Liver fibrosis in NASH: A Roadmap for Drug Discovery and Pharmacotherapy

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I have no conflict of interest to declare
Relevance of advanced liver fibrosis/cirrhosis as endpoint

- Noncirrhotic fibrosis
- Cirrhosis
- Fibrosis progression
- Induction of reversal
- Decompensation
- HCC
- Liver related death
Fibrogenesis is a Multicellular Process

**Normal Liver**
- Quiescent stellate cell
- Portal or perivascular fibroblast

**Activated Myofibroblast**
- Activated myofibroblast (HSC

**Macrophage**
- Macrophage
- TLR4
- Toxins
  - Toxic bile salts
  - (Auto-) Immunity
  - HBV, HCV
- Ox. stress, ROS
- Insulin resistance
- Microbiome, nutrients

**Endothelium**
- Endothelium

**Cytokines**
- Cytokines

**Chemokines**
- Chemokines

**ROS**
- ROS

**Genetic Predisposition**
- Progenitor cell, cholangiocyte, hepatocyte

**Repetitive Damage** (second hit)
- Toxins
- Ox. stress, ROS
- Insulin resistance
- Microbiome, nutrients

**Cirrhosis** and HCC
- Common pathways & immune environment

**Collagen Synthesis**
- MMP-1/3/13
- TIMP-1
- TIMP-2

**Cirrhosis**
- Organ failure
- Matrix accumulation

**Schuppan and Afdhal, Lancet 2008**
**Schuppan and Kim, JCI 2013**
Reversibility of advanced fibrosis after removal/suppression of the primary causal hit
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
Better 105/252
Worse 12/252

Baseline: cirrhosis
Better 71/96
(≥2 stages: 70/71)

Marcellin P et al, Lancet 2013
Antifibrotic approaches
complex interactions between cells and cytokines, chemokines, extracellular matrix
- anti- or profibrotic effects are context-dependent
- unambiguous targets are rare

Schuppan and Kim, J Clin Invest 2013
Mehal and Schuppan, Sem Liver Dis 2015
Role of activated cholangiocytes in fibrosis progression
Progenitors ("activated cholangiocytes") as driving force of fibrogenesis

Proliferation stimulus

2\textsuperscript{nd} hit

E.g. oxidative stress in NAFL

Hepatocyte growth arrest / lipoapoptosis

Rise of fibrogenic progenitor cells

Ductular reaction

EMT-like changes

Activated myofibroblasts

Activated progenitor cells/ cholangiocytes

Integrin $\alpha\beta_6$ $\uparrow\uparrow$

$\alpha\beta_6$ inhibitors

General mechanism in fibrosis $\geq F2$

FCH: Davies SE et al, Hepatology 1991
ALH: Ray MB et al, Liver 1993
Various CLD: Lowes KN, Am J Pathol 1999
HCV: Clouston A et al, Hepatology 2005
NASH: Richardson MM et al, Gastroenterology 2007

Wang et al, Hepatology 2007; Popov Y et al, J Hepatology 2008; Patsenker E et al, Gastroenterology 2008

Nanotechnology, activated myofibroblasts

EMT-like changes

Activated myofibroblasts

Cirrhosis/HCC
Pharmacological repolarization of macrophages

- IL-12, IFNγ
  - classically activated M1 macrophage

- IL-4, IL-13, Stat-6
  - alternatively activated M2 macrophage

Monocyte

Phagocytosis of debris and apoptotic cells
Generation of pro-inflammatory cytokines

“Resolution of fibrosis”
Immune-activating & cancer-suppressive

Pro-resolution macrophage
CD11b+ F4/80+ Ly6Clow

Targeted modulation

Promotion of angiogenesis
Generation of anti-inflammatory cytokines

“Promotion of fibrosis”
Immune-suppressive & cancer promoting

Macrophages of „wounds that do not heal“ = fibrosis- and tumor-associated macrophages

References:
- Duffield JS et al, JCI 2005
- Fallowfield JA, J Immunol 2007
- Duffield JS et al, JCI 2007
- Popov Y et al, Am J Physiol 2010
- Ramachandran S et al, PNAS 2012
- Schuppan and Kim, JCI 2013
- Mehal and Schuppan, Sem Liver Dis 2015
Mechanism based antifibrotic therapies in clinical development
Drugs in phase I-II clinical trials to address fibrosis (1)

ECM, EMT, Progenitor activation (inflammation, hepatocyte apoptosis)

- Gilead: GS6624 (Simtuzumab): α-Loxl2 Mab (>700 patients with ≥ stage 2 NASH or PSC)
- Gilead: GS9654 (Selonisertib): Ask1 (apoptosis signaling kinase 1) inhibitor (70 patients with ≥ stage 2 NASH)
- Biogen-Stromedix: STX-100: α-Integrin αVβ6 Mab
- Biogen-Idec: anti-Tweak
- Sanofi-Genzyme: Fresolimumab: α-TGFβ Mab
- Pfizer-Fibrogen: FG3019: α-CTGF Mab
- Novartis: Seculizumab, α-IL-17 Mab
- Conatus: Emricasan: Caspase inhibitor (>250 pts with NASH)
- Boehringer-Ingelheim: VAP-1 antagonist (>200 pts with NASH)

Drugs in phase I-II clinical trials to address fibrosis (2)
(M2) Macrophages, hepatic stellate cells

- Janssen: Carlumab: \(\alpha\)-MCP-1/CCL2 Mab
- Pfizer: Selzentry: CCR5 antagonist
- Tobira/Allergan: Cenicriviroc: CCR2/CCR5 antagonist (289 patients with NASH)
- BMS: Peg-FGF21
- Novartis: QUAX576: \(\alpha\)-IL-13 Mab
- Sanofi: SAR156597: \(\alpha\)-IL-4/IL-13 Mab
- Isis and own group: IL4R\(\alpha\)1, IL13R\(\alpha\)1……. antisense-DNA
- Promedior: RM-151: rec. Pentraxin-2 (SAP)
- Novo Nordisc: GLP-1 agonist/Semaglutide) (300 pts with NASH)
An anti-inflammatory agents are not necessarily antifibrotic, examples:

• anti-TGFβ1: blocks fibrosis, enhances inflammation

• anti-CCR2, CCR5… : blocks inflammatory and restorative macrophage infiltration/activation, HSC activation
Current and evolving options for fibrosis assessment

Liver Biopsy – crude assessment, not dynamic

Fibrosis serum markers → validated markers of fibrogenesis and fibrolysis

Imaging → targeted and quantitative imaging of fibrogenesis

Elastography – crude assessment, not dynamic
## Sampling variability in staging & grading

HCV, laparoscopic biopsy of right and left liver, n=124, Metavir-score

<table>
<thead>
<tr>
<th>Difference</th>
<th>n</th>
<th>%</th>
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<tbody>
<tr>
<td>≥ 1 stage</td>
<td>41/124</td>
<td>33.1</td>
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<tr>
<td>≥ 2 stages</td>
<td>3/124</td>
<td>2.4</td>
</tr>
<tr>
<td>≥ 1 grade</td>
<td>30/124</td>
<td>24.2</td>
</tr>
<tr>
<td>≥ 2 grade</td>
<td>2/124</td>
<td>1.6</td>
</tr>
<tr>
<td>cirrhosis vs. stage 3</td>
<td>18/124</td>
<td>14.5</td>
</tr>
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Regev et al., Am. J. Gastroenterol. 97, 2614, 2002

≥ 1 stage discordance

NASH > 40%  
PBC/PSC > 60%

Ratziu V, Gastroenterology 2005; Merriman RB et al, Hepatology 2006


Liver biopsy samples only 1/50,000 of the whole liver
Comparison of 6 biomarker scores in 180 pts with CHC (F0-1 vs. F2-4)

- **MP3**: PIIINP, MMP-1
- **APRI**: AST / platelets
- **Forns**: γGT, cholesterol, platelets, age
- **Fibrotest**: age, Bili, γGT, γ-globulin, haptoglobin, α2M
- **Hepascore**: age, gender, α2M, HA, Bili
- **Fibrometer**: age, INR, platelets, AST, urea, α2M, HA

All with AUROCs 0.80-0.85 = only modest tests to distinguish no/mild vs. significant fibrosis

*Leroy V et al, J Hepatol 2007*
**Biological plausibility:**

**Direct markers of fibrosis dynamics**

*precursor synthesis* → *propeptide-cleavage*

**Best current marker of fibrogenesis:** *ProC3*

**Marker(s) of fibrolysis needed**

- *P3NP*, *ProN5*, *TIMP-1*, *hyaluronic acid*
- *fibrogenesis*
- *matrix degradation/turnover*: *C3M*, *C4M*, *C5M*, *C6M*, *lumican*, *laminins*, *tenascin*

*But: degradation fragments also derive from freshly synthesized matrix proteins*

**ELF-Panel**

NB biomarkers - established assays – validation for liver:
C5M (MMP-mediated type V collagen degradation)
C6M (MMP-mediated type VI collagen degradation)
ProC3 (type III collagen formation) - fibrogenesis
ProN4 (type IV collagen formation)
ProC5 (type V collagen formation)
ProC6 (type VI collagen formation, adipokine) – fibrogenesis, adipose tissue fibrosis (NASH)

UMCM biomarkers of progression or reversal derived from serum iTRAQ and Somascan proteomics:
WDR85, WDR90, Ephrin B3, A9*, IB3*, PR8*, MK3* - fibrogenesis
IB3*, PR8*, MK3*, A2*, A14*, CS17*, CS26*, TP2* - fibrolysis

* in validation, listed for patent protection

Nielsen M et al, Liver Int 2015
Leeming D et al, submitted
Surabattula R et al, submitted
Procollagen type III processing

each variant of the same protein carries unique information
Nonresponders to HCV-antiviral therapy
Ishak fibrosis score 2-4
Treatment duration: 52 weeks with follow up biopsies (209/265=79%)
Placebo controls n=89
Farglitazar 0.5 mg/d: n=88
Farglitazar 1.0 mg/d: n=88
Histological quantification:
\( \alpha \)-SMA, collagen (SR morphometry)

Non-TZDD PPAR-\( \gamma \) agonist with 100-1000fold higher activity than Pio-/Tro-/Rosi-glitazone

<table>
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<tr>
<th>Treatment</th>
<th>Collagen</th>
<th>( \alpha )-SMA</th>
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<tr>
<td>Placebo controls</td>
<td>+49%</td>
<td>27%</td>
</tr>
<tr>
<td>Farglitazar 0.5 mg/d</td>
<td>+58%</td>
<td>27%</td>
</tr>
<tr>
<td>Farglitazar 1.0 mg/d</td>
<td>+52%</td>
<td>31%</td>
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no overall effect on fibrosis!
Pro-C3 identifies subjects who responded to antifibrotic therapy

Follow up on Pro-C3 levels

- > 20.2 ng/ml: selection criterion for responders
- a decline in serum levels indicates antifibrotic effect

Markers of fibrogenesis and fibrolysis: A2, A9 and A14: cell membrane molecules involved in ECM remodeling

Fibrolysis

Fibrogenesis

NS, healthy ctr

LTX: post transplant with progression to cirrhosis within 5 yr

BAV: before antiviral Tx for HCV

AAV: 24 w after highly effective antiviral Tx for HCV

Surabattula R et al, unpublished
Summary (1)

- (early) **Cirrhosis is reversible** when the major fibrogenic (inflammatory) trigger is eliminated (HepB, HepC, ai-Hep)
- This may even be possible for (decompensated) cirrhosis
- Most NASH drugs target the **hepatocyte** and its metabolic derangement, possibly with secondary antifibrotic effects
- Some drugs target **inflammation**, but this does not necessarily correlate with antifibrotic activity
- Other drugs address **multiple cells** and net effects are difficult to predict
• Major antifibrotic targets are related to fibrogenic cholangiocytes, macrophages and hepatic stellate cells

• Several (pharmacological) therapies that may inhibit progression and speed up reversal have entered the clinic

• Biologically plausible markers of fibrosis, fibrogenesis and fibrolysis to stratify patients and noninvasively monitor treatment response are being developed

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