Study Design to Validate Biomarkers of Therapeutic Response in NASH Due to Cirrhosis

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Fibrosis Progression and Reversal in NASH
Inflammation, the reparative response and evolution of fibrosis/cirrhosis in NASH

Fibrogenesis
- is a waxing and waning process
- follows inflammation (reparative response)

Schuppan et al, J Hepatol 2018

Antifibrotic therapy
Fibrogenesis in NASH

**normal liver**

- macrophage
  - cytokines
  - chemokines
  - TGFβ
  - TLR4
  - ROS

- quiescent stellate cell

- portal or perivascular fibroblast

**fibrotic liver**

- activated myofibroblast
  - MMP-1/3/13
  - TIMP-1
  - TIMP-2

- endothelium

**Ox. stress, ROS**

- Insulin resistance, FFA
  - Toxins
    - Toxic bile salts
      - (Auto-) Immunity
        - HBV, HCV
        - Microbiome, nutrients
  - genetic predisposition

- repetitive damage (multiple hits)

- (lipoapoptotic) hepatocytes
  - activated cholangiocytes

- Oxidative stress, ROS

- Insulin resistance, FFA

- Toxins
  - Toxic bile salts
    - (Auto-) Immunity
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- repetitive damage (multiple hits)

- (lipoapoptotic) hepatocytes
  - activated cholangiocytes

**Cirrhosis and HCC**

- common pathways & immune environment!

- Cirrhosis
  - organ failure
  - matrix accumulation

Schuppan and Afdhal, Lancet 2008
Schuppan and Kim, JCI 2013
Inhibition of fibrogenesis - induction of fibrolysis

**normal liver**

- quiescent stellate cell
- portal or perivascular fibroblast
- macrophage

**Causal therapy**

- Ox. stress, ROS
- Insulin resistance
- Toxins
- Toxic bile salts
- (Auto-) Immunity
- HBV, HCV
- microbiome

**Reversal of liver failure?**

- (lipoapoptotic) hepatocytes
- activated cholangiocytes
- endothelium

**Stimulation of (hepatocyte) regeneration**

- HGF
- FGF19...

**Antifibrotic agents**

- Inhibition of fibrogenesis
- induction of fibrolysis
- MMP-1/3/9/13
- TIMP-1
- TIMP-2

**fibrotic liver**

- collagen degradation
- Reversal of distorted (vascular) architecture?
Reversibility of Advanced Fibrosis by Addressing the Prominent "Hit"
Clinical Disease Progression in CHB Cirrhotics

- Placebo (n=215)
- Lamivudine (n=436) $P = .001$

HCC: 7.3% → 3.9%
Increase in Child’s-class: 8.8% → 3.4%

**Cirrhosis Regression with Longterm Tenofovir Treatment**

1-5 yr extension of 48 week tenofovir trial (*Marcellin P et al, NEJM 2008*)

489/615 pts (76%) included  
5 yr biopsy: 348/489 (71%)

**Baseline: no cirrhosis**

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<td>Better</td>
<td>105/252</td>
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<tr>
<td>Worse</td>
<td>12/252</td>
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**Baseline: cirrhosis**

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<tr>
<td>Better</td>
<td>71/96</td>
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<td>(&gt;2 stages: 70/71)</td>
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**Progression**

BMI ≥25: OR 7.4

**Incidence of HCC/CCC**

4/585 (0.8%)  
(>2%/yr in untreated cirrhotics)

*Marcellin P et al, Lancet 2013*
Design of currently largest study in NASH cirrhotics aimed at reversal

A Phase 3, Randomized, Double-Blind, Placebo-Controlled Study Evaluating the Safety and Efficacy of Selonsertib in Subjects With Compensated Cirrhosis Due to Nonalcoholic Steatohepatitis (NASH)

Gilead, International, 283 sites

883 patients enrolled, study start January 30 2018

• Selonsertib (GS-4997, Ask1 inhibitor) 6mg or 18mg per day (1 tablet p.o.) vs placebo

• Duration: 1. 48 weeks 2. 240 weeks (continuation of 1.)

• Completion: 1. January 2019, 2. November 2022
Primary endpoints

- Proportion of Participants Who Achieve a ≥ 1-Stage Improvement in Fibrosis According to the NASH Clinical Research Network (CRN) Classification Without Worsening of NASH [Time Frame: Week 48]

- Event-Free Survival (EFS) at Week 240 as Assessed by Time to the First Clinical Event [Time Frame: Week 240]
Secondary endpoints

• Proportion of Participants Who have a $\geq 1$-Stage Improvement in Fibrosis Without Worsening of NASH at Week 240

• Proportion of Participants Who Have a $\geq 1$-Stage Improvement in Fibrosis at Week 48

• Proportion of Participants Who Have a $\geq 1$-Stage Improvement in Fibrosis at Week 240

• Proportion of Participants Who Have NASH Resolution at Week 48

• Proportion of Participants Who Have NASH Resolution at Week 240
Key Inclusion Criteria:
• Liver biopsy consistent with NASH and cirrhosis F4 (central reader)
• The following laboratory parameters at the screening visit (central lab):
  ALT $\leq 8 \times$ ULN, Creatinine Clearance $\geq 30$ mL/min, HbA1c $\leq 9.5\%$ (or serum fructosamine $\leq 381 \mu$mol)

Key Exclusion Criteria:
• Prior history of decompensated liver disease (clinical ascites, hepatic encephalopathy (HE), or variceal bleeding)
• Child-Pugh score $> 7$, MELD score $> 12$, as determined at screening, unless due to therapeutic anti-coagulation
• Other causes of liver disease based on medical history and/or centralized review of liver histology.
• History of liver transplantation
• Current or history of hepatocellular carcinoma (HCC)
Other (noninvasive) markers and techniques to increase plausibility of cirrhosis reversal

- Broad panel for liver function (LFTs, albumin, INR, platelets……)
- Metabolic markers (lipids, phospholipids, metabolome, …)
- Immune & inflammation related markers (CD163, sCRP, …)
- Adipokines (adiponectin,……)
- PROs (QoL, SF36……)
- Elastography (MRE, USE, ARFI……)
- MRI-PDFF, Liver Multiscan

- Serum fibrosis markers
- Targeted fibrosis imaging
Current serum fibrosis markers

1. Direct tests: markers related to the pathophysiology of fibrosis (components from the scar matrix)

2. Indirect tests: parameters related to liver function and metabolism (ALT, Bili, INR, Alb, platelets…….)

3. Tests combining 1 and 2

These markers reflect liver function/ perfusion and/or dynamics of fibrogenesis rather than the extent of fibrosis

Direct and MOA-based serum markers are needed that truly reflect either fibrogenesis or fibrolysis
Development of Noninvasive Biomarkers for Rapid Clinical Efficacy Testing and a Personalized Medicine
Novel Direct Fibrosis Markers

**Fibrogenesis:** P3NP, ProC3, ProC5, A9, T2, TIMP-1, hyaluronic acid

- precursor synthesis
- myofibroblasts
- matrix degradation or turnover: C3M, C4M, C5M, C6M, lumican, laminins.....

**Fibrolysis (noncollagen):** A2, A14

- Degradation fragments may also derive from freshly synthesized matrix proteins and reflect enhanced turnover during fibrogenesis

Karsdal et al, Adv Drug Del Rev 2017
Schuppan et al, J Hepatol 2018; Schuppan et al, Matrix Biol 2018
Discovery of novel candidate fibrolysis markers

SOMAScan (DNA aptamer) proteomics comparing sera of
a) non fibrotic controls
b) rapid progressors (cirrhosis in 5 y)
c) super-rapid progressors (cirrhosis within 1 y post LTX)

B3 fibrolysis

![Graph showing B3 fibrolysis results]

Before and after highly effective antiviral therapy for Hep C

![Graph showing BAV and AAV results]

Surabattula, Zimmermann, Liberman, Schuppan, unpublished
Novel Direct Serum Markers of fibrogenesis and fibrolysis (MOA based)
A2, A9: shed cell membrane molecules involved in ECM remodeling
Exploratory validation in pts with rapid progression vs regression

NS: healthy ctr
LTX: post transplant with rapid progression to cirrhosis in 3-5 yr

BAV: before antiviral Tx for HCV
AAV: 24 w after highly effective antiviral Tx for HCV (regression in ~80%)

Surabattula R et al, unpublished
Development of Dynamic Imaging of Fibrogenesis
Quantitative imaging of liver fibrogenesis

Resting hepatic stellate cell or portal fibroblast

Coupled to radiotracer

$^{99}$Tc (SPECT) or $^{18}$F (PET) or MRI

Coupled to radiotracer

Fibrosis

$\alpha_v\beta_6$

Fibrogenesis

Col-P

Small molecular (receptor) ligands

Activated myofibroblast produces most of the scar tissue

Activated cholangiocyte major activators of myofibroblasts

Activated myofibroblast

Proliferation

Coupled to radiotracer

PDGF$\beta$R

FAP

TGF$\beta$, CTGF

Fibrosis

Small molecular (receptor) ligands

Activated myofibroblast produces most of the scar tissue
Bifunctional αvβ6-integrin imaging agent

PET-radiochelator

Cy5-DOTA-S-PepA
peptide monomer
N-terminus coupled peptide
bimodal chelator for NIR and radiolabelling

NIR-dye

αvβ6-binding peptide

Kim, YO et al, EASL # FRI-108
Quantification of αvβ6 integrin binding (ex vivo analysis)
Mdr2KO mice, age 8 weeks, with spontaneous biliary fibrosis (3fold increased liver collagen) – iv injection of bimodal αvβ6 integrin-binding peptide

5-7 fold higher signal in fibrotic KO than WT
Liver specific binding

Kim, YO et al, EASL # FRI-108
Summary

• Progression to cirrhosis, decompensation and HCC is the most important hepatic endpoint in NAFLD

• Major antifibrotic targets are related to the ECM, fibrogenic hepatic stellate cells, cholangiocytes, macrophages and

• The currently largest study to assess the efficacy of a potential antifibrotic to halt further progression of cirrhosis or induce cirrhosis reversal is still primarily based on biopsy

• Biologically plausible markers of fibrosis, fibrogenesis and fibrolysis to stratify patients in need of treatment to and noninvasively monitor treatment response are being developed within the EPoS, LITMUS and NIMBLE consortia

• This should permit short and slim POC studies, testing of combinations and a personalized antifibrotic therapy