CYTOKERATIN 18 (CK18) AS A BIOMARKER IN NASH CLINICAL TRIALS

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NAFLD/NASH: THE LANDSCAPE

- NAFLD: Non-alcoholic fatty liver disease
- NASH: Non-alcoholic steatohepatitis
- NAFLD is the most common cause of liver disorder in Western countries
- It is strongly connected to the epidemic increase of obesity and type 2 diabetes
- This year (2020), NASH is projected to overtake Hepatitis C as the leading cause for liver transplants in the U.S.

US Prevalence:
- NAFLD: ~65M
- NASH: ~15M
- Cirrhosis: ~1-3M
- Hepatocellular Carcinoma: ~70-200K

Source: cn-bio.com
There are currently no FDA-approved pharmacologic therapies for NASH

NASH CLINICAL TRIALS

NASH Therapeutic Targets

NASH Drug Studies by Phase (2020)

<table>
<thead>
<tr>
<th>Phase</th>
<th>Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1</td>
<td>40+</td>
</tr>
<tr>
<td>Phase 2</td>
<td>~30</td>
</tr>
<tr>
<td>Phase 3</td>
<td>6</td>
</tr>
</tbody>
</table>

NASH CRN Scoring System:

• **NAS** = Steatosis + lobular inflammation + ballooning

• **NFS** = F0 (no fibrosis) → F4 (cirrhosis)

Surrogate endpoints for accelerate/conditional approval:

i. Resolution of NASH without worsening of fibrosis

ii. Improvement in fibrosis without worsening of NASH

iii. Both

Source: Kleiner et al., Hepatology 2005
Liver Biopsy
- Invasive
- Costly
- Medical complications
- Sampling errors

HEPATOCYTE CELL DEATH IN NASH

- Hepatocyte apoptosis is considered a hallmark of NASH
- Hepatocyte apoptosis contributes to inflammation, liver injury, fibrosis
- Hepatocyte apoptosis is thought to be a driver in the progression of NAFLD
Cytokeratin 18 (CK18, Keratin 18, K18) is the major intermediate filament protein in the liver.

- **Cytoprotective function**
- **Present in hepatocytes and cholangiocytes**
- **Hepatocyte ballooning is associated with a loss of cytoplasmic CK18 immunostaining**
- **Component of Mallory-Denk bodies (MDBs)**
- **During hepatocyte apoptosis, CK18 is cleaved by caspases (9, 3, and 7)**
- **Upon hepatocyte cell death, CK18 is released into the blood**

**CK18 is a serum biomarker for hepatocyte cell death**

*Source: proteinatlas.org*
CK18 CELL DEATH ASSAYS

Total Cell Death (M65®)
- M65® ELISA
- M65® EpiDeath® ELISA
- M5 and M6 antibodies
- Measures total CK18
- Total cell death
  - Apoptosis and necrosis

Apoptosis (M30®)
- M30® Apoptosense®
- M30® CytoDeath™
- M30® Antibody
- Measures CK18 fragments (cCK18)
- Apoptosis only
CK18 elevations may represent active NASH
- CK18 levels have been shown to increase with increases in the NAS and fibrosis stage
- Monitoring changes in serum CK18 levels may be useful for assessing treatment response in NASH clinical trials
- A reduction in cell death markers (e.g., CK18) was recommended as a secondary endpoint for NASH clinical trials by an AASLD-FDA Joint Workshop (2015)
• 115 clinical trials have applied CK18 as a biomarker
• 60 clinical trials with a NAFLD/NASH indication

CK18 Biomarker Use in NASH
Clinical Trials by Phase

Source: Clarivate, Cortellis search CK18, filtered by NAFLD/NASH studies, 7-Aug-2018
## CK18 IN EARLY PHASE NASH DRUG TRIALS

<table>
<thead>
<tr>
<th>Drug</th>
<th>Selonsertib (Gilead)</th>
<th>Elafibranor (Genfit)</th>
<th>MGL-3196 (Madrigal)</th>
<th>PF-05221304 (Pfizer)</th>
<th>Tirzepatide (Eli Lilly)</th>
<th>MSDC-0602K (Cirius)</th>
<th>IMM-124E (Immuron)</th>
<th>AXA1125 (Axcella)</th>
<th>Emricasan (Conatus)</th>
<th>DUR-928 (Durect)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanism of Action/Target</td>
<td>ASK-1 inhibitor</td>
<td>PPAR a/d agonist</td>
<td>THRbeta agonist</td>
<td>ACC 1/2 inhibitor</td>
<td>GIP/GLP antagonist</td>
<td>MCP modulator</td>
<td>Antibody, multifactorial</td>
<td>Metabolic modulator</td>
<td>Pan-caspase inhibitor</td>
<td>Nuclear receptor modulator</td>
</tr>
<tr>
<td>Trial Phase</td>
<td>2</td>
<td>2b</td>
<td>2</td>
<td>2a</td>
<td>2b</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Length of study</td>
<td>24 weeks</td>
<td>52 weeks</td>
<td>36 weeks</td>
<td>16 weeks</td>
<td>26 weeks</td>
<td>26 weeks</td>
<td>24 weeks</td>
<td>12 weeks</td>
<td>48 hours</td>
<td>24 hours</td>
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- Statistically significant changes in CK18 have been observed as early as 6 weeks of treatment.
- For direct caspase inhibitors, changes can occur as early as 12 hours post-dose.
- The observed time course is dependent on the mechanism of action of the drug.

Source: clinicaltrials.gov
Decreases in CK18 were strongly associated with improved liver histology.
CORRELATION BETWEEN CHANGES IN CK18 AND CHANGES IN LIVER HISTOLOGY (CONT.)

- Selonsertib/simtuzimab in subjects with NASH (NAS ≥5, F 2-3)
- 24 weeks

Histologic responders had greater reductions in CK18 (M30® and M65®)

Change in CK18 according to Fibrosis Response

Change in CK18 according to Lobular Inflammation Response

- Histologic responders had greater reductions in CK18 (M30® and M65®)

CK18 ASSAYS AS A PRESCREENING TOOL IN NASH CLINICAL TRIALS

- Problem: high screen-failure rate in NASH clinical trials (>55%)
  - Many screened subjects to not have NASH upon biopsy, or they do not meet study enrollment criteria (NAS ≥4 and F1-F3)
  - Adds to cost and duration of studies
- CK18 levels correlate with ballooning
- Subjects with high CK18 levels are more likely to have ballooning, inflammation, and steatosis, and therefore are more likely to have a NAS of ≥4
- CK18 assays may be useful for prescreening subjects recruited for NASH clinical trials prior to biopsy
NASH is a global epidemic. It is a slowly progressing chronic liver disease that can lead to cirrhosis, hepatocellular carcinoma, and other medical complications.

There are currently no approved drugs for the treatment of NASH, but many clinical trials are underway to find an effective pharmacotherapy.

One of the biggest challenges for NASH drug development is the selection of surrogate endpoints that predict clinical outcomes.

Monitoring changes in serum CK18 levels treatment may be useful for assessing treatment response, especially in early phase NASH studies.

Improvements in CK18 correlate with improvements in liver histology.

Using CK18 assays as a prescreening tool prior to biopsy may decrease the screen fail rate in NASH clinical trials.
THANK YOU!

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