects undertaken by consortia have demonstrated the usefulness of this approach.”
Development of New Therapeutics for NASH

**Future Desired State**

### Phase 1
- **Safety & PK**
- 2-6 wks studies
  - LFC if anti-steatotic
  - Marker of inflammation
  - Marker of fibrosis turnover

### Phase 2
- **POC - Dose Ranging**
- Studies based on circ. markers or imaging to assess early inflammation and fibrosis effect → increased ability to properly dose range
- ↓ screen failure rates (<30%) @ Bx

### Phase 3
- **Ph3 pivotal studies**
- ↓ screen failure rates (<30%) @ Bx
- **LARGE INVESTMENT WITH HIGH CONFIDENCE**
- **Efficacy endpoints based on Non-invasive markers – No Bx**

### Conditional regulatory approval
- **Recruitment based on Non-invasive markers**
- **Outcomes Ph4 Study**
- ↓ screen failure rates (<30%) @ Bx
The Challenge is **NOT** Lack of Potential Biomarkers but Rather Lack of Properly Qualified Biomarkers

Many blood-based and imaging-based biomarkers being developed to diagnose and stage NASH

Wang et al., Nature Reviews, 2018
NIMBLE AND LITMUS: INDEPENDENT BUT COMPLEMENTARY PUBLIC:PRIVATE PRECOMPETITIVE BIOMARKER CONSORTIA

Liver Investigation: Testing Marker Utility in Steatohepatitis

Academic Lead: Prof. Quentin Anstee (Newcastle)
Industry Lead: Dr. Julia Brosnan (Pfizer)
Session 2: Panel Discussion

Early Discovery and Development—Treatment Mechanisms, Molecular Targets, and Biomarkers in Early Development of Therapies for NASH and Cholestatic Liver Diseases
Measuring the Liver’s Function

Metabolism Dependent
Flow Dependent
Functional tests can assess hepatocyte function, systemic inflow, portal inflow, and quantifies portal-systemic spillover.

HepQuant’s products are not FDA-approved and are for investigational use only in clinical trials under FDA IDE guidelines.

Figure is from Poster at AASLD Liver Meeting 2016
Drugs Taken by UCDenver HALT-C Subjects:

<table>
<thead>
<tr>
<th>Category</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects assessed:</td>
<td>170</td>
</tr>
<tr>
<td>Encounters:</td>
<td>305</td>
</tr>
<tr>
<td>Herbals/OTC Supplements at Encounter:</td>
<td>201</td>
</tr>
<tr>
<td>Prescription medications at Encounter:</td>
<td>217</td>
</tr>
<tr>
<td>Smoking at Encounter:</td>
<td>79</td>
</tr>
</tbody>
</table>
### Function Values for Dual Clearance Test

| Clearance | 5.00 | 6.00 | 7.00 | 8.00 | 9.00 | 10.00 | 11.00 | 12.00 | 13.00 | 14.00 | 15.00 | 16.00 | 17.00 | 18.00 | 19.00 | 20.00 | 21.00 | 22.00 | 23.00 | 24.00 | 25.00 | 26.00 | 27.00 | 28.00 | 29.00 | 30.00 |
|-----------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Clearance 1 | 28.48 | 29.26 | 30.13 | 31.60 | 32.20 | 32.80 | 33.46 | 34.16 | 34.93 | 35.77 | 36.50 | 37.72 | 38.88 | 39.93 | 41.1 | 42.43 | 43.99 | 45.63 | 47.17 | 48.92 | 50.41 | 52.57 | 56.58 | 62.00 | 68.92 | 77.79 | 90.00 |
| Clearance 2 | 22.66 | 23.67 | 24.54 | 25.25 | 25.85 | 26.37 | 26.86 | 27.37 | 27.86 | 28.37 | 28.85 | 29.85 | 30.98 | 31.98 | 33.32 | 33.95 | 34.37 | 35.09 | 36.52 | 37.92 | 40.02 | 41.49 | 45.06 | 50.19 | 60.00 | 72.00 | 87.00 |

### Portal-Systemic SHUNT Values for Dual Clearance Test

| Clearance | 5.00 | 6.00 | 7.00 | 8.00 | 9.00 | 10.00 | 11.00 | 12.00 | 13.00 | 14.00 | 15.00 | 16.00 | 17.00 | 18.00 | 19.00 | 20.00 | 21.00 | 22.00 | 23.00 | 24.00 | 25.00 | 26.00 | 27.00 | 28.00 | 29.00 | 30.00 |
|-----------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Clearance 1 | 28.48 | 29.26 | 30.13 | 31.60 | 32.20 | 32.80 | 33.46 | 34.16 | 34.93 | 35.77 | 36.50 | 37.72 | 38.88 | 39.93 | 41.1 | 42.43 | 43.99 | 45.63 | 47.17 | 48.92 | 50.41 | 52.57 | 56.58 | 62.00 | 68.92 | 77.79 | 90.00 |
| Clearance 2 | 22.66 | 23.67 | 24.54 | 25.25 | 25.85 | 26.37 | 26.86 | 27.37 | 27.86 | 28.37 | 28.85 | 29.85 | 30.98 | 31.98 | 33.32 | 33.95 | 34.37 | 35.09 | 36.52 | 37.92 | 40.02 | 41.49 | 45.06 | 50.19 | 60.00 | 72.00 | 87.00 |
Should Function be the New Gold Standard?

- Treatment should improve how a person/patient FEELS, FUNCTIONS, or SURVIVES.

- A person with a disease that progressively damages the liver - FEELS, FUNCTIONS, or SURVIVES based upon how the liver functions; and not based upon the surrogate of scar/fibrosis.

- If treatment improves the liver, the test must detect and quantify this improvement.
Change in Ishak Fibrosis Stage (IFS) from Baseline to Yr 2
(174 Paired Liver Biopsies from HALT-C Subjects)

\[ \Delta \text{IFS} = 0.02 \pm 1.10 \]

\[ p = 0.84 \]
Change in Function from Baseline to Yr 2
(188 Paired Function Tests in HALT-C subjects)

19.37 ± 5.42
ΔTest = 1.64 ± 5.21
p < 0.00003
21.00 ± 7.39

HepQuant’s products are not FDA-approved and are for investigational use only in clinical trials under FDA IDE guidelines.
Can Function Testing become a Clinical Reality?

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Function Testing Clarifies Liver Health

Cutoff for Significant Clinical Risk

- Clinical outcomes (variceal bleed, ascites, encephalopathy, liver-related death)
- High likelihood of cirrhosis
- Hepatocellular carcinoma (HCC)

Defines Global Liver Health

- Patients and providers can track function over time or with treatment
- Improved function leads to significant improvement in liver disease
- Improvement in function defines improvement in Pharma trials
- Worsening of function implies worsening of liver disease

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Session 2: Panel Discussion

Early Discovery and Development—Treatment Mechanisms, Molecular Targets, and Biomarkers in Early Development of Therapies for NASH and Cholestatic Liver Diseases