Drug Disposition in Non-Alcoholic Fatty Liver Disease: 
*Implications for Therapeutics and Drug Development*

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Non-Alcoholic Fatty Liver Disease (NAFLD)

- First described in 1980.
- Presence of intracellular fat (triglyceride) droplets in hepatocytes in the absence of significant alcohol consumption.
- A disease of nutrient excess.
- Imbalance between hepatic fatty acid input (from adipose or lipogenesis) with output (beta-oxidation and lipoprotein export).
- Economic burden ~$103 Billion.

Cohen et al., Science 332; 1519-23 (2011)
Prevalence

• Most common liver disease in the U.S. and Canada
• 30% of adult North Americans (45% Hispanics, 30% Caucasians, 25% Blacks or 25% Asians)
• 10% of children, 20% adolescents and young adults

Risk Factors

• Obesity
• Type 2 diabetes
• Metabolic syndrome
• Increased age
• Males > Females
• Genetics (PNPLA3 and TM6SF2 genes)
NAFLD Disease Spectrum

Prevalence

Simple steatosis
• Fat >5%
• Asymptomatic
• Without serum biochemical changes

NASH
• Non-alcoholic steatohepatitis
• Increased transaminases
• Histologic features:
  - Steatosis
  - Inflammation
  - Ballooning
• Fibrosis in advanced stage

Cirrhosis
• Irreversible hepatocellular damage
• Fibrosis
• Regenerative nodules

HCC
• Hepatocellular carcinoma

~25 %
5%
0.2 %
NAFLD Prognosis

- Most common cause of death in NAFLD is cardiovascular disease.
- Simple steatosis (NAFL) has a benign course, no survival risk.
- NASH has a more progressive natural history.
- Hepatic fibrosis is the most important predictor of poor outcome:
  - 20% risk of cirrhosis
  - Increased risk of all-cause mortality
  - Increased risk of liver-related mortality
- NASH cirrhosis will soon become the leading cause for liver transplantation.
Evaluation of NAFLD

NAS Score Feature  | Scoring
--- | ---
Steatosis | 0 - 3
Lobular Inflammation | 0 - 3
Hepatocyte Ballooning | 0 - 2

Fibrosis Score Feature  | Stage
--- | ---
Perisinusoidal or periportal fibrosis | 1
Mild perisinusoidal fibrosis (zone 3) | 1A
Moderate perisinusoidal fibrosis (zone 3) | 1B
Portal/periportal fibrosis | 1C
Perisinusoidal and portal/periportal fibrosis | 2
Bridging fibrosis | 3
Cirrhosis | 4

Rinella, JAMA 313; 2263-73 (2015)
Kleiner et al., Hepatology 41; 1313-21 (2005)
Evaluation of NAFLD

Steatosis
- Ultrasound
- CT Scan
- MR Imaging
- MR Spectroscopy

Fibrosis
- Transient Elastography
- MR Elastography
Current Management and Treatment of NASH

Lifestyle/Surgery
- Weight loss
- Bariatric surgery

Manage co-morbidities
- Hypercholesterolemia
- Diabetes
- Hypertension

Non-approved drugs for NASH
- Vitamin E
- Pioglitazone
- Liraglutide
NASH Drugs in Development

Table 1 | Selected NASH products in development

<table>
<thead>
<tr>
<th>Drug</th>
<th>Developer</th>
<th>Mode of action</th>
<th>Highest phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obeticholic acid (OCA/Ocaliva)</td>
<td>Intercept Pharmaceuticals/Sumitomo Dainippon Pharma</td>
<td>FXR agonist and semi-synthetic bile acid analogue</td>
<td>Phase III</td>
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<tr>
<td>Elafibranor (GF-505)</td>
<td>Genfit</td>
<td>PPAR α/δ agonist</td>
<td>Phase III</td>
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<td>Aramchol</td>
<td>Galmed Pharmaceuticals</td>
<td>Synthetic fatty acid–bile acid conjugate</td>
<td>Phase II/III</td>
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<tr>
<td>Emricasan (IDN-6556)</td>
<td>Conatus Pharmaceuticals</td>
<td>Caspase inhibitor</td>
<td>Phase II</td>
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<tr>
<td>Sintuzumab (GS-6624)</td>
<td>Gilead Sciences</td>
<td>Anti-LOXL12 monoclonal antibody</td>
<td>Phase IIb</td>
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<tr>
<td>GR-MD-02</td>
<td>Galectin Therapeutics</td>
<td>Galectin 3 inhibitor</td>
<td>Phase II</td>
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<tr>
<td>GS-4997</td>
<td>Gilead Sciences</td>
<td>MAPK5 inhibitor</td>
<td>Phase II</td>
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<tr>
<td>Liraglutide (Victozza/Saxenda)</td>
<td>Novo Nordisk</td>
<td>GLP1R agonist</td>
<td>Phase II</td>
</tr>
<tr>
<td>Cenicriviroc (TBR-652)</td>
<td>Allergan (formerly Tobira Therapeutics)</td>
<td>Dual CCR2 and CCR5 antagonist</td>
<td>Phase II</td>
</tr>
<tr>
<td>BMS-986036</td>
<td>Bristol-Myers Squibb</td>
<td>FGF21 agonist</td>
<td>Phase II</td>
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<tr>
<td>Tipelukast</td>
<td>MediciNova</td>
<td>LTD4 receptor antagonist</td>
<td>Phase II</td>
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<td>ARI 3037MO</td>
<td>Arisaph Pharmaceuticals</td>
<td>Niacin analogue</td>
<td>Phase II</td>
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<tr>
<td>Volixibat (SHP 626)</td>
<td>Shire/Sanoﬁ</td>
<td>ASBT inhibitor</td>
<td>Phase II</td>
</tr>
</tbody>
</table>

ACC, acetyl-CoA carboxylase; ASBT, apical sodium bile acid cotransporter; CCR2, chemokine receptor agonist 2; CCL11, chemokine (C–C motif) 11; CYP7A1, cholesterol 7α-monooxygenase; FGF21, fibroblast growth factor 21; FXR, farnesoid X receptor; GLP1R, glucagon-like peptide 1 receptor; LTD4, leukotriene D4; LOXL2, lysyl oxidase homologue 2; MAPK5, mitogen-activated protein kinase 5; PPAR, peroxisome proliferator-activated receptor.

Figure 1 | Estimated NASH global sales ($US, 2015 and 2025). T2DM, type 2 diabetes mellitus.

Cassidy and Syed, Nat Rev Drug Discovery 15; 745-6 (2016)
Altered Drug Response in NAFLD?

• Patients with NAFLD are commonly treated with drugs for co-morbidities.

• Do patients with NAFLD have altered drug response or risk for drug toxicity?

• Does NAFLD alter the pharmacokinetics of drugs?

• New drugs are being developed to treat NASH.

• Should we consider NAFLD during drug development for other diseases?
Altered Drug Elimination in NAFLD?

Simple steatosis
- Unclear impact on hepatic drug clearance

NASH
- Decreased hepatic drug metabolism suspected
- Increased hepatocyte sinusoidal efflux suspected

Cirrhosis
- Decreased activity of most drug metabolizing enzymes
- Decreased hepatic blood flow
- Hepatic endothelial capillarization
- Intra- and extra-hepatic shunting of liver blood flow

HCC
Antipyrine Clearance in NASH

- Antipyrine clearance test is a classic method to evaluate liver function.
- Antipyrine undergoes oxidative metabolism by several CYP enzymes.
- Patients with NASH have reduced antipyrine clearance when compared to a historical group of healthy control subjects.

Engel et al., Clin Pharmacol Ther 59; 613-23 (1996)
Fiatarone et al., J Gastroenterol Hepatol 6; 585-590 (1991)
**In Vivo CYP2E1 Activity in NAFLD**

- CYP2E1 expression is induced in NAFLD.
- CYP2E1 may contribute to the pathogenesis of NASH.
- Chlorzoxazone is an *in vivo* probe for CYP2E1 activity.
- *In vivo* chlorzoxazone metabolism is increased in NASH and correlates with hepatic CYP2E1 expression and disease.

*In Vivo CYP2E1 Activity in NAFLD*

- Aubert et al., Clin Res Gastroenterol Hepatol 35; 630-7 (2011)
- Orellana et al., Hepatol Res 34; 57-63 (2006)
Acetaminophen Pharmacokinetics in Pediatric NASH

Barshop et al., J Ped Gastroenterol Nutr 52; 198-202 (2011)
Canet et al., Drug Metab Dispos 43; 829-35 (2015)
Morphine Pharmacokinetics in NASH

- IV Morphine PK not different among groups.
- Morphine-3- and -6-glucuronide AUC were 58% greater in NASH.
- Morphine-glucuronides AUC correlated with disease severity.
- Consistent with increased hepatic MRP3 expression in NASH.

Ferslew et al., Clin Pharmacol Ther 97; 419-27 (2015)
Hepatic Uptake Transporter Activity in NASH

- Gadoxetate is a liver MR contrast agent used to identify focal hepatic lesions (cancer).
- Transported by OATPs and MRP2.
- \textit{SLCO1B1} genetic polymorphisms influence hepatic gadoxetate concentrations.
- Gadoxetate liver contrast enhancement is 40\% lower in NASH vs. simple steatosis.

van Montfoort et al., J Pharmacol Exp Ther 290; 153-7 (1999)
Nassif et al., Radiology 264; 741-50 (2012)
Bastati et al., Radiology 271; 739-47 (2014)
Research Theme

To determine the effect of NAFLD on the pharmacokinetics of drugs and the underlying drug metabolism and transport mechanisms involved.

Initial Objectives

1. To determine cytochrome P450 3A4 (CYP3A4) mediated drug metabolizing enzyme activity in NAFLD, in vivo.

2. To explore mechanisms involved in CYP3A4 regulation in NAFLD.
Cytochromes P450 3A (CYP3A)

- CYP3A4 and CYP3A5
- Metabolize 50% of all marketed drugs
- High interpatient variation in hepatic/intestinal activity
- Expression is significantly dependent on environmental factors
- Genetics determines a relatively small proportion of variable CYP3A activity

<table>
<thead>
<tr>
<th>Indication</th>
<th>CYP3A Metabolized Drugs</th>
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<tbody>
<tr>
<td>Hypertension</td>
<td>Amlodipine, Felodipine, Nifedipine, Diltiazem, Verapamil</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>Simvastatin, Atorvastatin</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Glyburide, Rapaglinide, Sitagliptin</td>
</tr>
</tbody>
</table>
Single-Point, Oral Midazolam Microdose for CYP3A Phenotyping

- Midazolam is the most commonly used CYP3A probe drug
- Oral midazolam (vs. IV)
  - CYP3A phenotype for common route of drug administration
- Microdose (100 µg)
  - No sedation
  - Decreased observation time
  - PK linearity
- Single-time point
  - Simplicity/efficiency
  - Validated previously

Gong et al., Eur Heart J 33; 2856-64 (2012)
CYP3A4 Activity in NAFLD: Midazolam Test

Healthy control subjects
• n=20
• 65% female
• 43 yrs (28-58)
• BMI = 24 (18-35)

Biopsy-proven NAFLD
• n=10
• 50% female
• 51 yrs (27-63)
• BMI = 35 (28-45)
• 1 Simple Steatosis / 9 NASH
• Not taking CYP3A inhibitors/inducers

Woosley et al., Drug Metab Dispos 43; 1484-90 (2015)
4β-Hydroxycholesterol as an Endogenous CYP3A Biomarker

CYP3A Induction/Inhibition

Contribution of CYP3A Activity to Variability in Drug Concentrations

Multivariable Regression of Atorvastatin Plasma Concentrations

<table>
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<tr>
<th>Variable</th>
<th>Effect</th>
<th>P-value</th>
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<td>Age (yr)</td>
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<td>0.002</td>
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<tr>
<td>SLCO1B1 521T&gt;C</td>
<td>0.339</td>
<td>0.02</td>
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<tr>
<td>SLCO1B1 388A&gt;G</td>
<td>-0.278</td>
<td>0.009</td>
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<tr>
<td>4βHC</td>
<td>-0.015</td>
<td>0.006</td>
</tr>
</tbody>
</table>

R² = 0.47

DeGorter et al., Circ Cardiovasc Genet 6: 400-8 (2013)
CYP3A4 Activity in NAFLD: Plasma 4β-HC Test

Healthy control subjects
- n=20
- 65% female
- 43 yrs (28-58)
- BMI = 24 (18-35)

Biopsy-proven NAFLD
- n=30
- 37% female
- 49 yrs (27-69)
- BMI = 30 (23-45)
- 23% Simple Steatosis
- 77% NASH
- Not taking CYP3A inhibitors/inducers

Woosley et al., Drug Metab Dispos 43; 1484-90 (2015)
CYP3A4 Activity in NAFLD: Plasma 4β-HC Test

Fibrosis

Genetics

Insulin Resistance

Woosley et al., Drug Metab Dispos 43; 1484-90 (2015)
CYP3A4 mRNA Expression in NAFLD Liver

Control Livers
- Obtained from LTCDS
- n=9
- 3 male : 6 female
- Avg. age 45 yrs
- Histologically normal
- Negative Oil Red O Staining

NAFLD Liver Biopsies
- n=17
- 10 male : 7 female
- Avg. age 46 yrs
- 5 Simple Steatosis
- 12 NASH

Woosley et al., Drug Metab Dispos 43; 1484-90 (2015)
CYP3A4 in a Mouse Model of Simple Steatosis

Normal Diet

High Fat Diet

H&E

Trichrome

Oil Red O

Liver CYP3A4 Luciferase Activity (Relative Units)

Woosley et al., Drug Metab Dispos 43; 1484-90 (2015)
CYP3A in a Cellular Model of Hepatic Steatosis

Huh7 Human Hepatoma Cells

Lipid Accumulation

CYP3A Activity

CYP3A4 Expression

Control Fatty Acid

1-OH MDZ (ng/ml)

CYP3A4 mRNA (Relative Expression)

Woosley et al., Drug Metab Dispos 43; 1484-90 (2015)
Conclusions on CYP3A in NAFLD

1. *In vivo* CYP3A activity is reduced in human NAFLD.
   - Two phenotyping methods
   - Both in simple steatosis and NASH
   - Associated with fibrosis

2. Cultured cell model of NAFLD recapitulates humans CYP3A4 activity.

3. CYP3A4 mRNA is downregulated in human NAFLD and cell model.
   - CYP3A4-luciferase activity is reduced in mouse NAFLD model

4. A transcriptional mechanism may be involved in the regulation of CYP3A4 in NAFLD.
Potential Mechanisms for CYP3A Downregulation in NAFLD

- Hepatic inflammatory response occurs in NAFLD.
- Inflammatory signaling crosstalks with Pregnane X Receptor (PXR) signaling.
- In one report, hepatic PXR protein expression was downregulated in human NAFLD.

Xie and Tian, Cell Metab 4:177-8 (2006)
Bitter et al., Arch Toxicol 89:2089-103 (2015)
Fibroblast Growth Factor 21

- FGF21 is a paracrine / endocrine hormone.
- Produced by liver, fat, heart.
- Regulates lipid and glucose homeostasis.
- Tissue-dependent actions.
- Acts on a receptor complex containing FGF receptors with Klotho-beta.
- FGF21 mimetic drugs are in development.
- Circulating FGF21 concentrations are associated with degree of hepatic steatosis in humans.

Dushay et al., Gastroenterology 139:456-63 (2010)
FGF21 in Patients/Models of NALFD with Lower CYP3A Activity

Human

Mouse

Huh7 Cells

Woosley et al., Mol Pharmacol 90; 437-46 (2016)
FGF21 Receptors in NAFLD Liver

Klotho-β

FGFR1c

FGFR2c

FGFR3c

Relative Gene Expression

Control  NAFLD  Adipose

Liver

Control  NAFLD  Adipose

Liver

Control  NAFLD  Adipose

Liver

Control  NAFLD  Adipose

Liver

Woosley et al., Mol Pharmacol 90; 437-46 (2016)
FGF21 Downregulates CYP3A4 Expression *In Vivo*

Woosley et al., Mol Pharmacol 90; 437-46 (2016)
FGF21 Reduces CYP3A4 Expression and Activity In Vitro

Woosley et al., Mol Pharmacol 90; 437-46 (2016)
FGF21 Signaling and CYP3A4 Expression

Woosley et al., Mol Pharmacol 90; 437-46 (2016)
Pregnane X Receptor (PXR) Localization in NAFLD Models

Woosley et al., Mol Pharmacol 90; 437-46 (2016)
PXR Localization with FGF21 Overexpression

Woosley et al., Mol Pharmacol 90; 437-46 (2016)
Experimental Steatosis and FGF21 Reduces PXR Binding to the CYP3A4 Promoter

Woosley et al., Mol Pharmacol 90; 437-46 (2016)
Working Model for CYP3A4 Expression in NAFLD

- Fatty Acids
- FGF21
- PPARα
- ER Stress
- FGF21
- MEK1
- ERK1/2
- PXR
- RXR
- CYP3A4
Are Other PXR Target Genes Similarly Affected?

Woosley et al., Mol Pharmacol 90; 437-46 (2016)
Implications

• Patients with NAFLD may be more sensitive to CYP3A drugs
  • Lower dose requirement?
  • More susceptible to adverse drug effects?

• Drug development
  • Better prediction of drug dosing in target populations with NAFLD.

• FGF21 therapeutics for NASH, T2DM, obesity, dyslipidemia
  • Drug interaction liability?

• CYP3A activity in other conditions with increased circulating FGF21?
  • Chronic kidney disease, CVD, atrial fibrillation, pancreatitis, fasting, lactation, high fructose consumption
Challenges of Studying NAFLD

• NAFLD is a heterogeneous disease. Requirement to examine pharmacokinetics in various NAFLD subtypes/stages.

• Need better non-invasive measures of NAFLD stages that replace liver biopsy.

• NAFLD is confounded by obesity, diabetes, hypertension and chronic kidney disease.
Challenges of Studying NAFLD

- Animal models may not completely reflect human disease.

<table>
<thead>
<tr>
<th>Model</th>
<th>Obesity</th>
<th>Insulin resistance</th>
<th>Steatosis</th>
<th>Steatohepatitis</th>
<th>Fibrosis</th>
<th>Ballooning</th>
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<td>MCD</td>
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<td>Atherogenic diet</td>
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<td>db/db</td>
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<td>SREBP-1c overexpression (adipocyte specific)</td>
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<td>ob/ob + MCD</td>
<td>+</td>
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</tr>
</tbody>
</table>

Challenges of Studying NAFLD

- Need for better *in vitro* models of human NASH.

NASH modeled by perfusion with high glucose, insulin and fatty acids

Feaver et al., JCI Insight 1(20):e90954 (2016)
Future Directions

- Additional studies with probe drugs (cocktails) are required to fully understand the in vivo activities of enzymes and transporters in NAFLD.
- Evaluate liver intracellular concentrations of drugs in relation to plasma concentrations, especially in the context of drug efficacy and potential harms.
- Use of quantitative gene expression studies together with in vivo activity data to model drug disposition in NAFLD during drug development.
- Population-based studies are needed to examine the safety and efficacy of currently prescribed drugs in NAFLD.
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