# Emerging biomarkers of liver injury: from miR-122 to liquid biopsies

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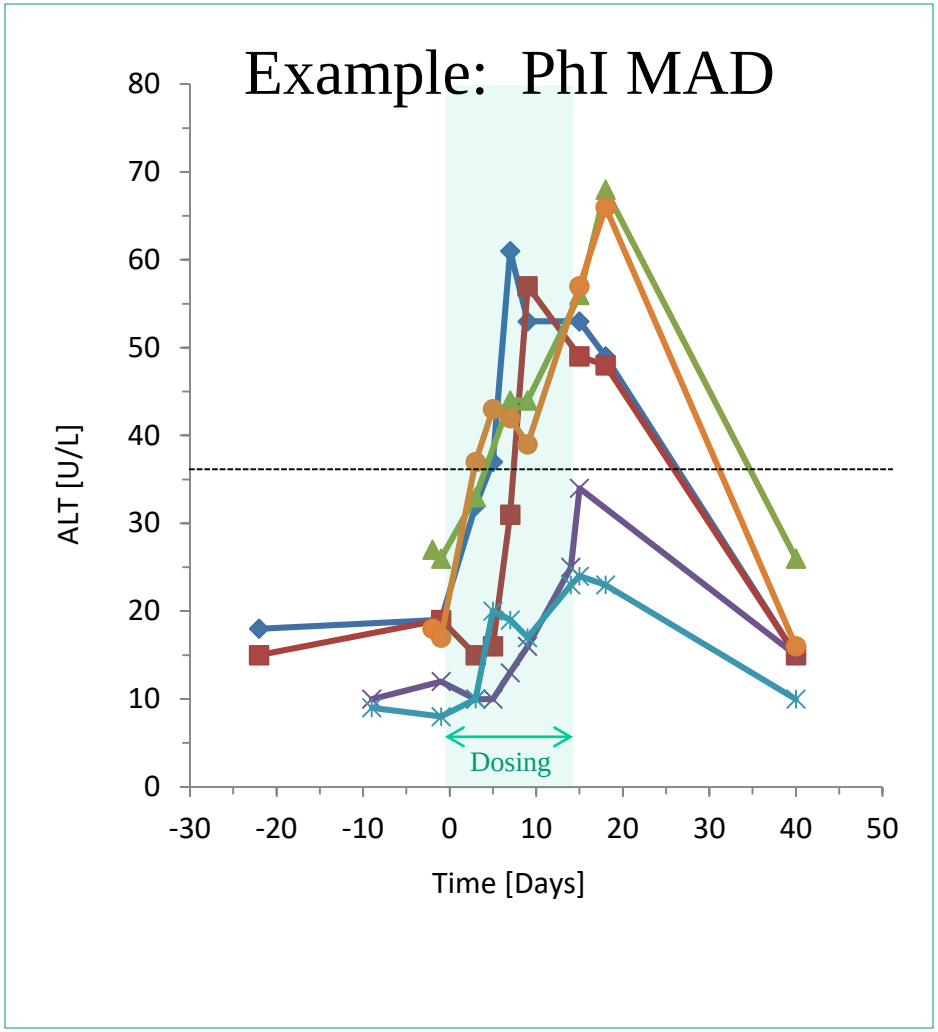
# Why do we need a new biomarker of liver injury in drug development?

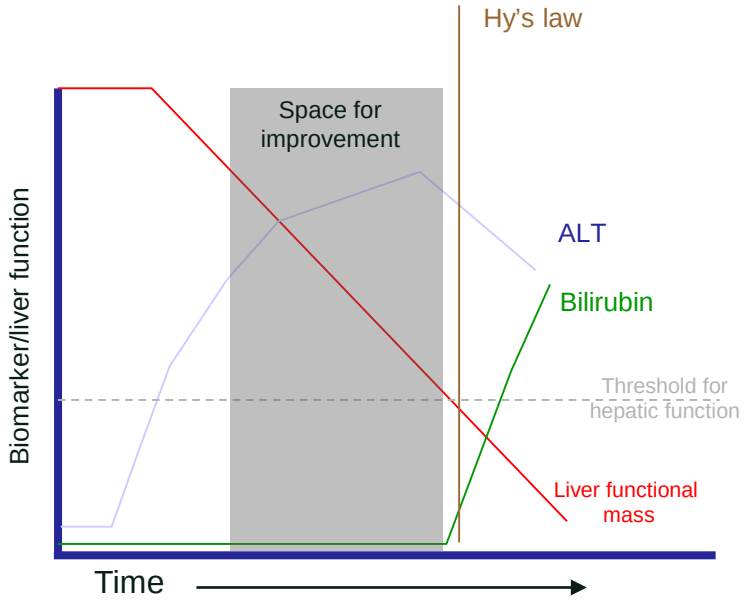



- Transient small ALT increases in clinical trials are relatively common
- Hepatic or extra-hepatic origin of ALT?
  - Underlying muscle disease eliminates ALT as biomarker of DILI
- Metabolism, life style
- Sensitive populations

# Challenges

## Current status

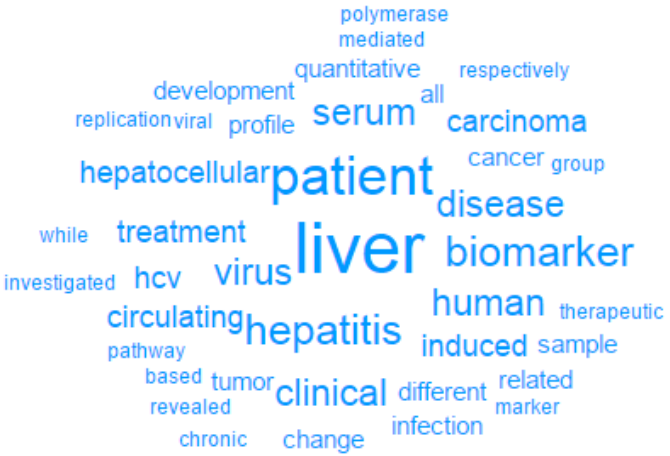
- Conventional biomarker-based DILI diagnostic paradigm detects liver injury only after substantial (sometimes irreversible) damage has occurred.
  - ALT is sensitive enough but not specific enough
  - Bilirubin is not sensitive enough but specific enough




## Gaps

- Sensitive and specific biomarkers that detect DILI before substantial or irreversible damage has occurred
- Biomarkers with better prognostic value (transient vs progressive increase/damage)
- Translational biomarkers (improve DILI risk assessment in preclinical species)
- Early identification of individuals susceptible to idiosyncratic DILI

# miR-122




Small non-coding RNAs that negatively regulate gene expression at the post-transcriptional stage

Serum miR-122 is liver-specific, not found in muscle

**Clinical Relevance:**

- Potentially more sensitive than ALT
- Elevated in patients with drug-induced liver injury
- Elevated in patients with disease-induced liver injury
- Correlates to histopathology severity score

**Current Clinical application:**

- Research/exploratory use only
- Requires broad clinical validation
- Clinical qualification by regulatory agencies needed for use in drug development

# Challenges in Clinical Translation of Emerging Safety Biomarkers

- Human studies mirroring preclinical toxicity studies generally cannot be conducted
  - Treatments with a wide variety of known toxicants is not possible
  - Regular histopathology (i.e., biopsy) of target organs would not be practical
- Assessing biomarker performance in human studies is difficult
  - Benchmarking against histopathology or current biomarkers is generally impossible or complicated
- Access to human samples of acute drug-induced organ failure is limited
- Funding for clinical translational studies
  - HESI, PSTC, IMI-SAFE-T

# Clinical Translation of Safety Biomarkers

- General themes that can be addressed
  - Baseline biomarker values across genders and age and ethnic groups
  - Assess prognostic / diagnostic threshold values
- Study considerations
  - Monitoring biomarker performance in human disease that approximates drug-induced injury
  - Monitoring biomarker performance in standard treatments that are known to carry a risk of injury
    - Acetaminophen hepatotoxicity
- Study design
  - Prospective
    - Clinical trial design required, consortia, large funding needed
  - Retrospective
    - Discard (left over) samples; close collaboration with clinicians, economical and relatively fast

# Study design

- Sample collections*
  - Healthy subjects - volunteers from PhI clinical trials
    - Medical exam at the time of sample collection
  - Healthy subjects (UoM) with normal levels of liver injury biomarkers and no signs of liver disease in medical history
  - Subjects with range of liver diseases
  - Subjects diagnosed with APAP overdose
- Analytical measurement of DILI biomarkers
  - Automated assays
- Data analysis
  - Effect of age, gender
  - ROC analysis
    - Liver injury defined using modified biochemical criterion of liver injury

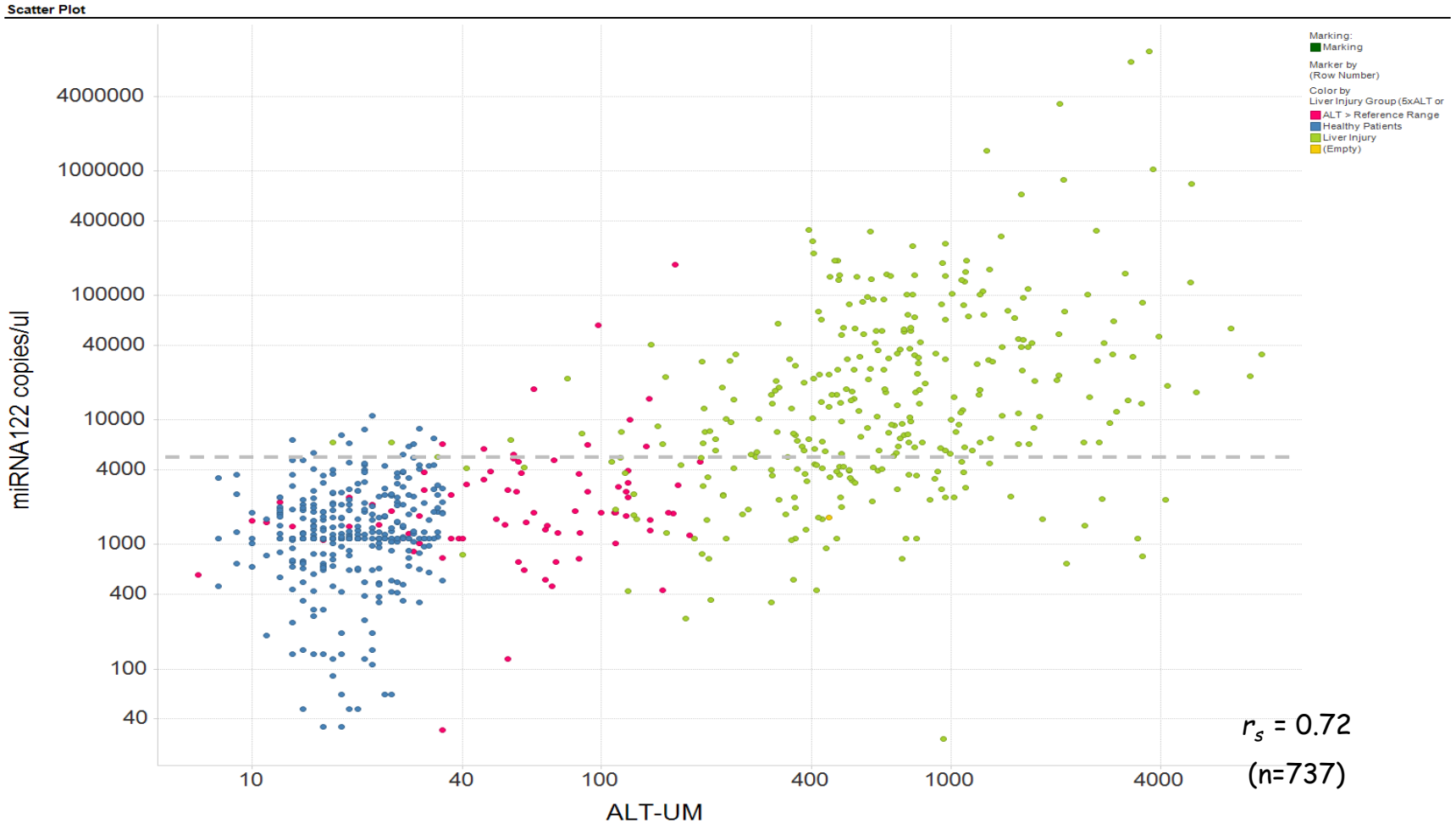
* Research on human clinical trial subjects/samples was conducted in accordance with all applicable Pfizer policies, including IRB/IEC approval.

# miR-122 levels in healthy subjects

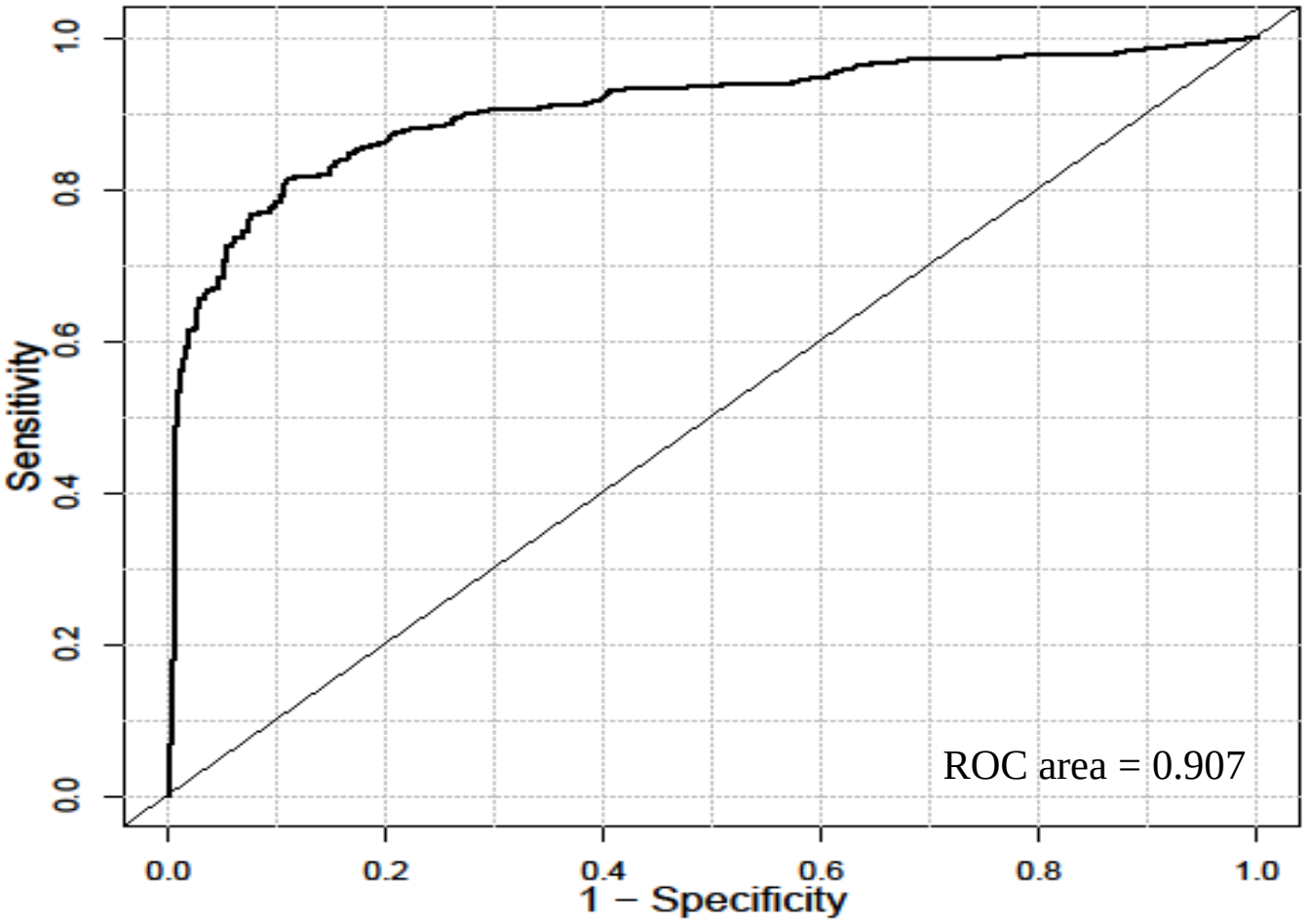
| Gender | All Ages | Age < 20 | Age 20-40 | Age 41-60 | Age > 60 |
|---|---|---|---|---|---|
| Male | 40-3602 | N = 0 | N =20 | N = 47 | N = 33 |
| | | | 40-5340 | 40-2697 | 40-3766 |
| Female | 40-6927 | N = 11 | N =69 | N = 97 | N = 55 |
| | | 40-9136 | 40-3470 | 40-4844 | 40-3502 |

Upper limit of normal = 6333 copies/ul
(n=333)

# Correlation of miR-122 and ALT

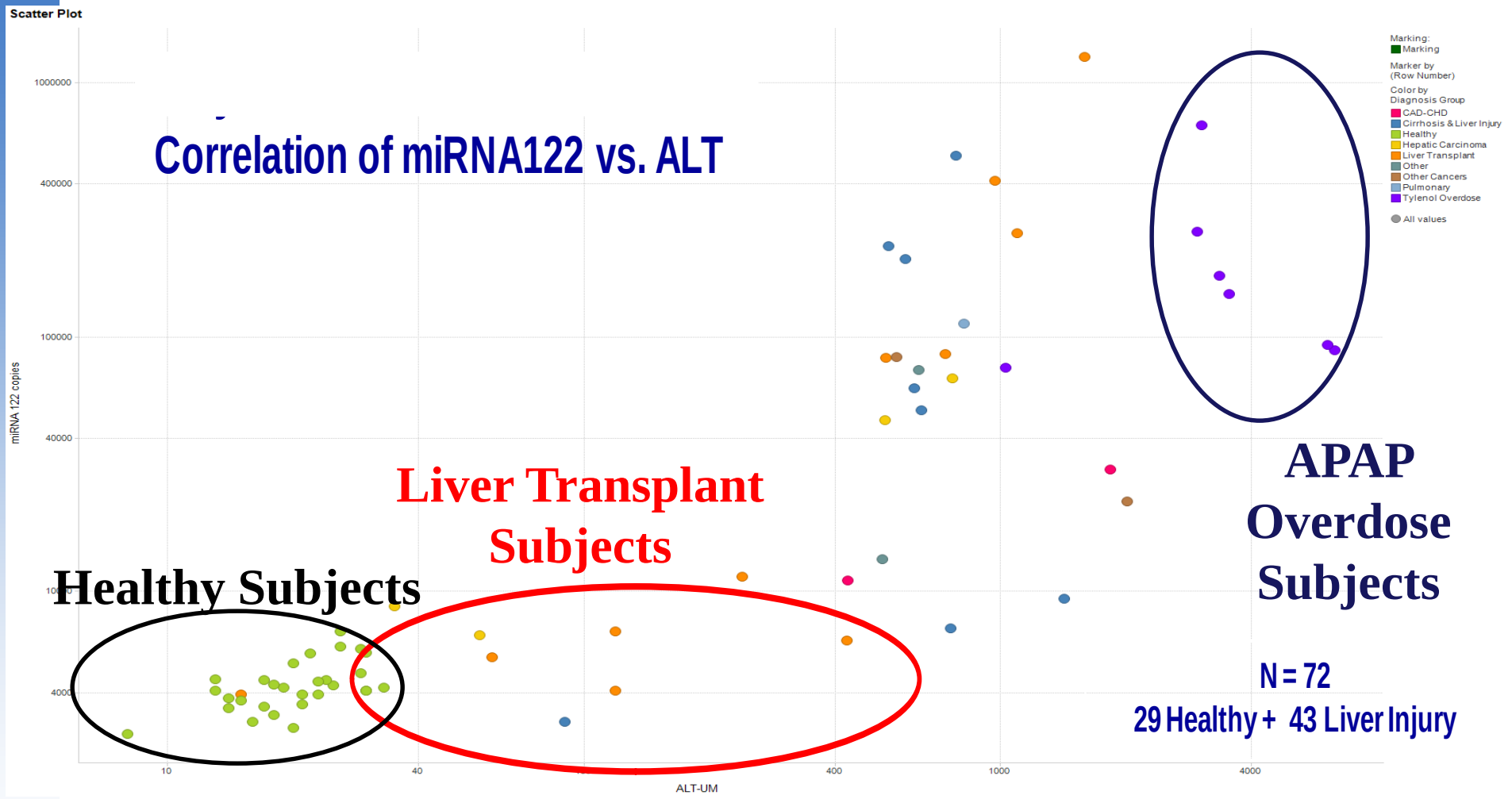


# Performance of miR-122 to detect liver injury

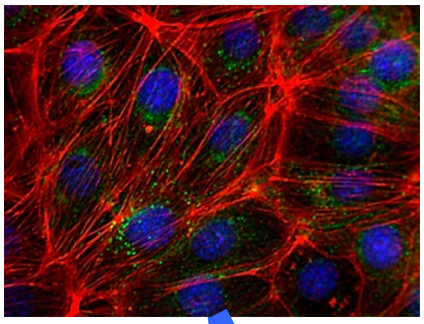


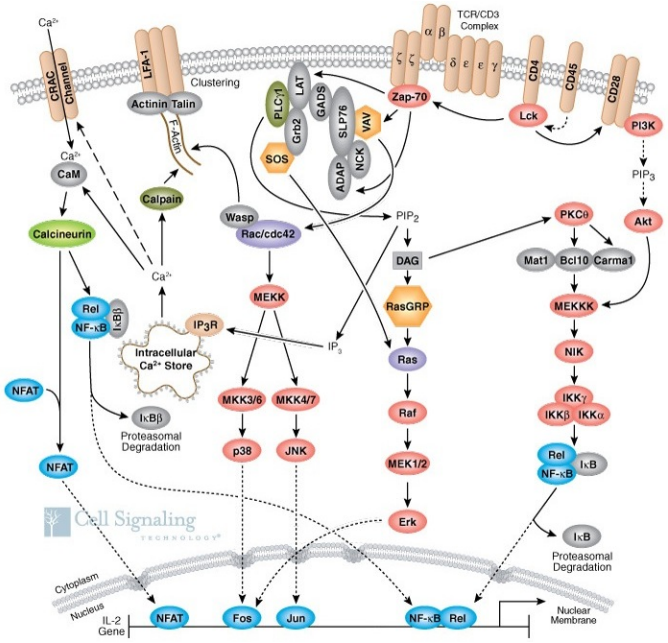

Liver injury defined as 5x ALT or 2x ALP or 3x ALT/2x Tbil.
n= 737

# miR-122 - potential biomarker of Liver Injury



# Liquid biopsy - Signatures of circulating miRs






From cells to animal studies to clinic

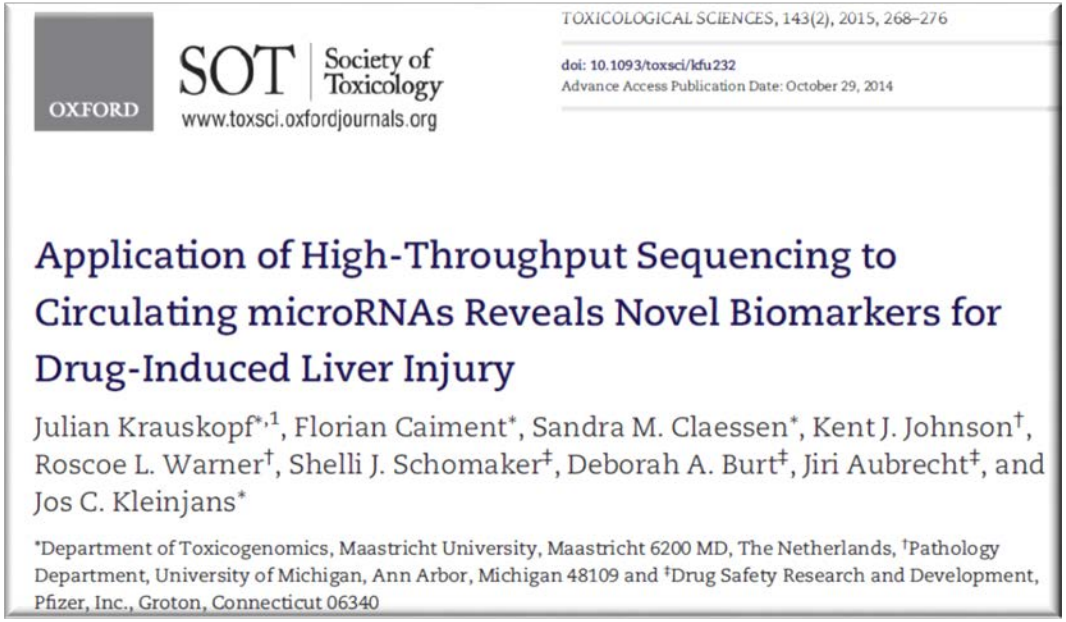
# Hypothesis

- Profiles (signatures) of circulating miRs reflect mechanistic information about toxicity, disease

- miR signatures might be useful for:
  - understanding tox effect
  - Diagnosis of disease
  - Susceptible populations
  - Patient stratification

# Proof of concept studies

1. miR signature of APAP overdose



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## Application of High-Throughput Sequencing to Circulating microRNAs Reveals Novel Biomarkers for Drug-Induced Liver Injury

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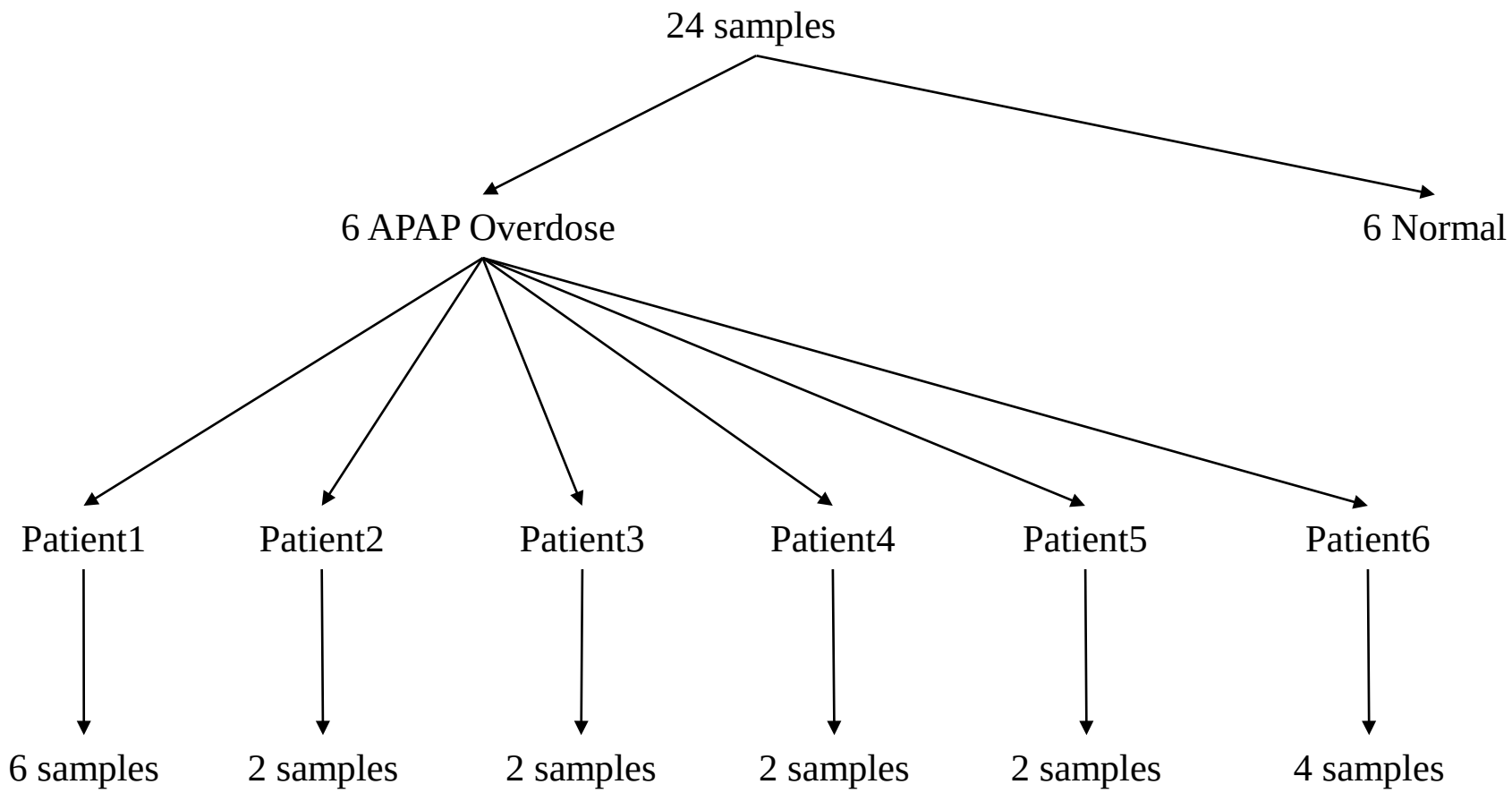
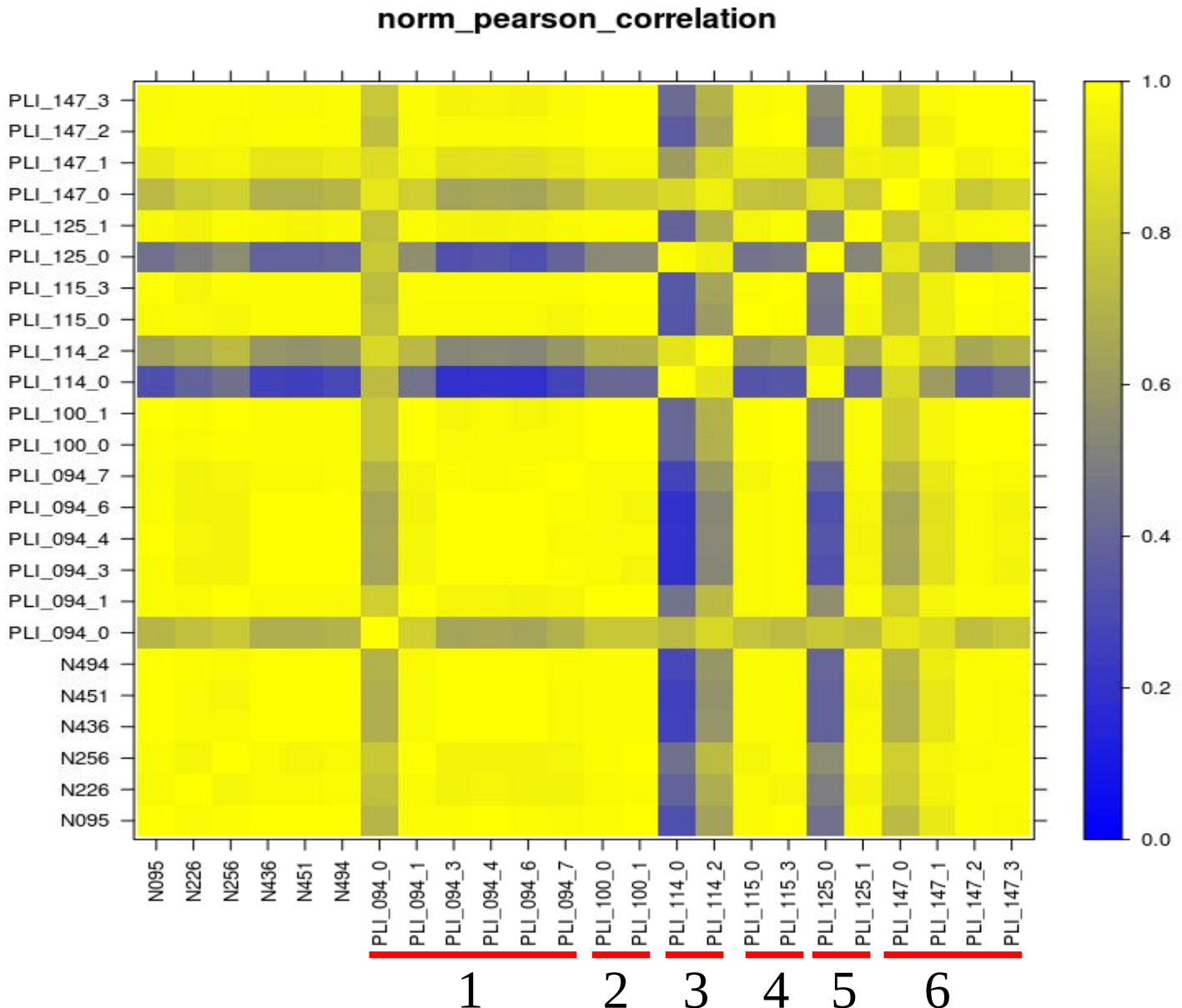
2. miR signatures of liver diseases

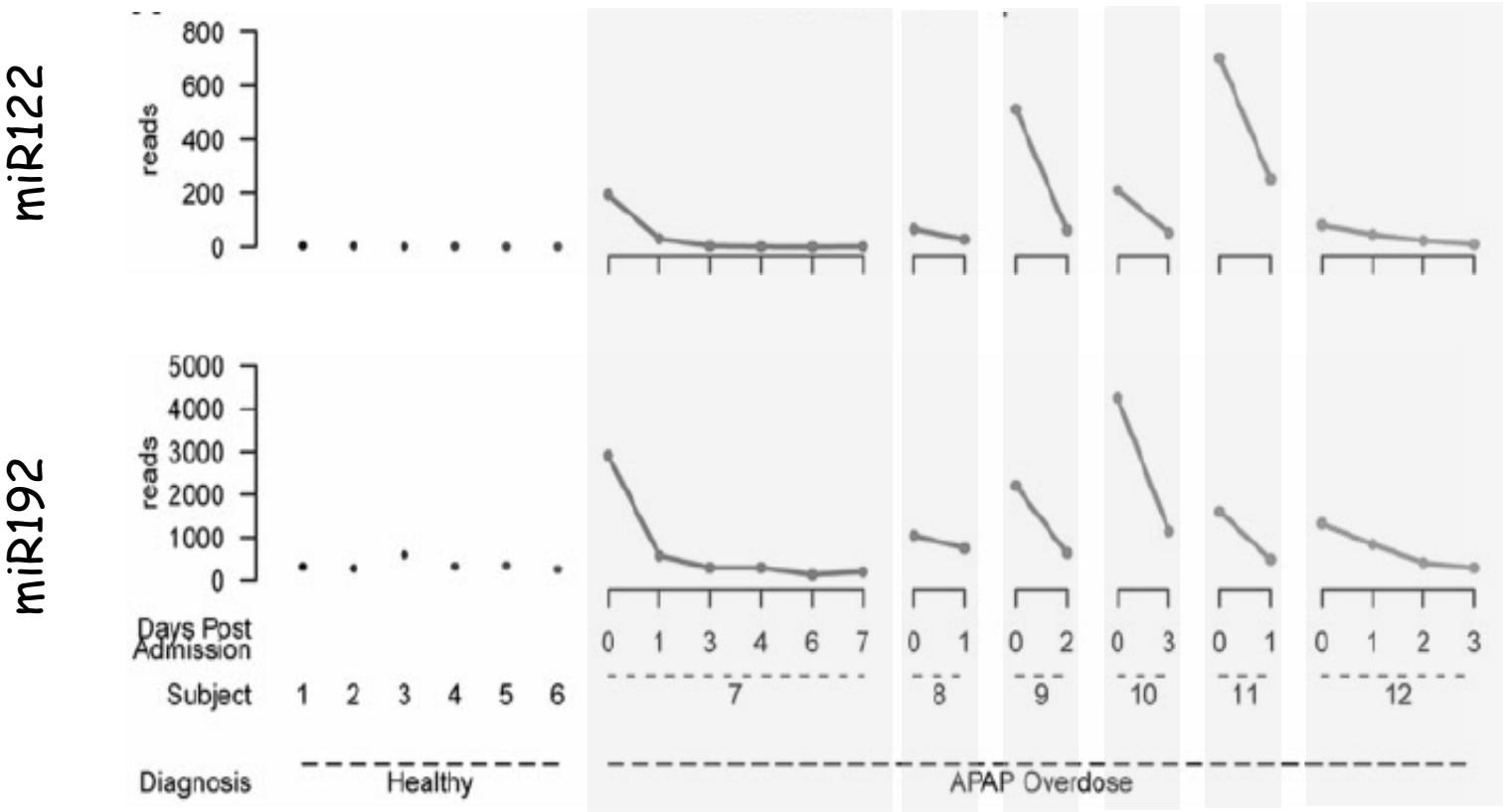
# 1. miR signatures of APAP Overdose- Study design



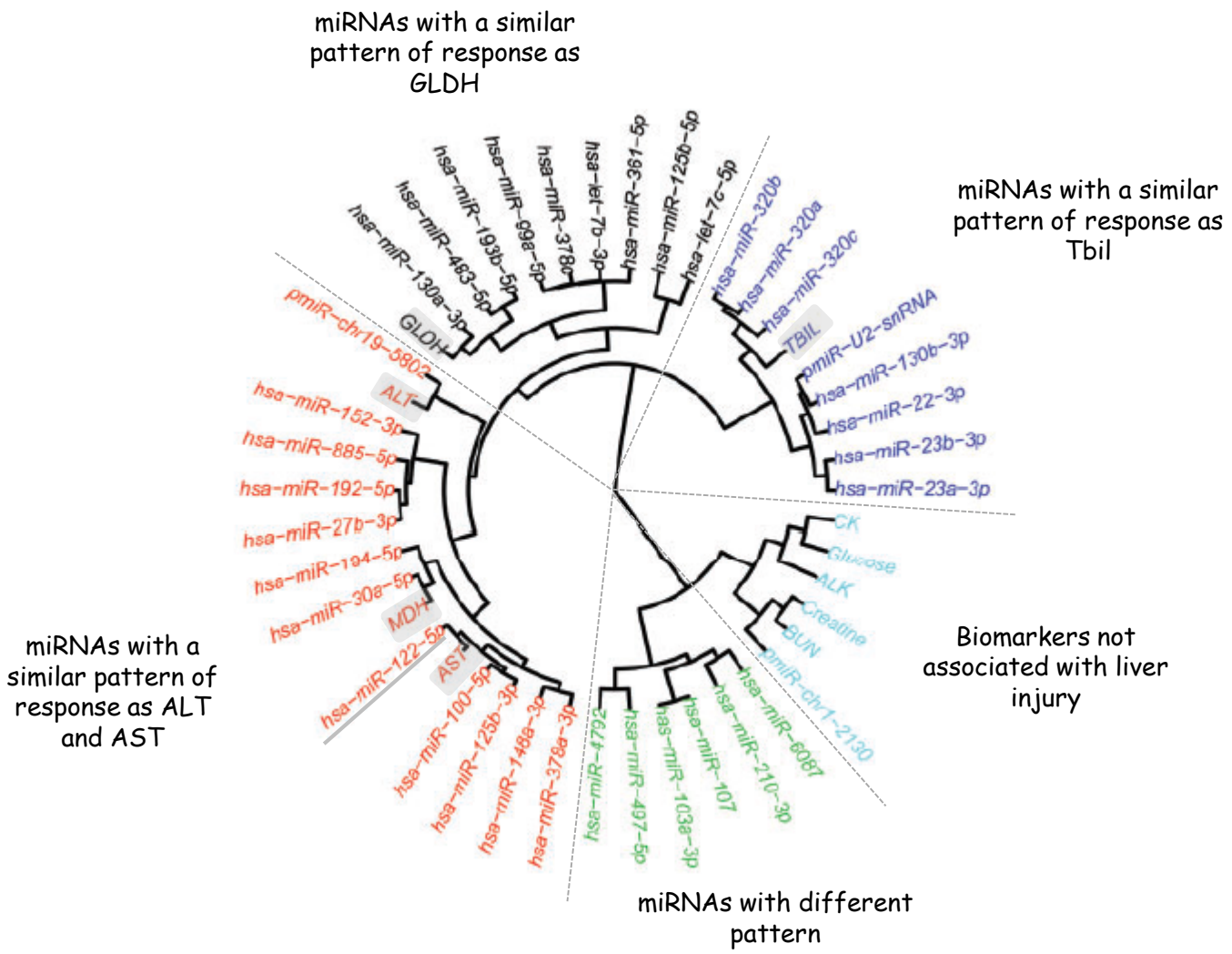
# Circulating miR profiles differentiate APAP-induced liver injury



# NGS Identified Known Liver Injury Associated miRs

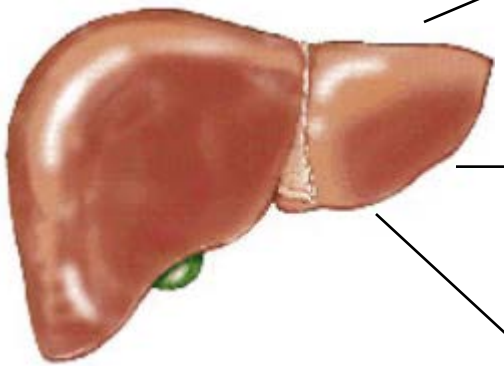


# miRs time course patterns cluster with conventional biomarkers




Hierarchical Clustering Based on Spearman Distance

# Biological significance of observed miRs



| Liver-specific Biological Function | miRNAs |
|---|---|
| Cancer/proliferation | |
| Liver regeneration | let-7b, miR-27b |
| Liver proliferation/cancer | let-7c, miR-100, miR-122, miR-130b, miR-148, miR-152, miR-192, miR-193, miR-22, miR-23, miR-30, miR-483, miR-497 |
| Wnt pathway | miR-130a |
| TGF-beta | miR-23a |
| Metabolism | |
| Glucose homeostasis | miR-103, miR-107, miR-22 |
| Insulin sensitivity/signaling | miR-130, miR-320a |
| Lipid metabolism | miR-122, miR-27b, miR-99a |
| PPAR pathway | let-7c, miR-27b |
| PXR, CYP3A | miR-148, miR-378 |
| Iron metabolism | miR-122 |
| Fibrosis | miR-483 |
| Hepatobiliary development | miR-30 |
| Oxidative stress | let-7b |
| ER stress | miR-107 |
| Not reported function in liver | miR-125, miR194, miR361, miR-6087, miR-885 |

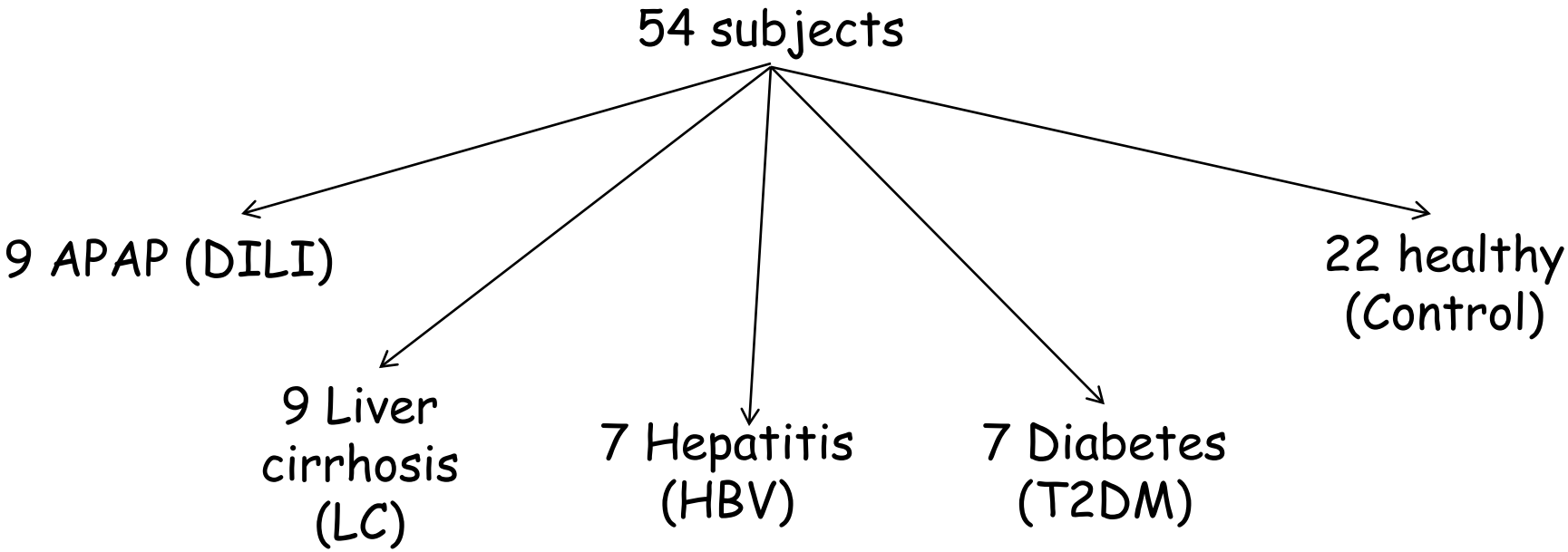
- Liver specific processes indicated by miRs are consistent with molecular mechanism of APAP toxicity

# 2. miR signatures of liver diseases

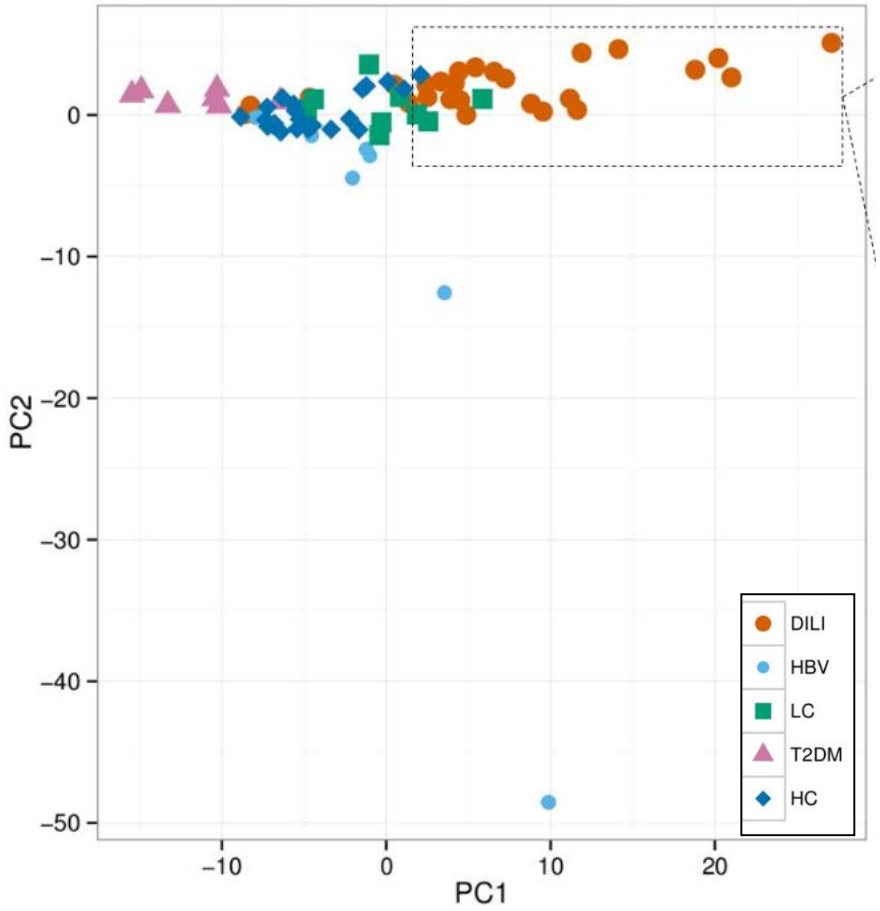
Hypothesis:

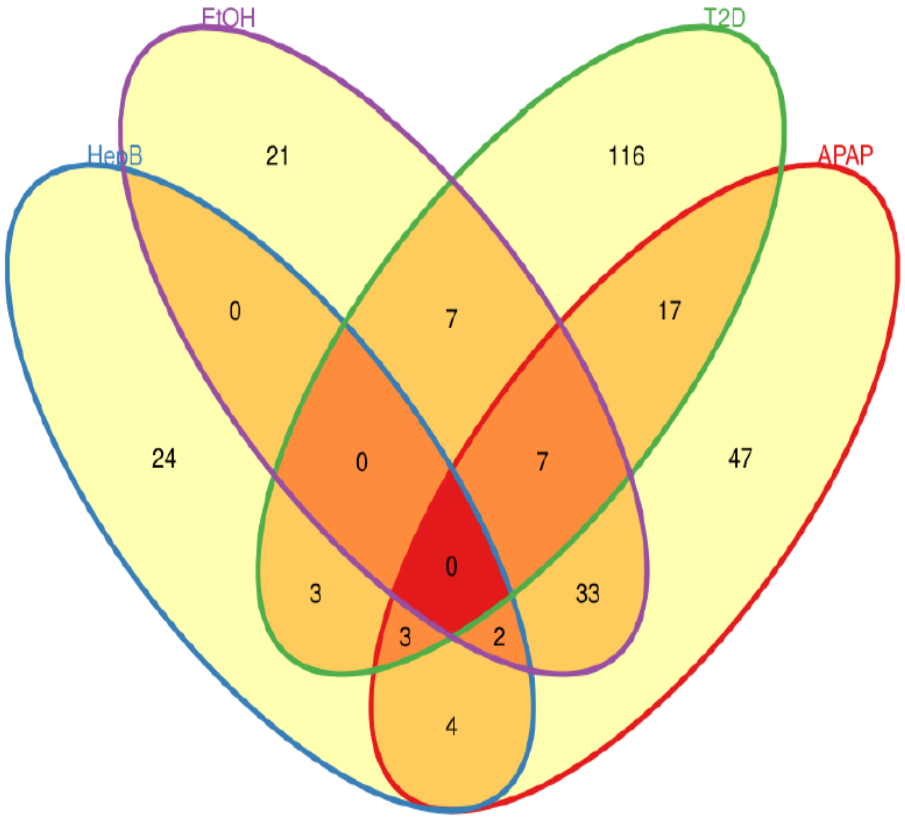
- miR "signatures" in serum can differentiate among variety of liver impairments including providing insights into pathophysiology of disease

- Study design:



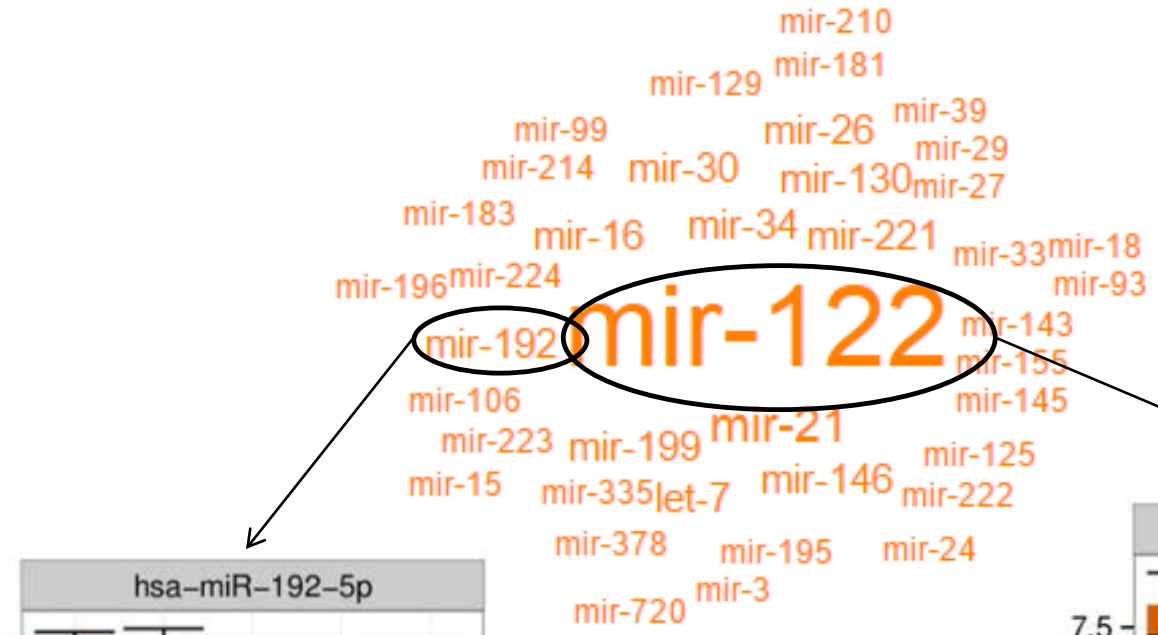
# miR profiles differentiate among variety of liver impairments

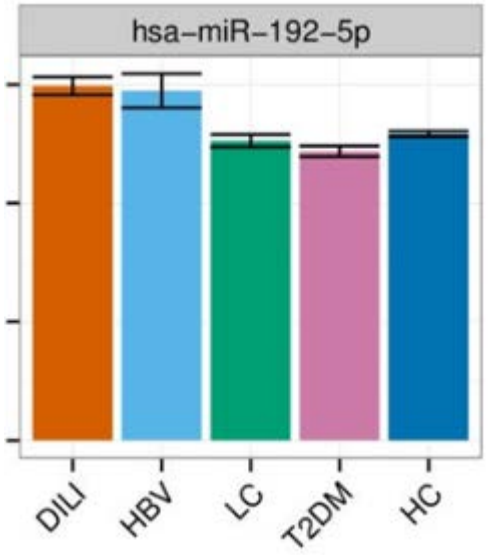


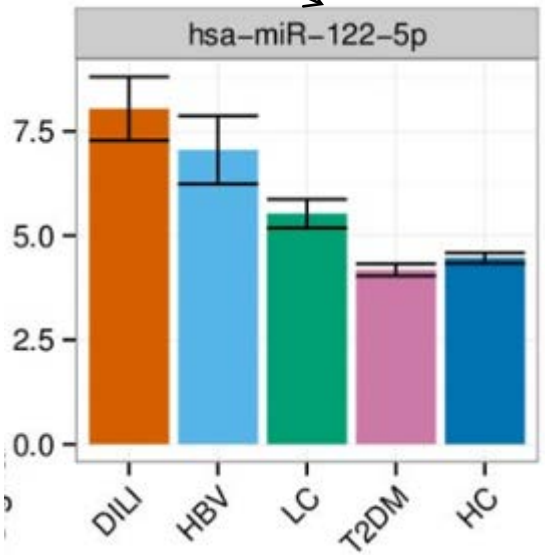




Individual impairments show distinct miR signatures

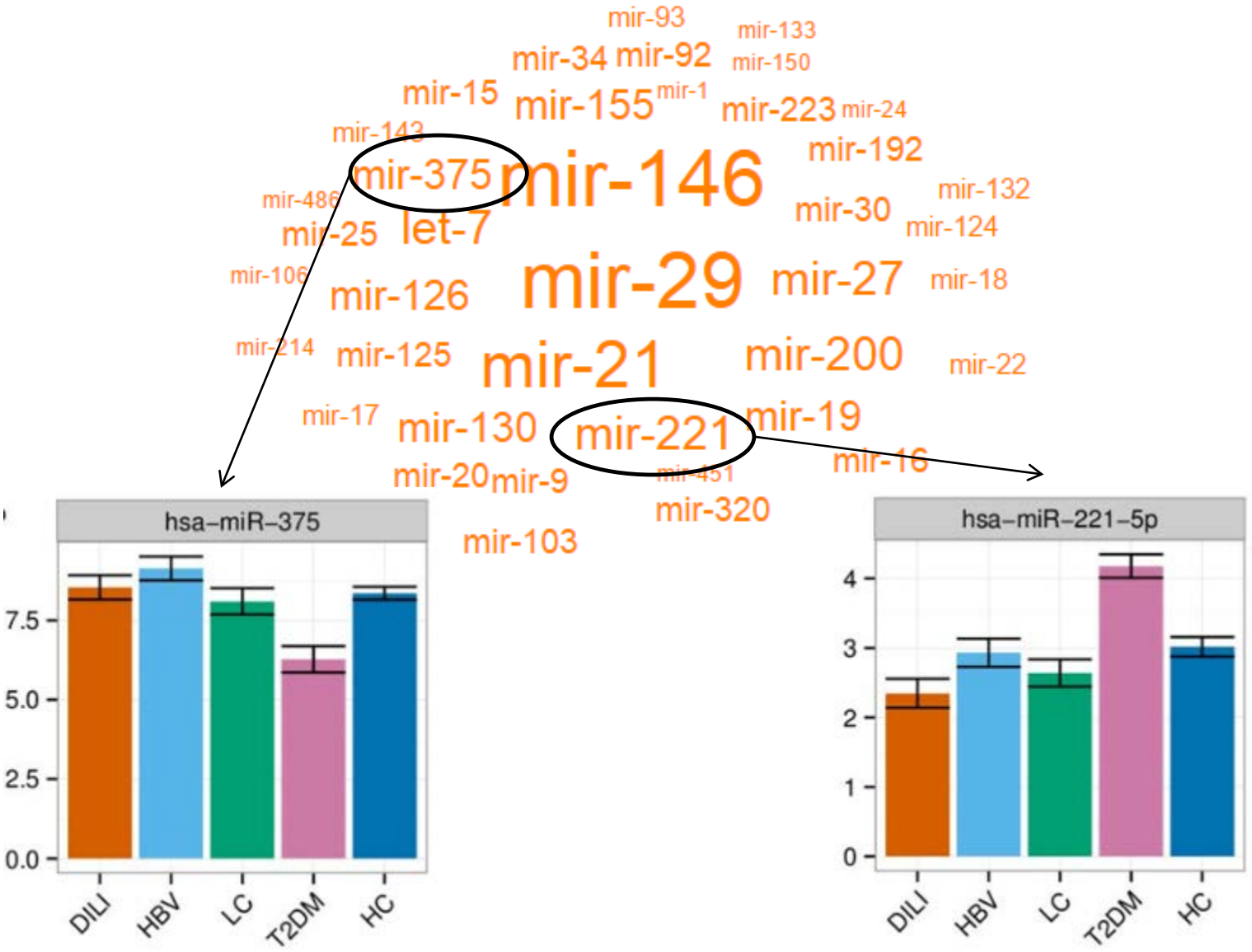
# miRs associated with Hepatitis



# miRs associated with Diabetes



# Conclusions

- miR-122 alone will not replace conventional biomarkers (ALT/AST) for detection of DILI in clinic
  - miR-122 might potentially complement conventional biomarkers
- miR signatures have a potential to provide a fundamental advancement (paradigm shift) in non-invasive tool for evaluating liver injury and liver disease in clinic

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