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.

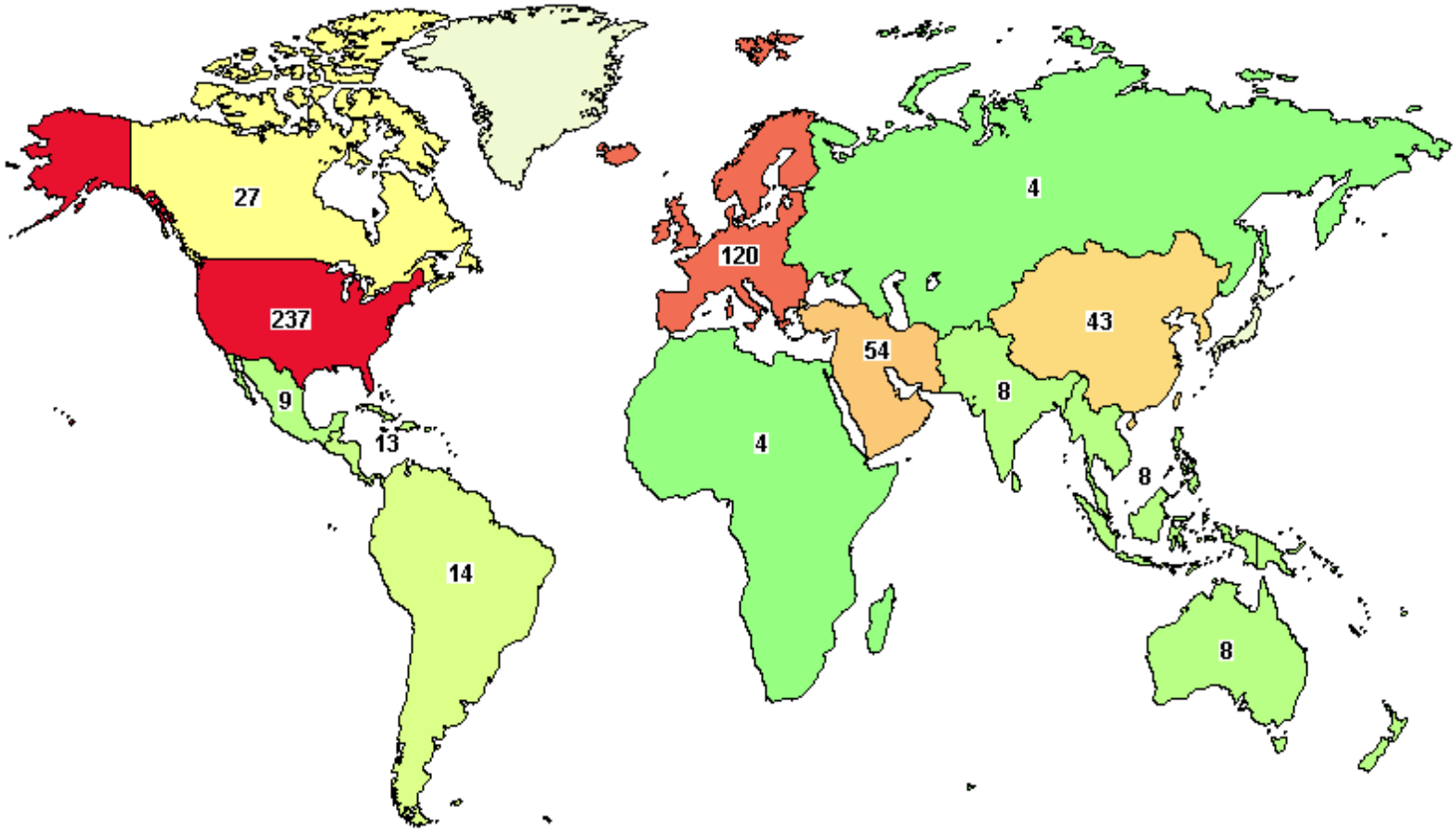
- **Composite biomarker**

- **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



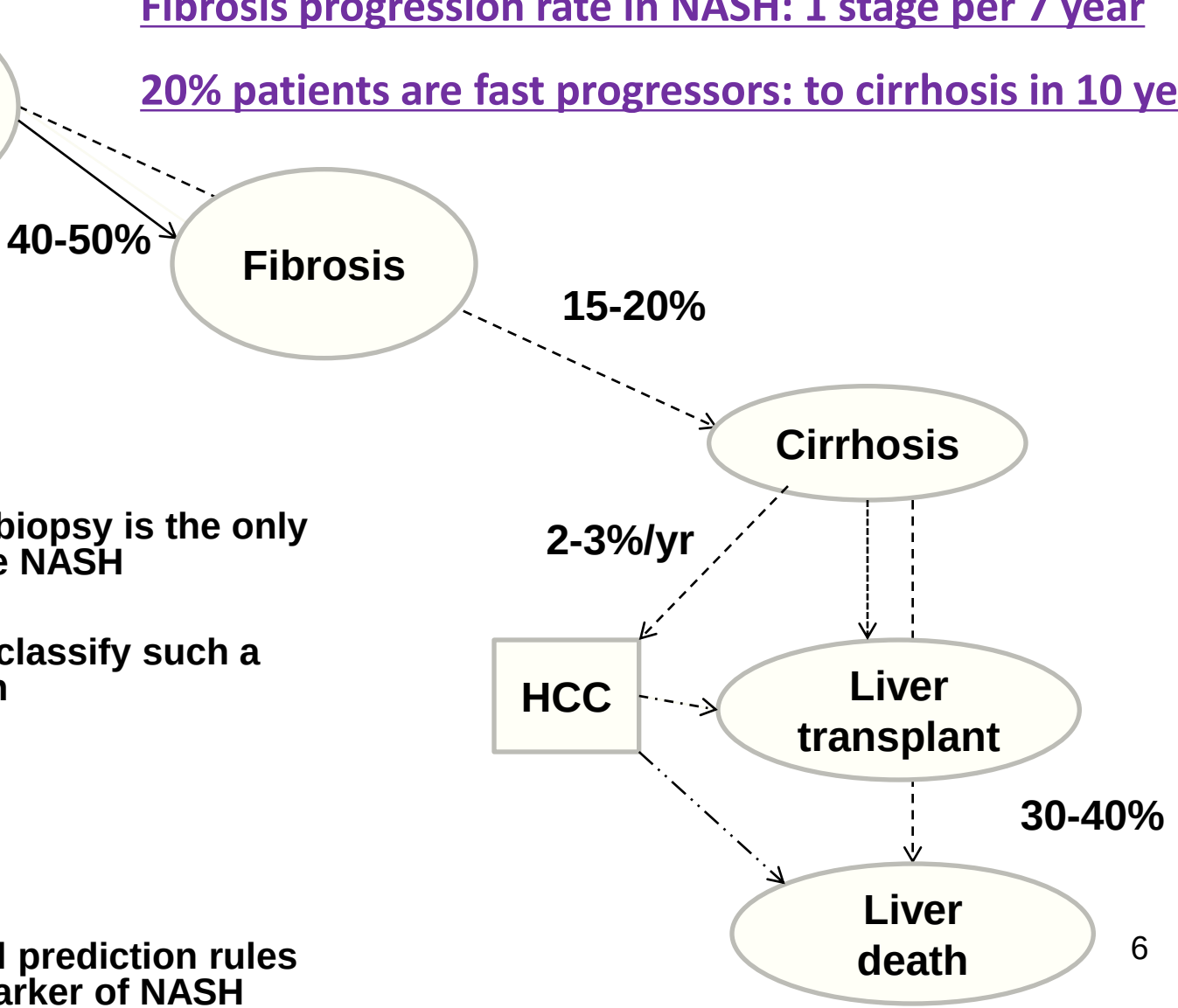
Clinicaltrials.gov

Natural history of NASH

18 million Americans

Fibrosis progression rate in NASH: 1 stage per 7 year

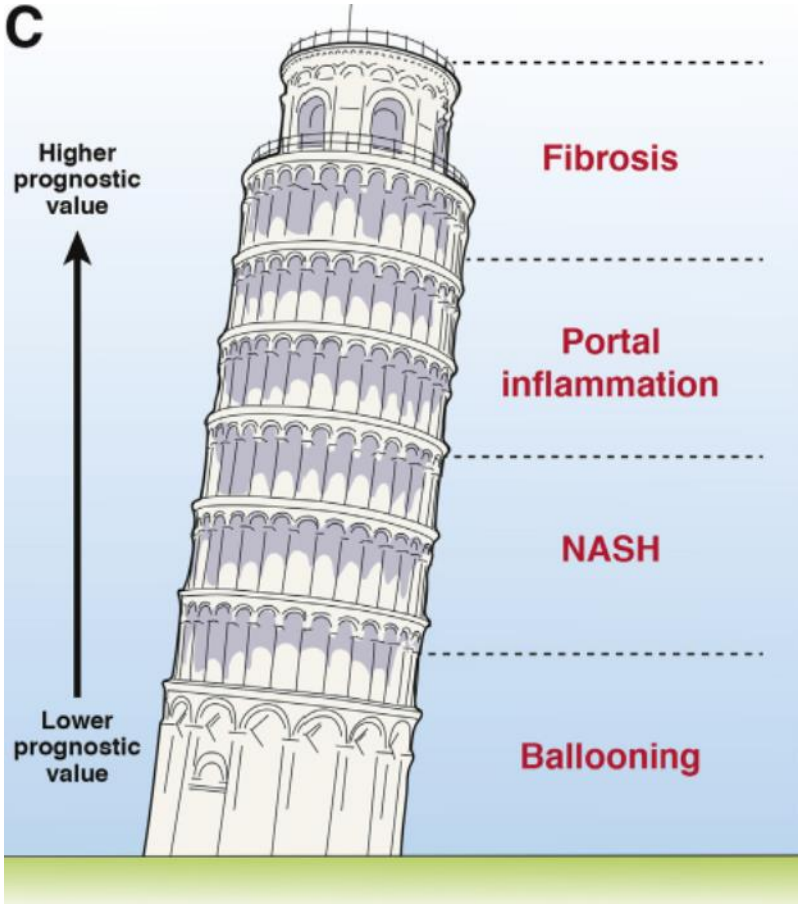
20% patients are fast progressors: to cirrhosis in 10 years



- **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**

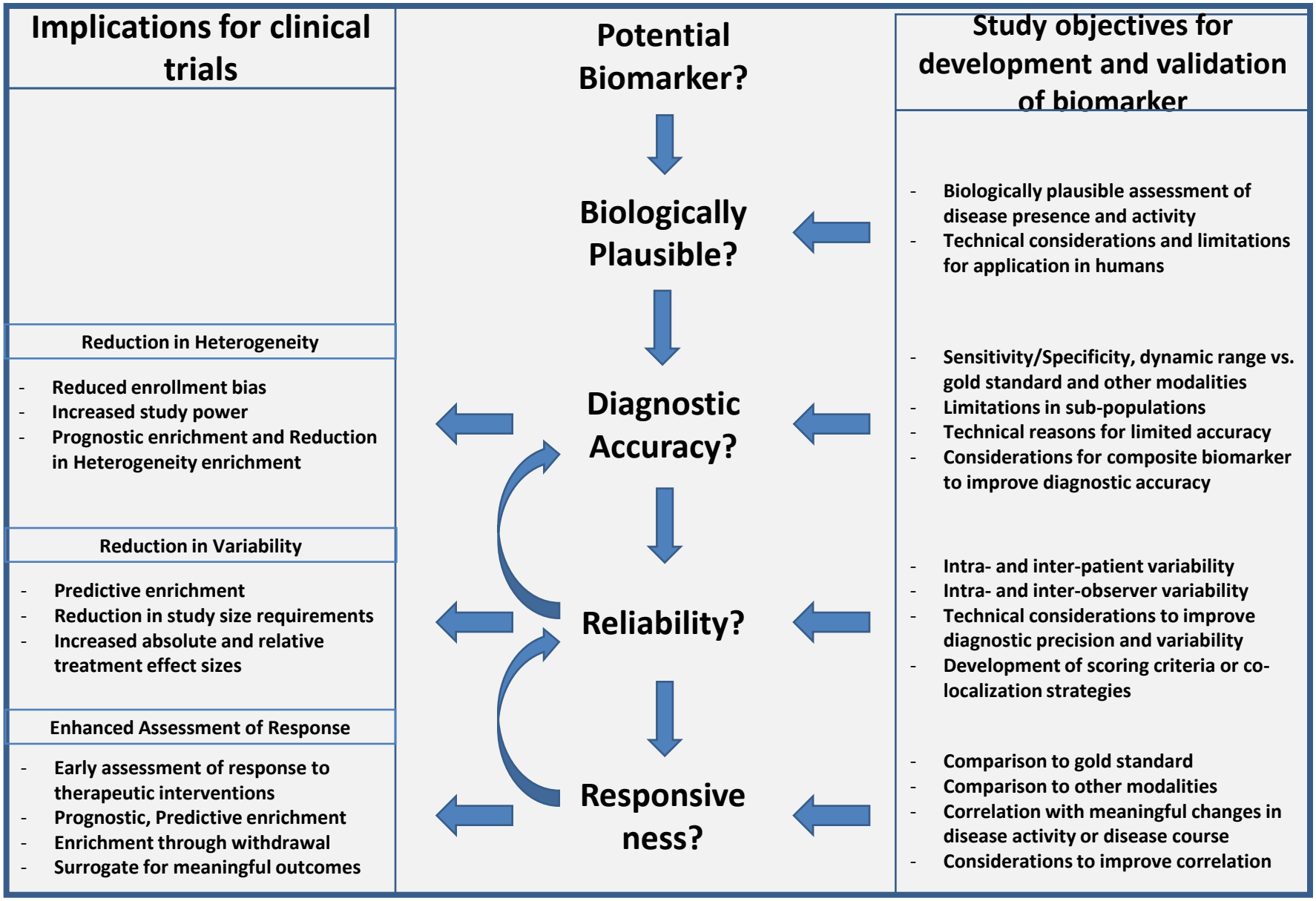
Key histologic predictors of mortality in NAFLD

- **Presence of advanced fibrosis**
- **Presence of fibrosis**
- **Presence of NASH**



Loomba et al. Gastroenterology 2015

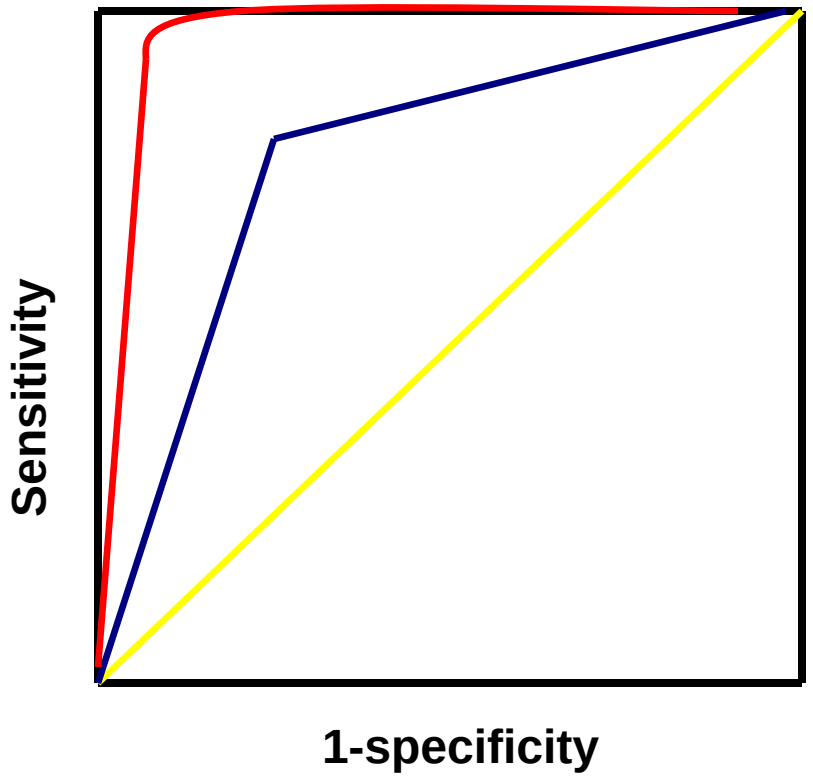
Building a biomarker program in NASH



AUROC

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

	NASH Yes	NASH No	
Test +	a	b	a + b
Test -	c	d	c + d
	a + c	b + d	

NPV = d / c + d
PPV = a / a + b

As prevalence of disease increases PPV increases

Performance of CPR

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

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

Outcome	Study	AUROC	AUROC
NASH	Feldstein et al. CK-18	**0.83**	**0.82**
	Feldstein et al. CK-18, sFasL	**0.93**	**0.79**
	Feldstein et al. oXNASH (13-HODE/LA, age, BMI, AST)	**0.83**	**N/A**
	Younossi et al. NASH Diagnostics	**0.98**	**0.72**
	Poynard et al. Nash Test	**0.79**	**0.78**
	Palekar et al. HA + Clinical model	**0.76**	**N/A**
	Loomba et al. Lipidomic	**1.00**	**N/A**
Advanced fibrosis (stage 3 or 4)	Guha et al. ELF	**0.9**	**0.82 (0.85*)**
	Corgenix Inc. HA (NASH-CRN*)	**0.83**	**0.82**
	Hepa score (NASH-CRN*)	**0.80**	**N/A**
	PIIINP	**0.69**	
	TIMP-1	**0.70**	

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/MulitScan
- Mechanism-based

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

Clinical trial – Phase 2/3 studies →

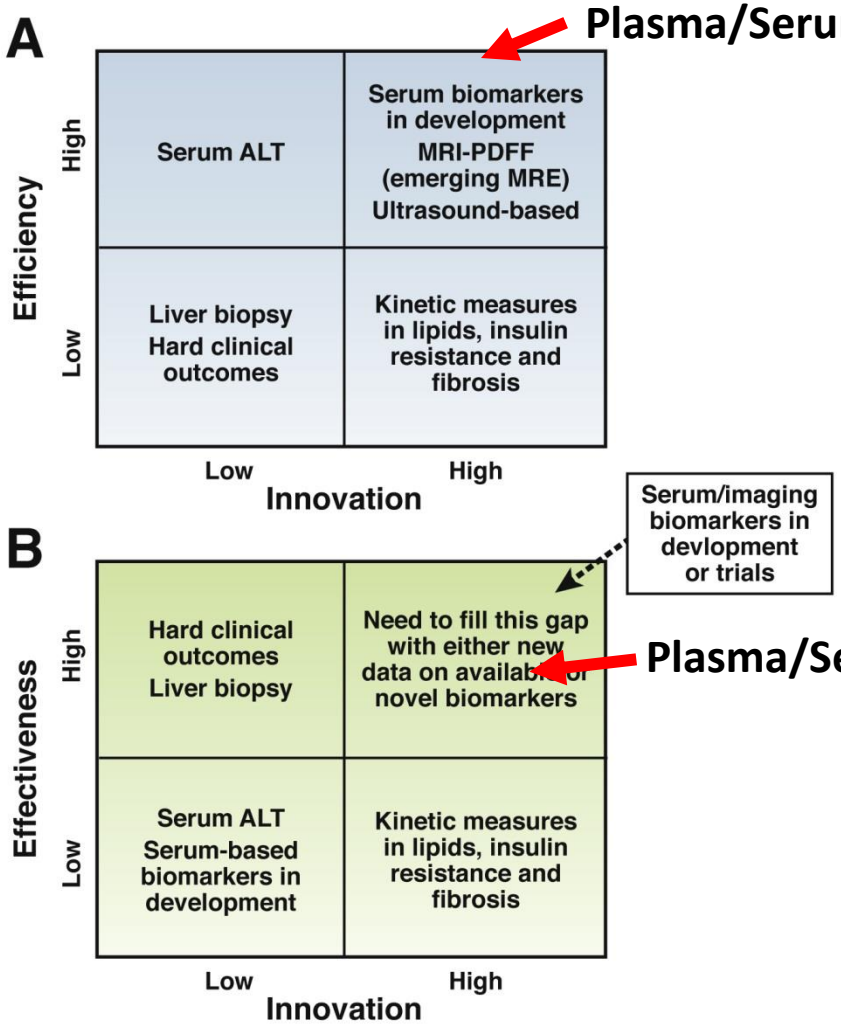
Primary end point
- Liver histology
  - NAS
  - Resolution
- HVPG
- Clinical

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



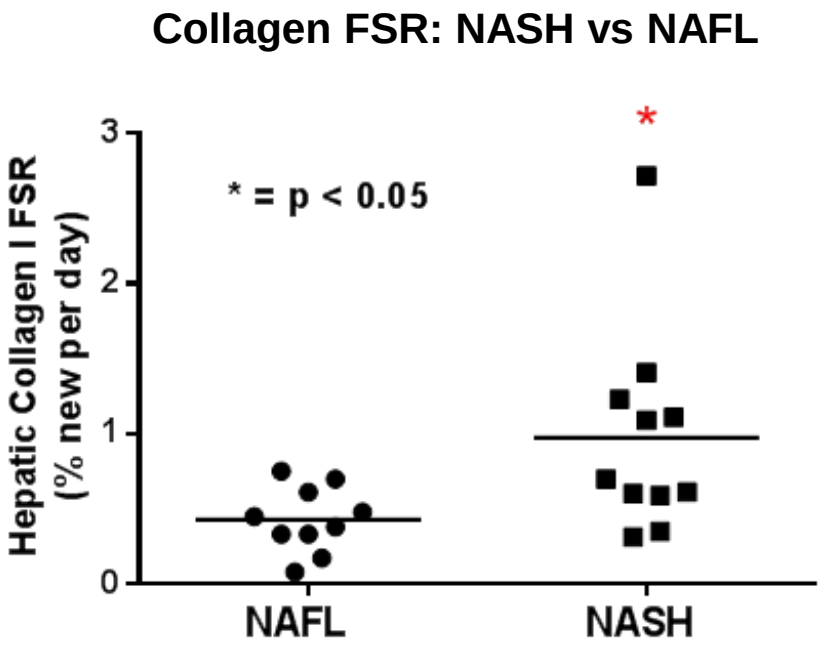
Caveats: Efficiency of phase 1 and 2, and effectiveness of phase 3 and 4

Loomba. CGH 2014

Hepatic Fibrogenesis Measured from Liver Biopsies

Collagen Synthesis Rate is Higher in Patients with NASH

- Hepatic collagen FSR was significantly higher in patients with NASH vs NAFL (with high variability in the NASH patient group)



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**

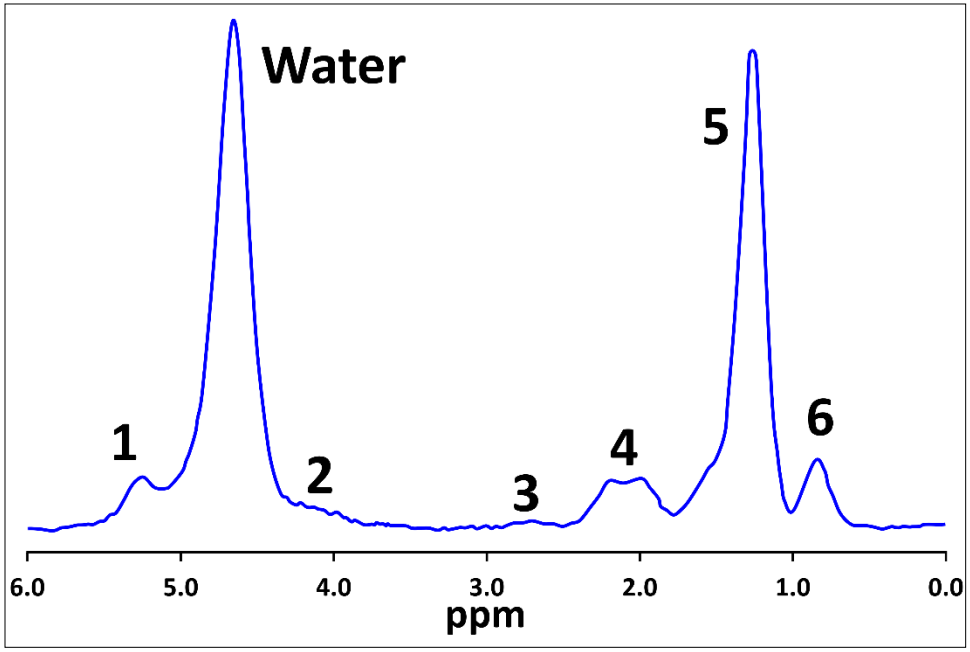
Goals of predicting treatment response in NASH

- **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]



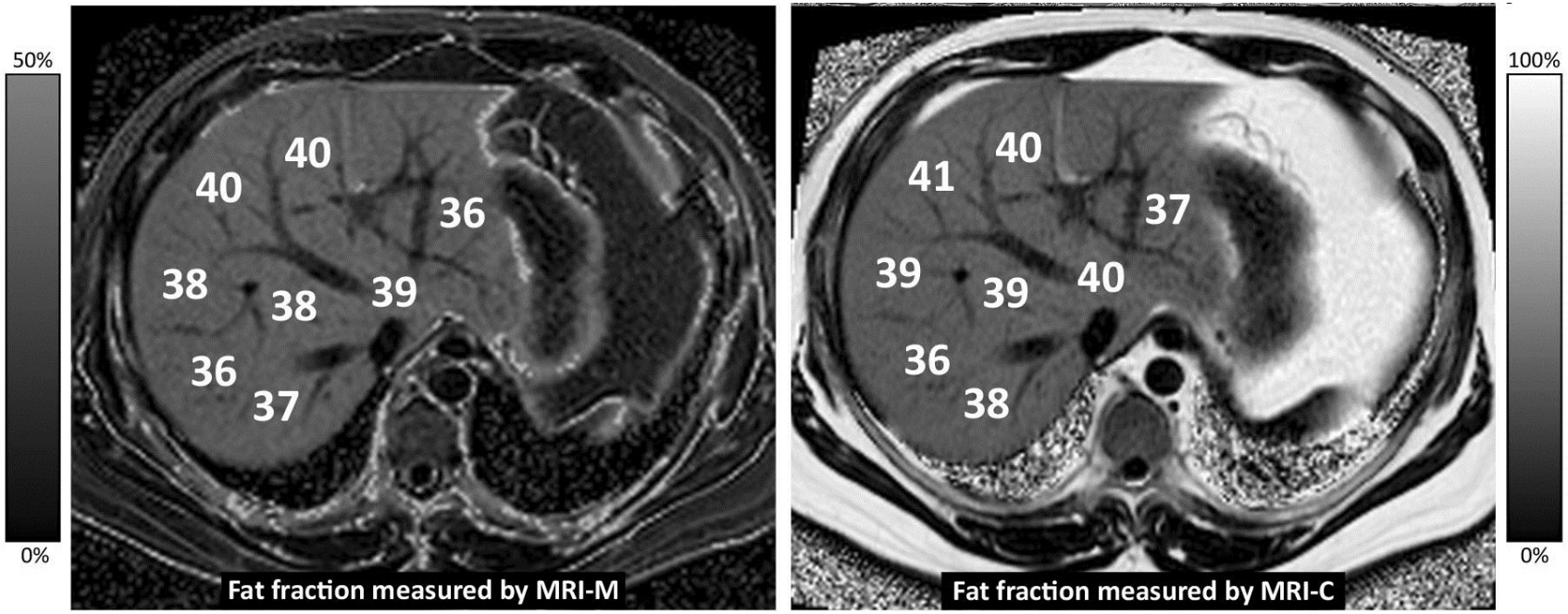
Imaging method to estimate PDFF would have advantages….

<u>Limitations of MRS</u>

- One 8cm^3 voxel
- Not available on routine scanners
- Requires expertise

Thomsen MRI 1994
Hamilton JMRI 2009
Hamilton NMR Biomed. 2011
Reeder JMRI 2011

MR 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

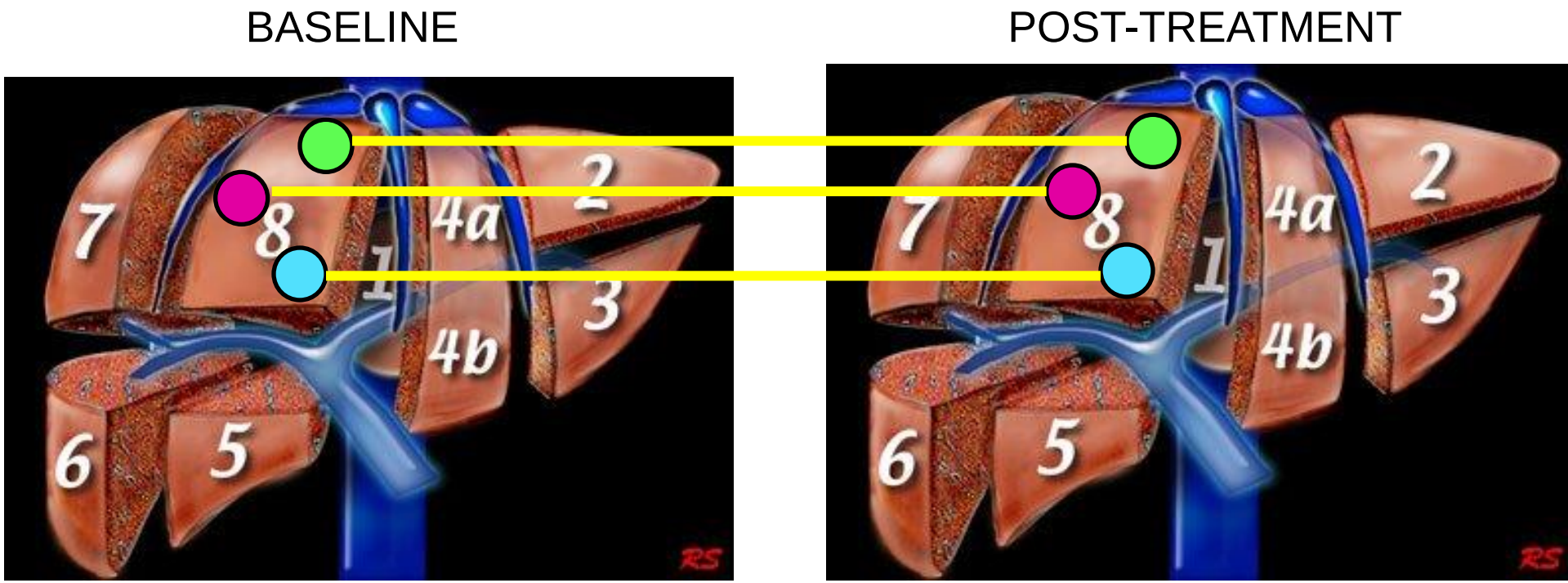
- Scanner field strength, manufacturer
- Patient factors: age, sex, BMI, etiology of liver disease
- Concomitant liver abnormalities: iron overload, necroinflammation

MRI-PDFF robust to parameter changes

Acquisition 12-25 seconds

Yu MRM 2008
Bydder MRI 2008
Bydder MRI 2010
Hansen MRI 2012
Kang Invest Radiol 2012
Kuhn Radiology 2012
Tang Radiology 2013

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

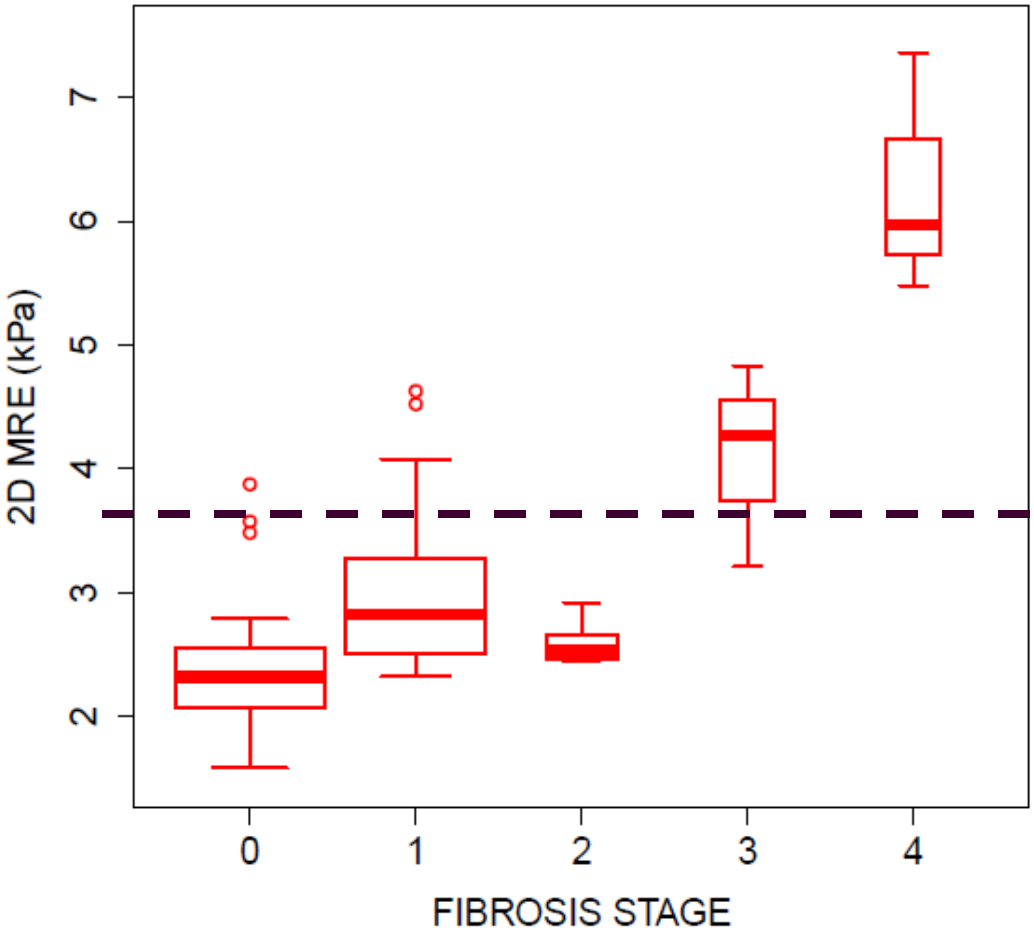
Le et al. Hepatology 2012

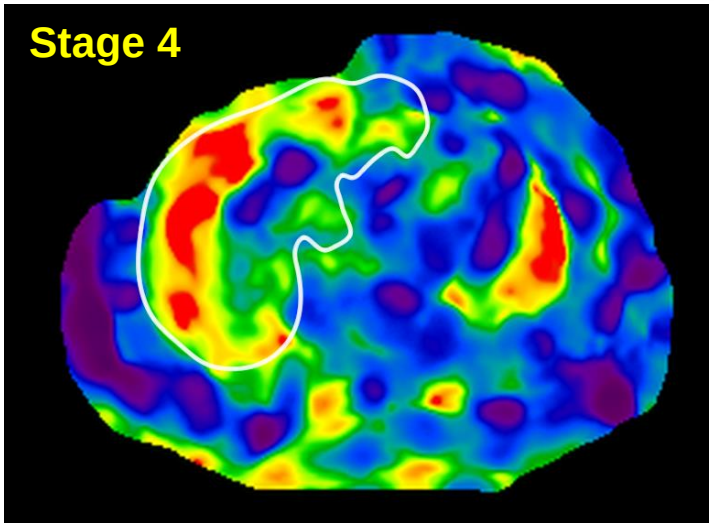
Imaging biomarkers of fibrosis: Overview

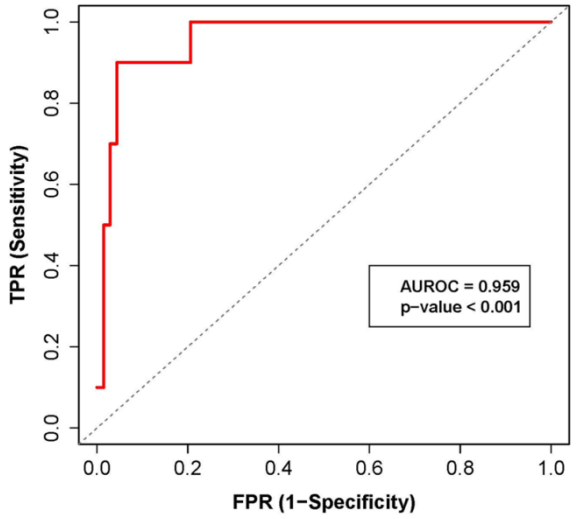
- Fibrosis has ***no molecular signature*** that can be detected by current imaging techniques
- All imaging tests for fibrosis attempt to detect fibrosis ***indirectly***.
- Many imaging biomarkers proposed: stiffness, diffusion, perfusion, metabolites, image texture,…
- Leading biomarker is liver “stiffness” (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 descriminates advanced fibrosis







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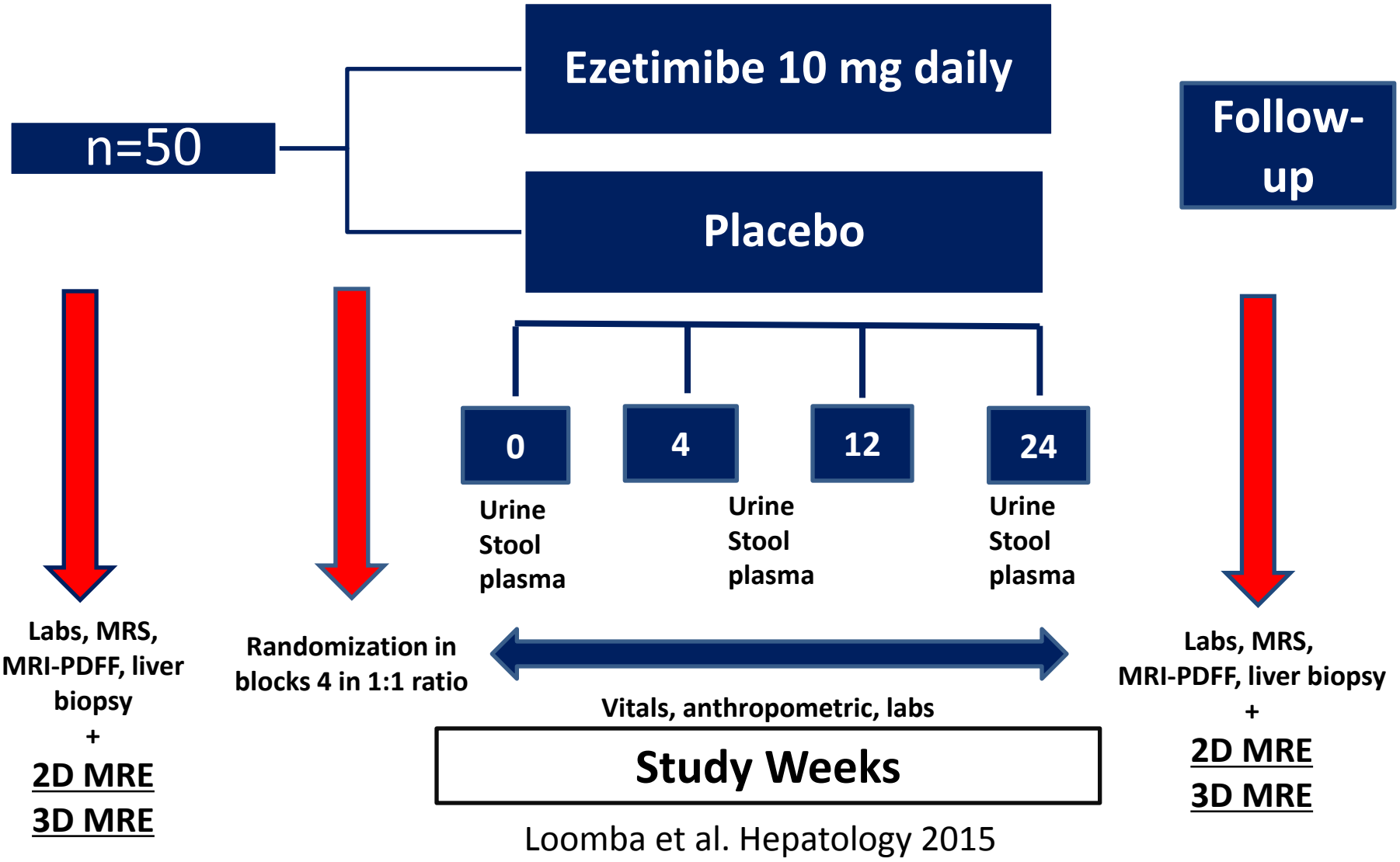
MOZART Trial Design: Ezetimibe vs Placebo

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

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



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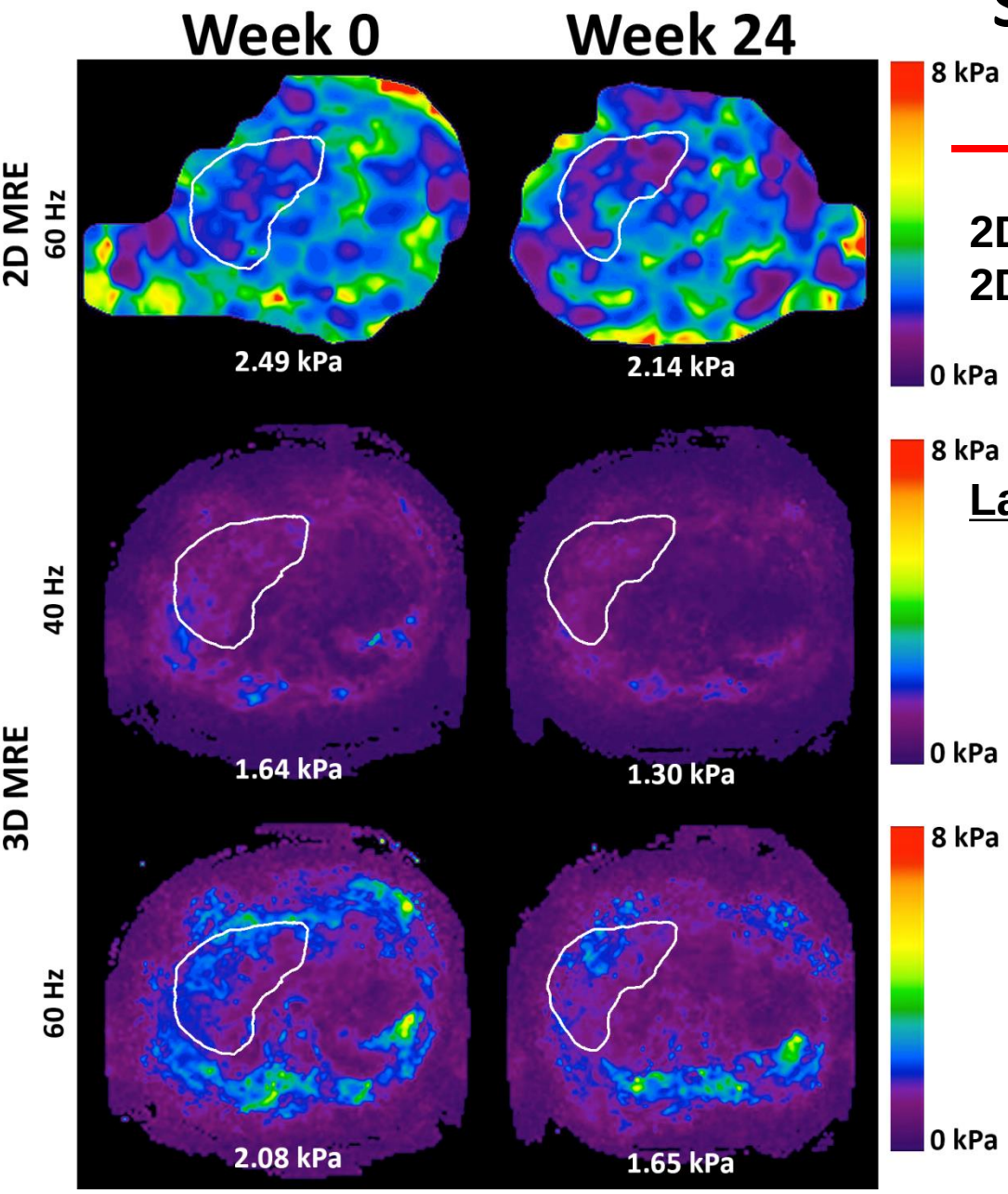


Stiffness-mapping before and after treatment

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

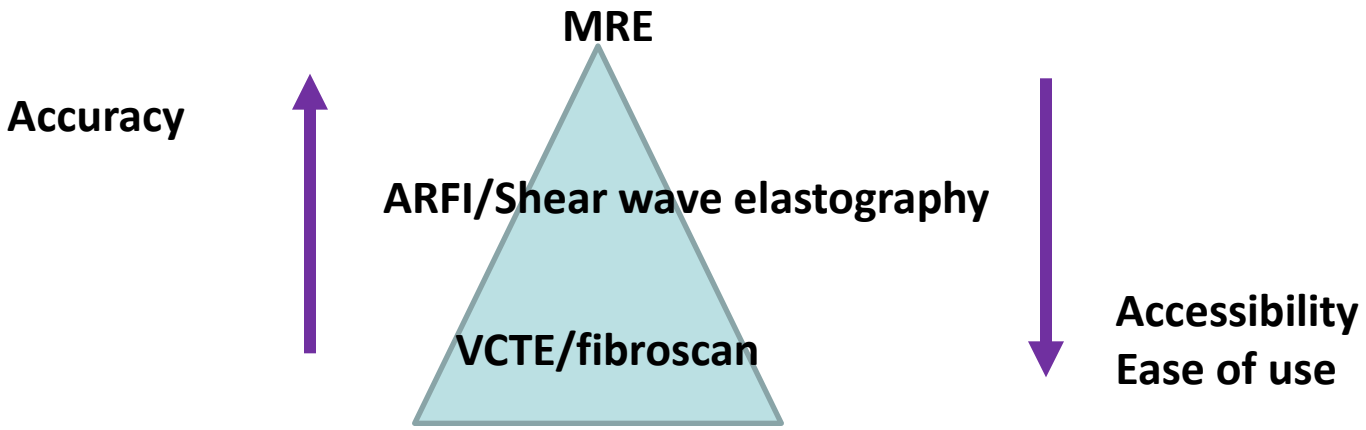
↓

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

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

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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