# Defining the gold standard in biomarker validation for NASH

Arun J Sanyal M.D.

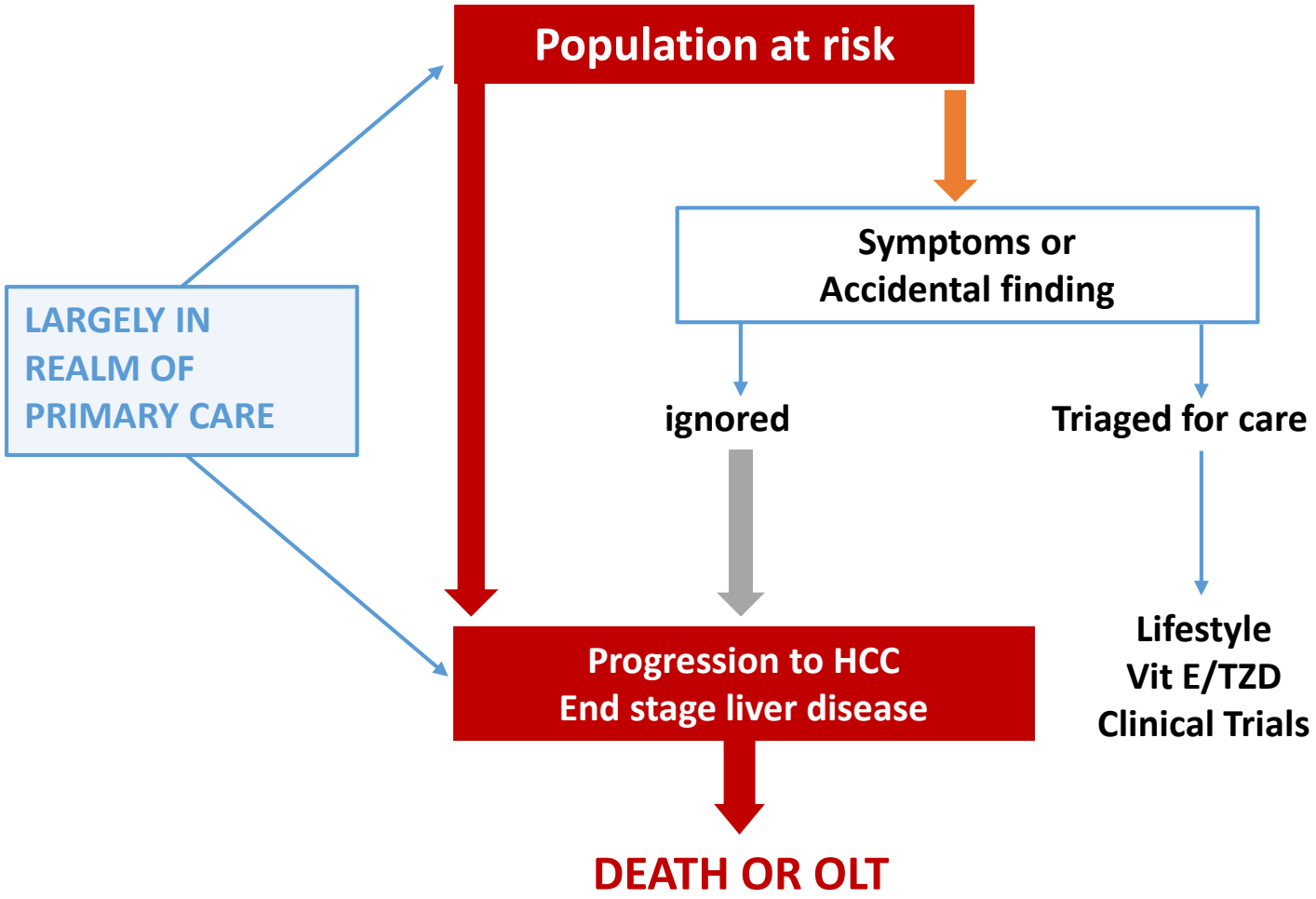
Professor of Medicine, Physiology and Molecular Pathology

Virginia Commonwealth University School of Medicine

# Conflicts of interest

- **Salaried employee: of VCU**
- Member of Board: McGuire VA Research Institute, unpaid member
- Ad hoc consultant:
  - Salix: < $ 5K
  - Ikaria: < $ 5K
  - Abbott: $5-10K
  - Bristol-Myers: < $ 5K
  - Genfit: $ 5-10 K
  - Genentech: < $ 5K
  - Bristol Myers: < $ 5K
  - Echosens: unpaid consultant
  - Gilead: unpaid consultant
  - Novartis: unpaid consultant
  - Takeda: unpaid consultant
- Royalties:
  - Elsevier- Boyers Textbook of Hepatology: < $ 5K
  - Uptodate: < $ 5K
- Grants (awarded to university):
  - NIH: $ 1.5 million annual direct costs
  - Roche, Gilead, Astellas: $ 25-50K total
  - Salix, Ikaria: $ 50-100000 each
  - Gilead: $ 150 K annual direct costs based on recruitment
  - Genfit: US PI for GFT505 trial
  - Galectin: $ 30000

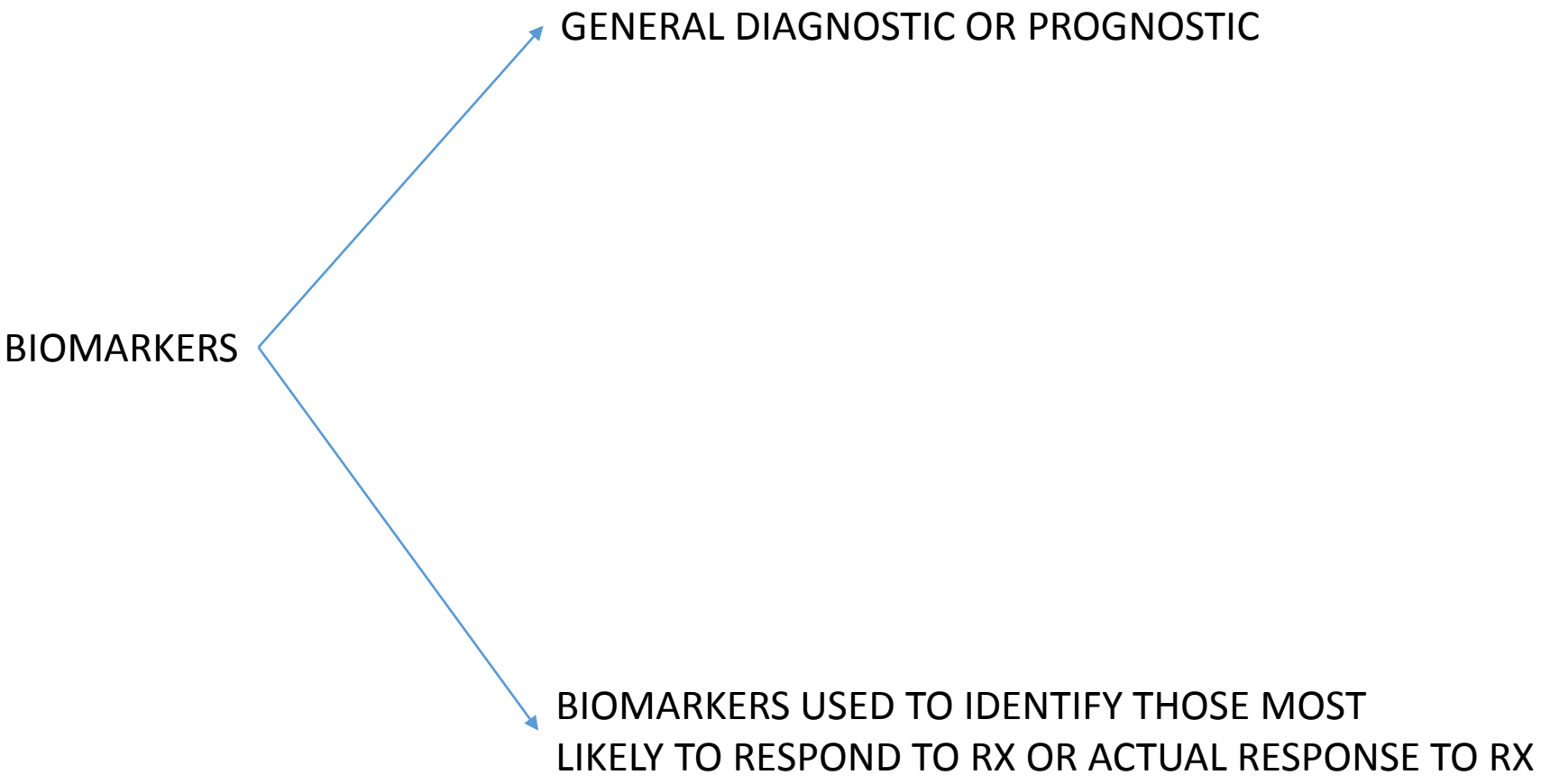
# The clinical perspective



# Barriers to better NASH-related care

- Awareness
- Lack of point of care diagnostics
- Lack of easy ways to risk stratify and triage subjects
- Lack of approved therapies
- Need for a liver biopsy to determine whether treatments are effective

# Types of biomarkers based on what they reflect



# What will make clinicians use a biomarker panel

- Validated to outcomes
- Biological plausibility
- Ease of use:
  - point of care trumps need to make a separate appointment
  - balance between ease of use versus accuracy
- Ability to guide actionable next step in clinical care
- Ability to reduce unnecessary testing, exposure to potentially harmful drugs
- Contribution of improving overall cost of clinical care

# Biomarker qualification

- Define the clinical question and use
- Identify the population to be studied
- Validation:
  - biological plausibility
  - validity
  - scale of measure
  - sensitivity to change
  - methodological quality controls
- Ability to predict the outcome of interest in rigorously designed trials

# Types of clinical questions relevant for NASH

| Clinical question | Target population | Provider population |
|---|---|---|
| Is NAFLD present | General population | Primary care providers |
| Will it lead to outcomes | Those where NAFLD present | Primary care providers<br>Diabetologists<br>Cardiovascular clinics |
| Is disease progressing, stable or regressing | Those with NAFLD | Primary care, Diabetologists, GI-Hep, Cardiovascular clinics |
| Is drug therapy warranted | Those with NAFLD and at risk for outcomes | Primary care, Diabetologists, GI-Hep, Cardiovascular clinics |
| What drug to choose | NASH with some fibrosis | Hepatology, primary care, diabetologists |
| How is the subject responding | NASH and fibrosis | Hepatologists, GI, Primary care, diabetes |

# Is NAFLD present?

- Relatively the most simple question
- Need to demonstrate:
  - Excess fat in the liver
  - inability to attribute the fat to alcohol consumption
- remember:
  - The spectrum of the steatosis-steatohepatitis syndrome ranges from pure alcoholic to nonalcoholic with the majority of subjects in the middle.

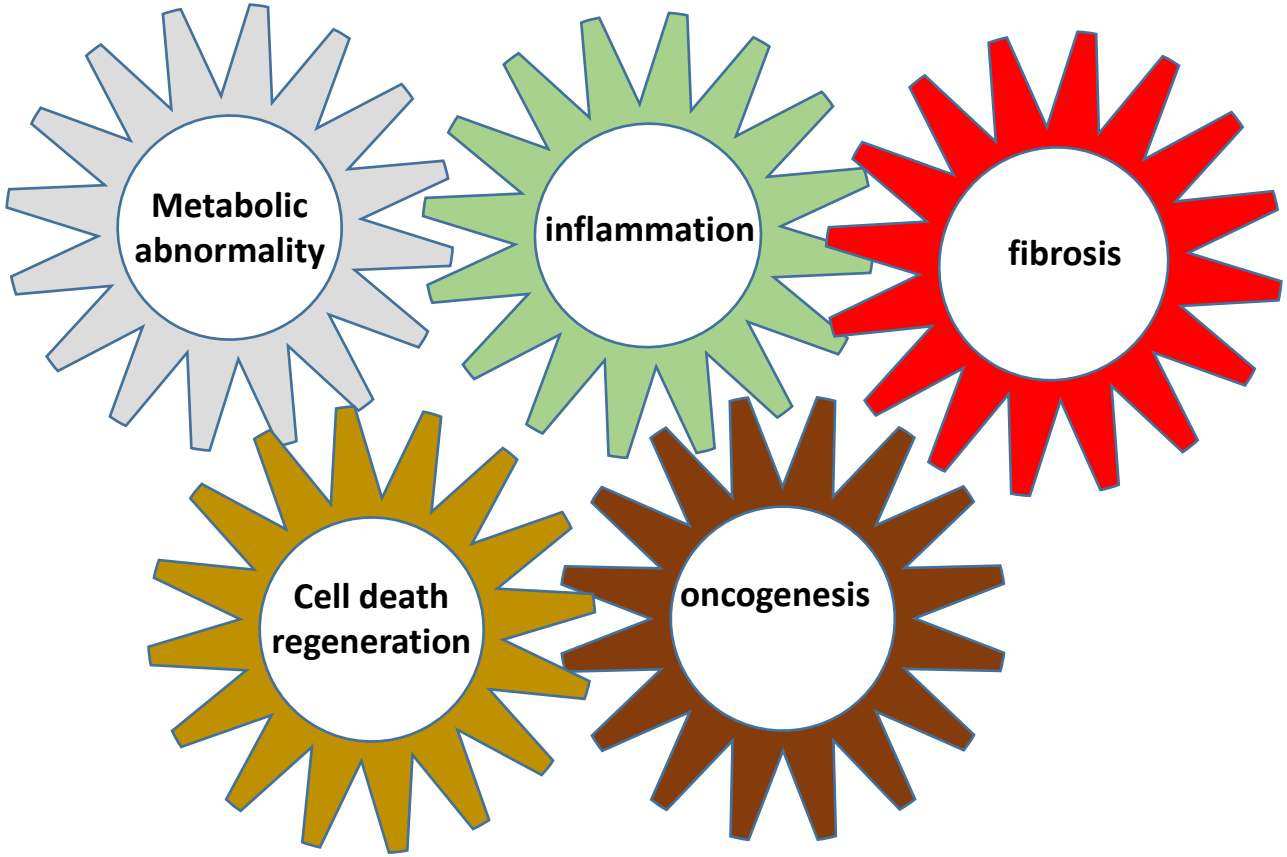
# Does the patient have fatty liver disease that will kill?

- Define the population to be studied:
  - subjects with risk factors
  - subjects with NAFLD
  - subjects with NAFLD and some fibrosis
  - subjects with NAFLD and cirrhosis

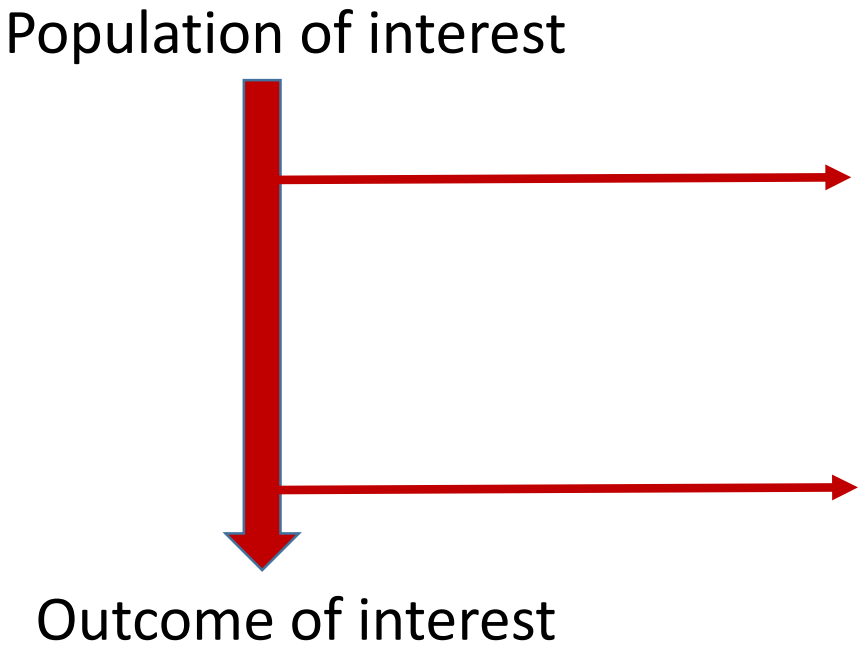
# Biological plausibility- the biomarker should measure something that is linked to progression to death

steatosis → steatohepatitis → cirrhosis

steatohepatitis → HCC



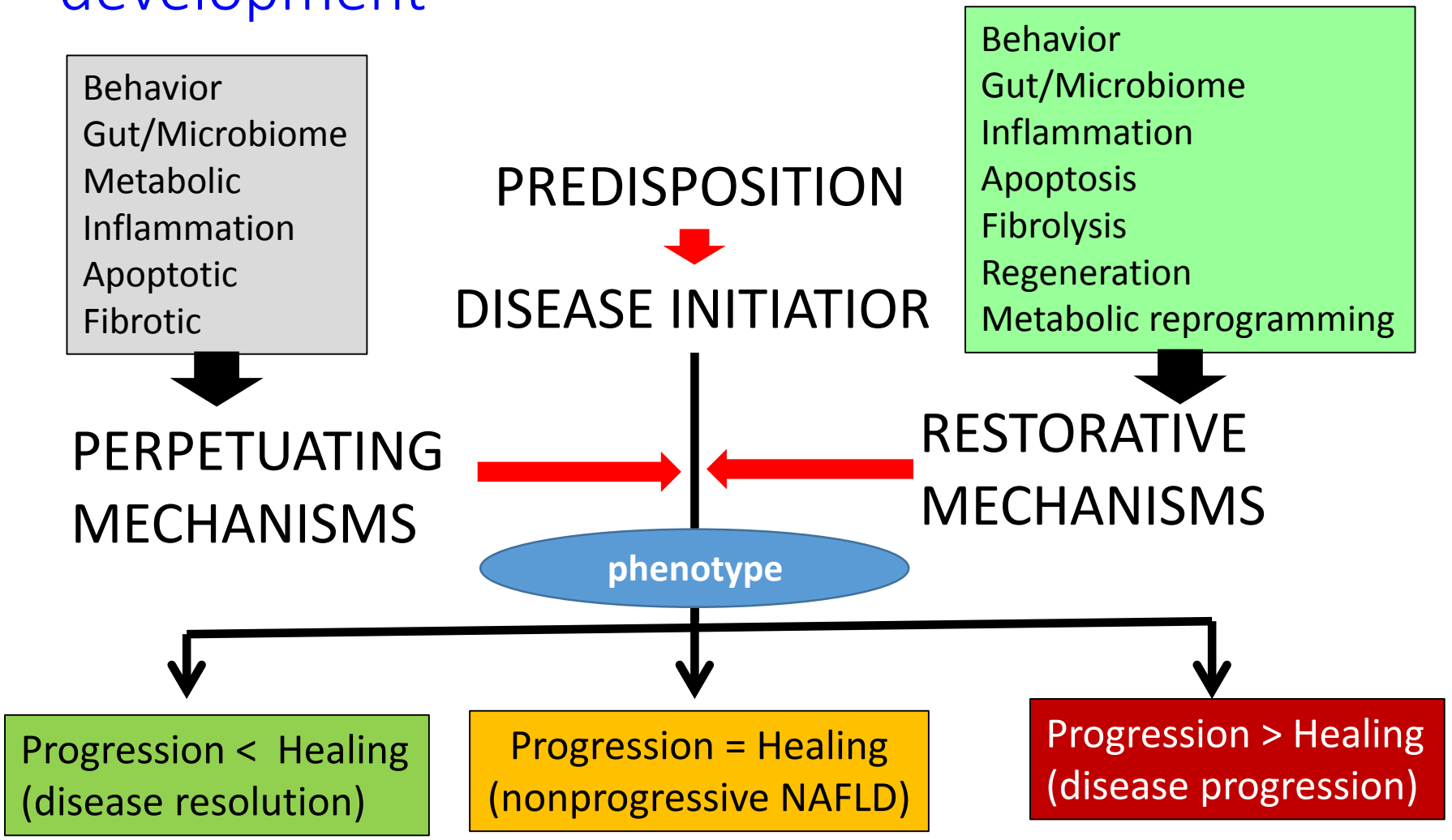
# Study Design




- Measure biomarker
- Measure other known confounders
- Define if intervention is planned

- How is outcome measured?
- When is it measured?
- Who it is measured in

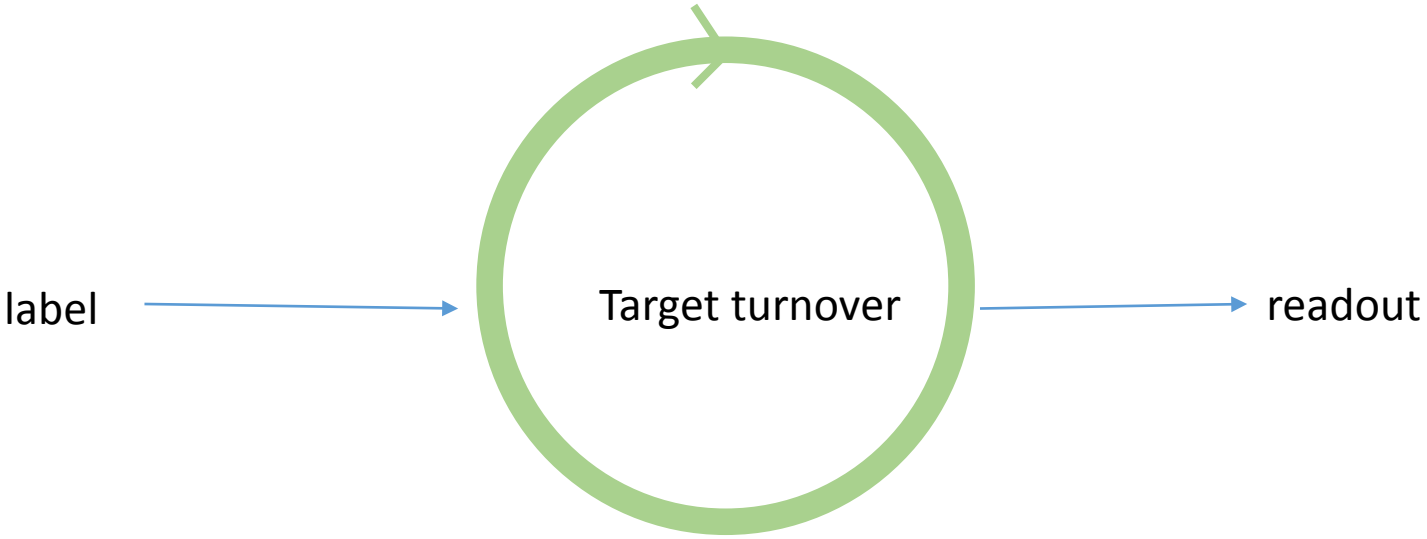
# NASH pathogenesis: relevance to biomarker development



# Methodological aspects

- What is measured?
- Type of sample:  plasma, serum, whole blood
- Method of collection:  time to spin, freeze down etc and documentation of quality control
- Sample storage
- Sample transport
- Sample- chain of custody
- Sample sequence on analyzer
- Analyzer quality controls- platform effects

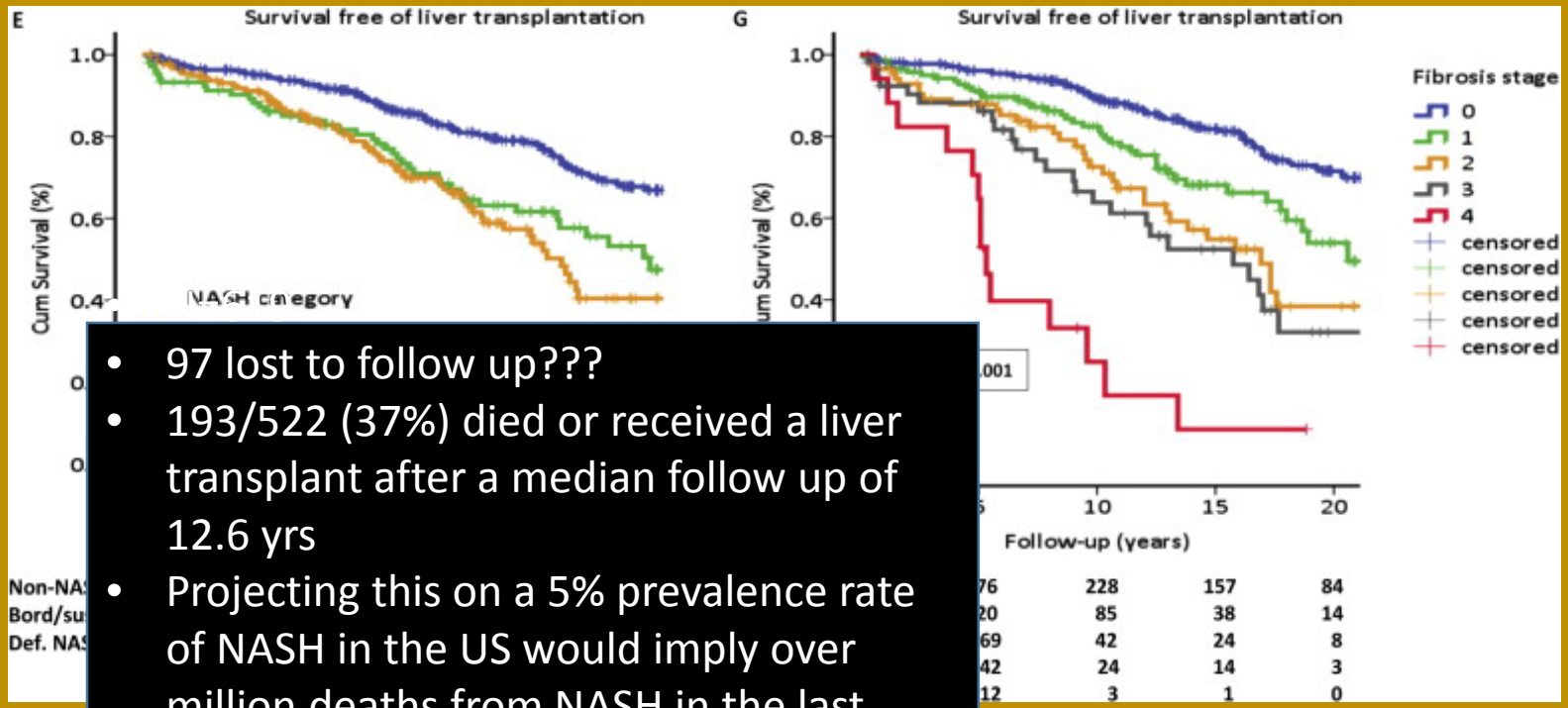
# Pitfalls: methodological



# Plan of analysis

- Relate biomarker to endpoint:
  - Categorical vs continuous scales for both biomarker and endpoint
- Account for confounders
- Sensitivity analysis to address generalizability to gender, race, subpopulations
- Multivariable analysis to demonstrate independent ability to predict outcome of interest
- Consider alternate strategies- consider probability of developing outcome at varying levels of biomarkers corrected for confounders, algorithm building strategies

# Pitfalls: face validity

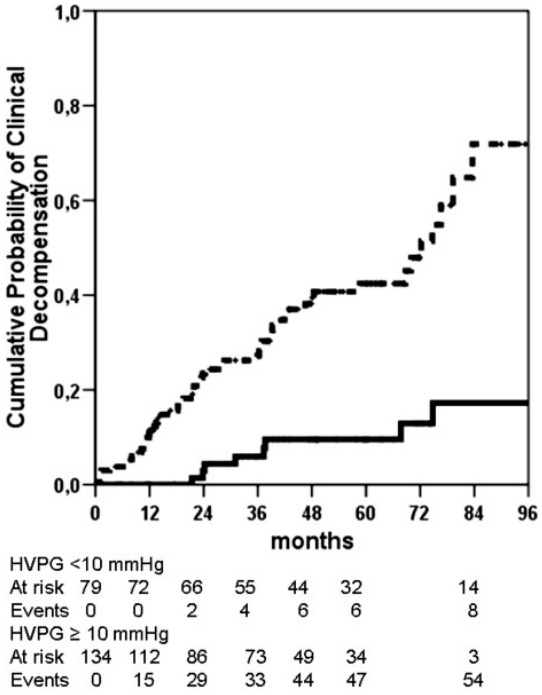



- 97 lost to follow up???
- 193/522 (37%) died or received a liver transplant after a median follow up of 12.6 yrs
- Projecting this on a 5% prevalence rate of NASH in the US would imply over million deaths from NASH in the last decade!!!!
- 74/522 (14%) died of heart disease

Angulo et al, Gastroenterology, Gastroenterology. 2015 Aug;149(2):389-397

# Pitfalls: another example

**A**



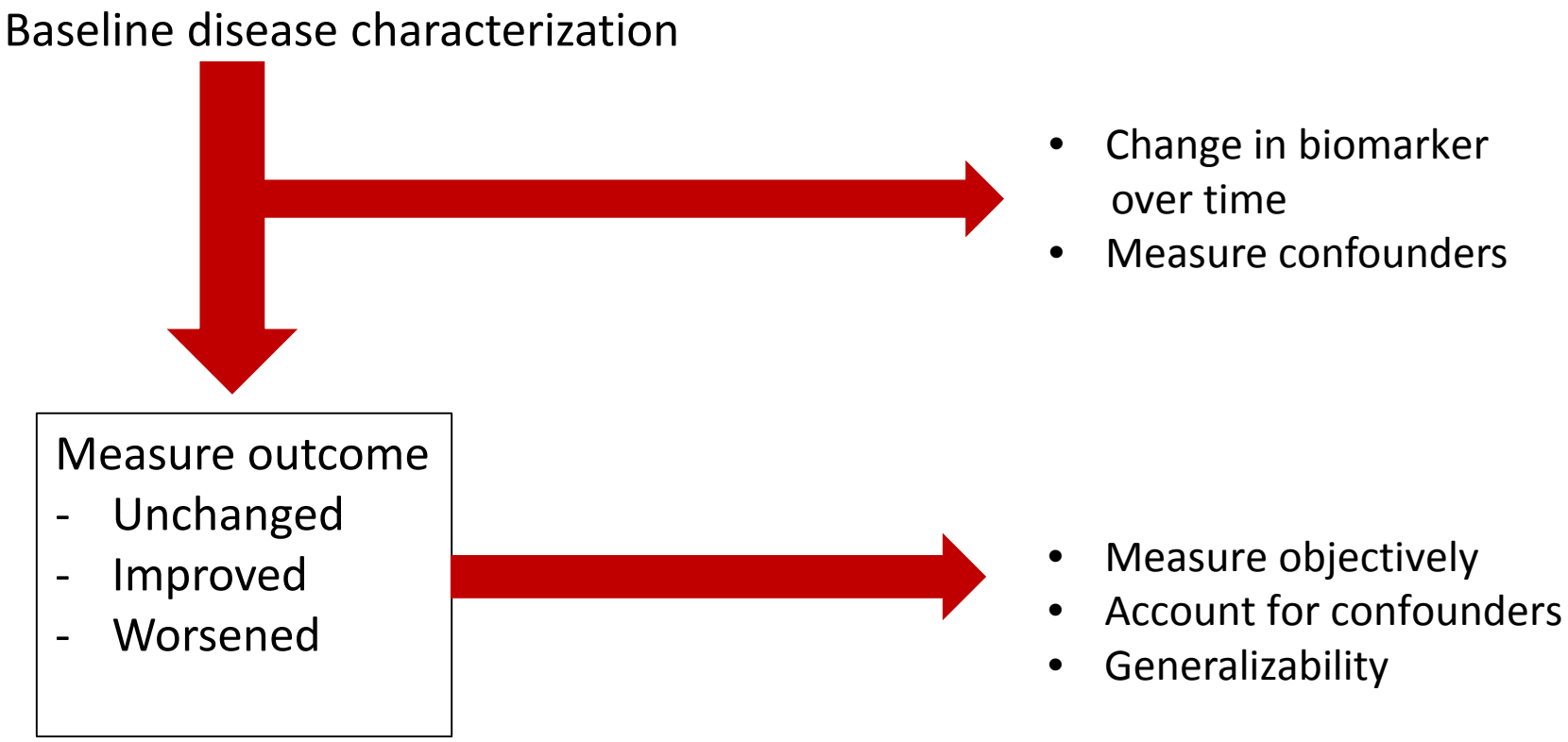

- *HVPG > 10 mm Hg is associated with increased risk of decompensation.*
- *However-TIPS decreases HVPG but may not improve outcomes so mechanism of HVPG decrease has to be considered*
- *Beta blockers lower HVPG but do not improve mortality*
- *Much of the data on HVPG are in alcoholic cirrhosis*
- *Is the drop in HVPG needed to improve outcomes related to etiology and mechanism of intervention*

*Ripoll et al, GASTROENTEROLOGY 2007;133:481–488*

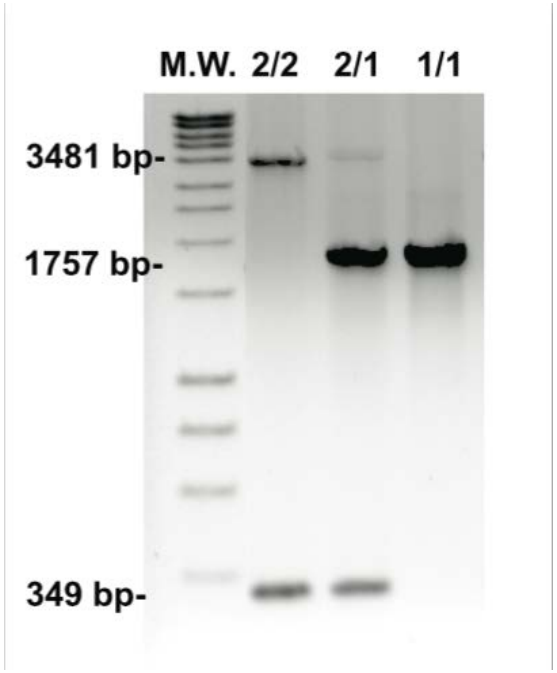
# Special considerations

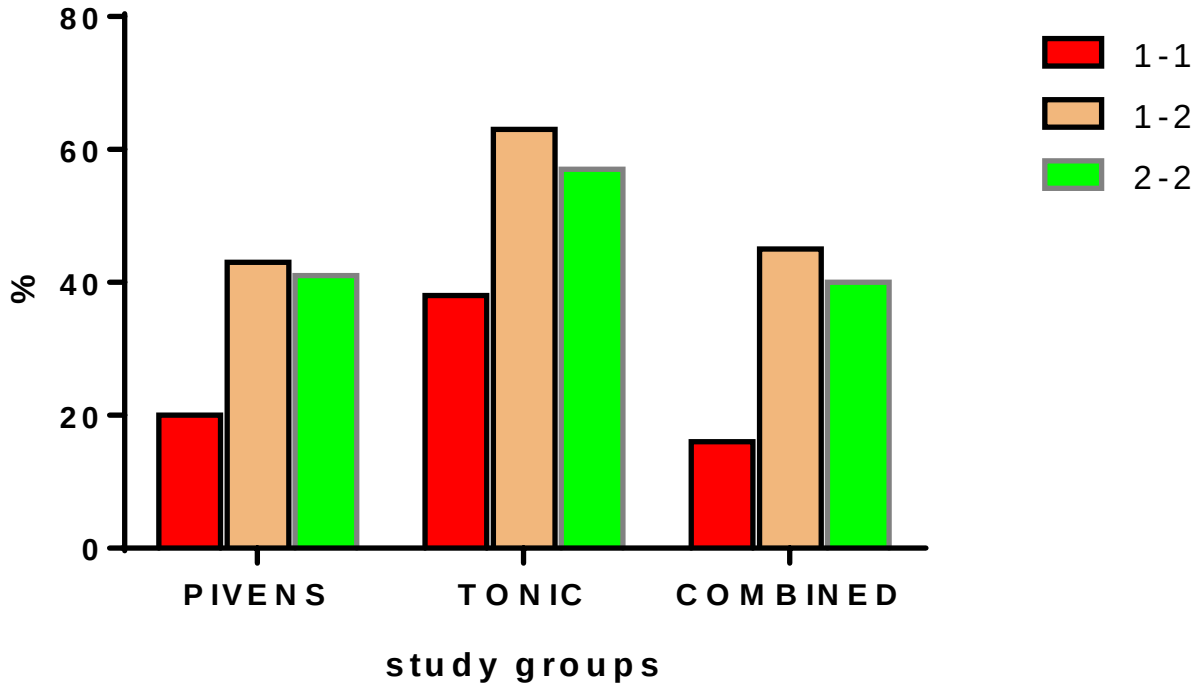
- Sensitivity to change:
  - From one risk stratum to another
  - Needs to be measurable and objective
  - Could be continuous, categorical or ordinal
  - Need to demonstrate that change in biomarker from one category to another or along a continuous scale relates to change in risk stratum which is in turn related to outcomes.

# Assessment of disease progression



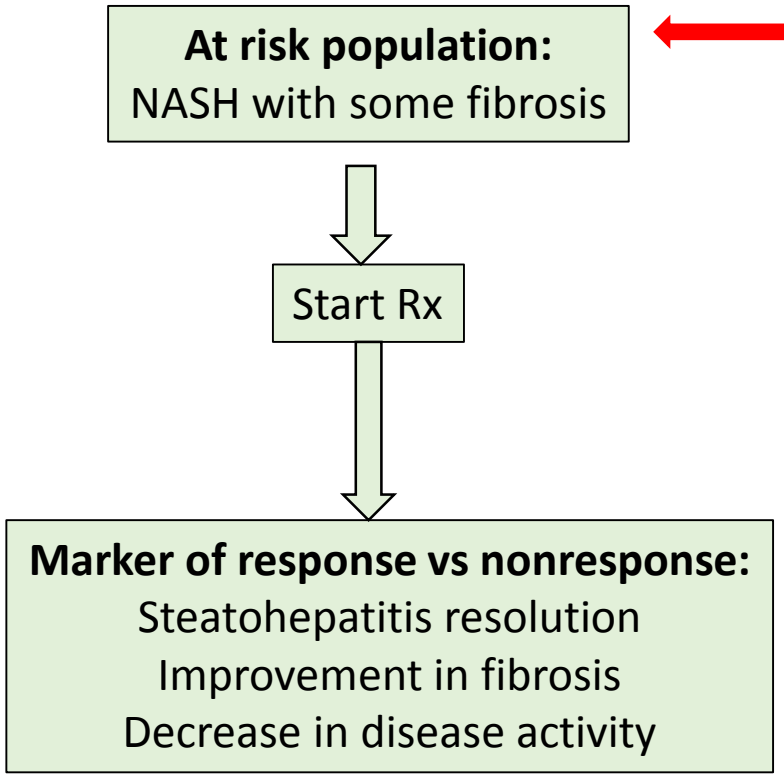
# Use of companion diagnostics to identify population to treat: Hp 2 allele was associated with likelihood of resolution of steatohepatitis




Combined Odds ratio for Hp2 vs Hp1: 3.75 (1.13,12.5) p< 0.03

# A critical role of companion diagnostics based endpoints

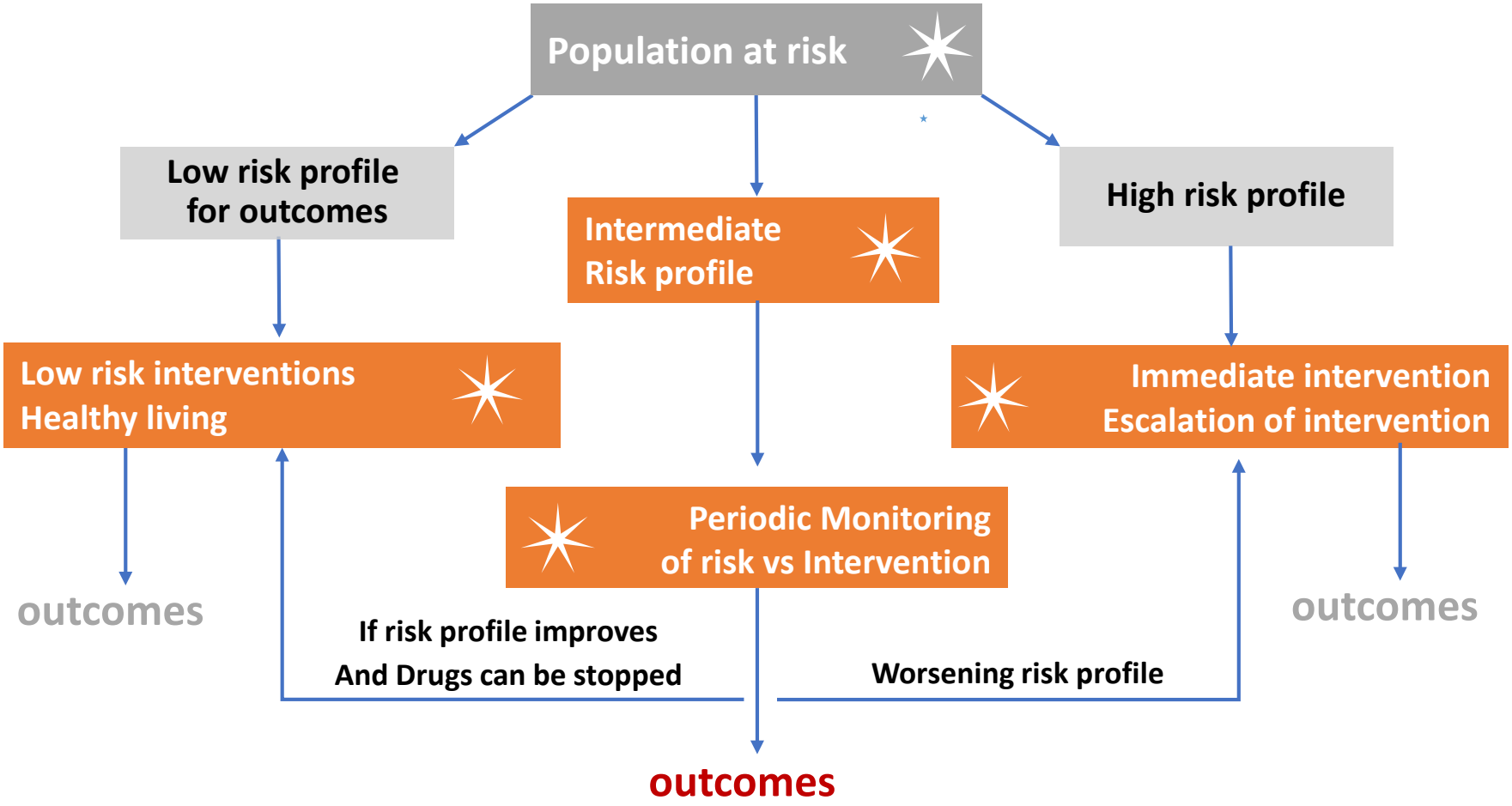



Need POC test to define

- Imaging-based studies
- Elastography-based methods
- Lipidomic/proteomic signatures
- Circulating miRNA
- Function based assays
- Cytokines
- Changes in APRI, FIB4, NAFLD fibrosis score

Validation in context of clinical trials

# How biomarkers may be used in the future



# Summary

- There is no single gold standard
- Biomarker validation must be considered in the context of the question posed and how it is to be used
- Key issues in the design, implementation and validation include:
  - population studied
  - objectivity of measures
  - assessment of confounders
  - methodological rigor
  - choice of endpoints against which biomarker is validated