Non-invasive diagnostic biomarkers

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Outline

• Definition of biomarker

• Clinical need

• Current status

• Approach to successful biomarker
Biomarker

• A **biomarker**
  – is a characteristic that is objectively measured and evaluated as an indication of normal biologic processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.

• Composite biomarker

• Surrogate biomarker or end-point
  – Prince Criteria (modified for understanding and application in NASH)
    • Positive or elevated or decreased only in disease state
    • Disease state gets worse it gets worse irrespective of the intervention
    • Disease state improves it improves irrespective of the intervention
    • Predicts long-term risk of clinical outcome
      – Change in biomarker predicts outcome
Types of biomarker

- Diagnostic
- Prognostic
- Predictive
- Pharmacodynamic
Number of trials in NASH/NAFLD: October 2015

Colors indicate the number of studies with locations in that region

Least  Least  Least

Most  Most  Most

Labels give the exact number of studies
Natural history of NASH

- 18 million Americans

Fibrosis progression rate in NASH: 1 stage per 7 year

20% patients are fast progressors: to cirrhosis in 10 years

- Currently, liver biopsy is the only way to diagnose NASH

- Liver biopsy to classify such a large population
  - Impractical
  - Expensive
  - Invasive
  - Variability

- Need for clinical prediction rules and novel biomarker of NASH

Liver death
Liver transplant
HCC
Cirrhosis
Fibrosis
NASH

40-50%
15-20%
2-3%/yr
30-40%
Key histologic predictors of mortality in NAFLD

- Presence of advanced fibrosis
- Presence of fibrosis
- Presence of NASH
## Building a biomarker program in NASH

<table>
<thead>
<tr>
<th>Implications for clinical trials</th>
<th>Study objectives for development and validation of biomarker</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>- Biologically plausible assessment of disease presence and activity</td>
</tr>
<tr>
<td></td>
<td>- Technical considerations and limitations for application in humans</td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>- Technical reasons for limited accuracy</td>
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<td></td>
<td>- Considerations for composite biomarker to improve diagnostic accuracy</td>
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<td></td>
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<td></td>
<td>- Intra- and inter-observer variability</td>
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<td></td>
<td>- Technical considerations to improve diagnostic precision and variability</td>
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<tr>
<td></td>
<td>- Development of scoring criteria or co-localization strategies</td>
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<td></td>
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<td></td>
<td>- Comparison to other modalities</td>
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<td>- Correlation with meaningful changes in disease activity or disease course</td>
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<td></td>
<td>- Considerations to improve correlation</td>
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<tbody>
<tr>
<td>Reduction in Heterogeneity</td>
<td>- Reduced enrollment bias</td>
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<td>- Increased study power</td>
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<tr>
<td></td>
<td>- Prognostic enrichment and Reduction in Heterogeneity enrichment</td>
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<tr>
<td>Reduction in Variability</td>
<td>- Predictive enrichment</td>
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<td>- Reduction in study size requirements</td>
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<td>- Increased absolute and relative treatment effect sizes</td>
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<tr>
<td>Enhanced Assessment of Response</td>
<td>- Early assessment of response to therapeutic interventions</td>
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<tr>
<td></td>
<td>- Prognostic, Predictive enrichment</td>
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<td></td>
<td>- Enrichment through withdrawal</td>
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<td>- Surrogate for meaningful outcomes</td>
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Discrimination between binary outcomes

- 0.9-1.0 = Excellent
- 0.8-0.9 = Good
- 0.7-0.8 = Fair
- 0.6-0.7 = Poor
- 0.5-0.6 = bad
# Diagnostic test characteristics

<table>
<thead>
<tr>
<th></th>
<th>NASH</th>
<th>NASH</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Test +</td>
<td>a</td>
<td>b</td>
<td>a + b</td>
</tr>
<tr>
<td>Test -</td>
<td>c</td>
<td>d</td>
<td>c + d</td>
</tr>
</tbody>
</table>

\[ \text{NPV} = \frac{d}{c + d} \]
\[ \text{PPV} = \frac{a}{a + b} \]

As prevalence of disease increases, PPV increases
# Performance of CPR

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Study</th>
<th>AUROC 1</th>
<th>AUROC 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>NASH</td>
<td>N-Tetri et al. (NASH-CRN)</td>
<td>0.71</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>AST+ALT+AST/ALT</td>
<td>0.79</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>36 variables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Advanced fibrosis (stage 3 or 4)</td>
<td>N-Tetri et al. (NASH-CRN)</td>
<td>0.73</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>AST+ALT+AST/ALT</td>
<td>0.85</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>36 variables</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Angulo et al. NAFLD Fib Score</td>
<td>0.88</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>Age, BMI, PLT, Alb, AST/ALT</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ratziu et al. BAAT (&gt;1)</td>
<td>ND</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>BMI, ALT, Age, TG</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Harrison et al. BARD (≥ 2)</td>
<td>0.81</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>BMI, AST/ALT, DM</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Cales et al.</td>
<td>0.92</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>Glu, AST, PLT, Fer, Weight, Age,</td>
<td></td>
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</tr>
</tbody>
</table>
Types of biomarkers

• Molecular
  – Genomic
  – Proteomic
    • CK-18
    • ELF
    • HA
    • RBP-4
    • IU panel
    • Younossi panel
  – Lipidomic
    • Oxidized FA
    • Non-HDL cholesterol
    • Small dense LDL
    • Eicasanoids
  – Metabolomic
  – Hybrid panels
    • NAFIC panel

• Imaging
  – MR-based
    • MRI-PDFF
    • MRS
    • MRE
    • Diffusion-weighted imaging
    • Multiscan
  – Ultrasound
    • USG
    • VCTE
    • ARFI/SWE
  – CT
## Performance of biomarkers

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<th>AUROC</th>
</tr>
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<tr>
<td><strong>NASH</strong></td>
<td>Feldstein et al. CK-18</td>
<td>0.83</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>Feldstein et al. CK-18, sFasL</td>
<td>0.93</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>Feldstein et al. oXNASH (13-HODE/LA, age, BMI, AST)</td>
<td>0.83</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Younossi et al. NASH Diagnostics</td>
<td>0.98</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td>Poynard et al. Nash Test</td>
<td>0.79</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>Palekar et al. HA + Clinical model</td>
<td>0.76</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Loomba et al. Lipidomic</td>
<td>1.00</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Advanced fibrosis (stage 3 or 4)</strong></td>
<td>Guha et al. ELF</td>
<td>0.9</td>
<td>0.82 (0.85*)</td>
</tr>
<tr>
<td></td>
<td>Corgenix Inc. HA (NASH-CRN*)</td>
<td>0.83</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>Hepa score (NASH-CRN*)</td>
<td>0.80</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>PIIINP</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TIMP-1</td>
<td>0.70</td>
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sFasL, soluble FAS ligand, HODA, hydroxy-octadecadienoic acid; LA, linoleic acid, hyaluronic acid (HA), amino-terminal propeptide of type III collagen (PIIINP), and tissue inhibitor of metalloproteinase 1 (TIMP-1)
Key clinical issues for assessing response

Clinical trial – Phase 1/2 studies

Primary end point
- MRI-PDFF/MRS/ALT/MultitScan
- Mechanism-based

Secondary end point
- Decline in ALT
- Decline in CK-18
- Kinetic biomarkers
- Omic-based

Clinical trial – Phase 2/3 studies

Primary end point
- Liver histology
  - NAS
  - Resolution
- HVPG
- Clinical

Secondary end point
- MRI-PDFF/MRE/Multiscan
- Fibroscan/ARFI/SWE
- Decline in ALT
- Decline in CK-18
- Omic-based

Clinical trial – Phase 4 studies

Long term clinical outcomes

Caveats: Efficiency of phase 1 and 2, and effectiveness of phase 3 and 4
Approach to biomarkers/endpoints

Caveats: Efficiency of phase 1 and 2, and effectiveness of phase 3 and 4
Hepatic collagen FSR was significantly higher in patients with NASH vs NAFL (with high variability in the NASH patient group).

Collagen FSR: NASH vs NAFL

* = p < 0.05

Kinemed and UCSD collaboration
Unmet need in NAFLD

• Initial assessment:
  – Need non-invasive biomarkers to answer following
    • Presence of NASH
    • Presence of NASH with fibrosis
    • Presence of advanced fibrosis
    • Risk of hepatic decompensation and mortality

• Predicting treatment response:
  – Need therapy to reverse NASH and biomarkers to predict response to treatment
    • Resolution of or improvement in NASH (inflammation/ballooning)
    • Improvement in one stage of fibrosis
Goals of predicting treatment response in NASH

• Predicting treatment response
  – Improvement in liver fat content (steatosis)
    – MRI/MRS (Most robust, precise accurate and quantitative measure)
  – Resolution of or improvement in NASH (inflammation/ballooning)
    – Biopsy
  – Improvement in fibrosis
    – Unreliable assessment so far
  – Reduction in the risk of hepatic decompensation (ascites, variceal bleeding, hepatic encephalopathy, and HCC) and mortality
    • Liver disease, CVD or cancer
    • NO DATA
Fat (TG) has a chemical signature

This chemical signature can be detected directly by magnetic resonance spectroscopy (MRS).

Performed properly, MRS quantifies the proton density fat fraction (PDFF), a standardized measure of liver tissue [TG].

Limitations of MRS
- One 8cm$^3$ voxel
- Not available on routine scanners
- Requires expertise

Imaging method to estimate PDFF would have advantages....

Thomsen MRI 1994
Hamilton JMRI 2009
Hamilton NMR Biomed. 2011
Reeder JMRI 2011
MRI Imaging Methods to Estimate PDFF

Magnitude data-based MRI

Complex data-based MRI

MRI-PDFF addresses confounding factors, unlike conventional in-phase and opposed-phase MRI-PDFF *not* affected by

- Scanner field strength, manufacturer
- Patient factors: age, sex, BMI, etiology of liver disease
- Concomitant liver abnormalities: iron overload, necroinflammation

MRI-PDFF robust to parameter changes

Acquisition 12-25 seconds
Co-localized MRI-PDFF and cross-validated with MRS

- PDFF recorded in regions of interests (ROI)s ~300-400mm$^2$
- The same 3 ROIs in each of the 9 liver segments measured at baseline and post-treatment.
- Each segment fat fraction = average 3 ROIs
- Total liver fat fraction = average 27 ROIs

Le et al. Hepatology 2012
Imaging biomarkers of fibrosis: Overview

- Fibrosis has **no molecular signature** that can be detected by current imaging techniques.
- All imaging tests for fibrosis attempt to detect fibrosis *indirectly*.
- Many imaging biomarkers proposed: stiffness, diffusion, perfusion, metabolites, image texture, ...
- Leading biomarker is liver “stiffness” (or “elasticity”) and its family of related parameters
  - shear wave speed, Young’s elastic modulus, shear elastic modulus, shear complex modulus, ...
- **Rationale:** collagen deposition associated with fibrosis imparts parenchymal rigidity.
- Imaging tests that assess stiffness = “elastography”
Accuracy of MRE in non-invasive diagnosis of advanced fibrosis in NAFLD

A threshold of 3.63 kPa discriminates advanced fibrosis.

Loomba et al. Hepatology 2014
MOZART Trial Design: Ezetimibe vs Placebo

**Design:** Randomized, double-blind, allocation-concealed, placebo-controlled, clinical trial

First trial to assess 2D and 3D MRE in NASH

- **Ezetimibe 10 mg daily:**
  - Study Weeks:
    - 0: Urine Stool plasma
    - 4: Urine Stool plasma
    - 12: Urine Stool plasma
    - 24: Urine Stool plasma

- **Placebo:**
  - Randomization in blocks 4 in 1:1 ratio
  - Study Weeks:
    - 0: Vitals, anthropometric, labs
    - 4: Urine Stool plasma
    - 12: Urine Stool plasma
    - 24: Urine Stool plasma

- Follow-up:
  - Labs, MRS, MRI-PDFF, liver biopsy + 2D MRE 3D MRE

Loomba et al. Hepatology 2015
Why do we need to co-localize?

- Need for precision
  - Higher precision and accuracy
  - Efficiency in clinical trial

Stiffness-mapping before and after treatment

2D and 3D MRE is feasible
2D and 3D MRE may change in 24 wks

Larger area of the liver:
  - More comprehensive assessment

Why do we need to co-localize?
  - Need for precision

Higher precision and accuracy

Efficiency in clinical trial

Loomba et al. Hepatology 2015
Caveats associated with available imaging modalities

- Transient elastography or ARFI or other ultrasound-based test have following limitations:
  - Obesity
  - Ascites
  - Acute Inflammation
  - Cirrhosis

- MRE improves upon all except
  - Iron Overload
  - Acute Inflammation

- Depth of assessment
- Total volume or surface area of the liver covered
- MRE is more precise, accurate, reproducible not affected by obesity, ascites
- US-based and fibroscan point-of-care, ease of use, more access
# Hepatic steatosis quantification as an example

<table>
<thead>
<tr>
<th></th>
<th>MRS</th>
<th>MRI/MRE</th>
<th>US</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Measurement</strong></td>
<td>Directly measures differences in water and fat peaks on a resonance frequency domain</td>
<td>Indirect CSI assessment of signal interface between water and fat peaks during OP and IP echoes</td>
<td>Assessment through proxies (i.e. attenuation and echogenicity)</td>
</tr>
<tr>
<td><strong>Dynamic Range</strong></td>
<td>Single area (8cm³ voxel) manually placed in liver parenchyma using 3-plane localizing imaging</td>
<td>Quantification over a full dynamic range (0 – 100%) throughout parenchyma</td>
<td>Limited when overall content of hepatic steatosis is &lt; 20%</td>
</tr>
<tr>
<td><strong>Application</strong></td>
<td>Not available on routine scanners and requires expertise</td>
<td>Readily applied to routine scanners with some expertise required</td>
<td>Readily available in routine practice for use</td>
</tr>
<tr>
<td><strong>Accuracy</strong></td>
<td>High diagnostic accuracy not significantly impacted by demographics, histologic activity, or co-existing hepatic conditions</td>
<td>High diagnostic accuracy not significantly impacted by demographics, histologic activity, or co-existing hepatic conditions</td>
<td>Modest diagnostic accuracy; significantly limited by demographics (obesity), and co-existing hepatic conditions</td>
</tr>
<tr>
<td><strong>Reliability</strong></td>
<td>High precision with minimal variability</td>
<td>Higher precision and lower variability than MRS and histologic assessments</td>
<td>Modest reliability and agreement with training</td>
</tr>
<tr>
<td><strong>Responsiveness</strong></td>
<td>Responsive to changes in steatosis in single area</td>
<td>Highly responsive to changes in steatosis throughout parenchyma</td>
<td>Limited responsiveness and unable to co-localize ROI for response</td>
</tr>
<tr>
<td><strong>Co-localization of fibrosis</strong></td>
<td>Requires alternative imaging modality for co-localizing elasticity</td>
<td>Co-localization with MRE</td>
<td>Potential to co-localize with ultrasound elastography techniques</td>
</tr>
</tbody>
</table>
Summary: What does the biomarker need to do?

- **Imaging/Omic/based biomarkers**
  - Cross-sectional association
    - Diagnostic intent
    - Screening population
  - Validation in a larger, multicenter-cohort
    - Diagnostic intent
    - Screening population
    - High-risk groups
  - Longitudinal changes with treatment
    - Change in biomarker accurately predicts change in disease states
  - Predicts treatment response
    - Biomarkers shows improvement or worsening of disease on intervention
  - Predicts long-term prognosis
    - Today’s level accurately predict the risk of hepatic decompensation in future
Thank you

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10. Investigator Initiated Research Grant- 2 Merck Inc