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

Example: PhI MAD

![Graph showing ALT over time with dosing and phasing](chart)
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

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</table>

Upper limit of normal = 6333 copies/ul (n=333)
Correlation of miR-122 and ALT

$r_s = 0.72$

(n=737)
Performance of miR-122 to detect liver injury

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

n= 737

ROC area = 0.907
miR-122 - potential biomarker of Liver Injury

Correlation of miRNA122 vs. ALT

Liver Transplant Subjects

Healthy Subjects

APAP Overdose Subjects

N = 72
29 Healthy + 43 Liver Injury

N = 72
29 Healthy + 43 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

![Image]

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

24 samples

- 6 APAP Overdose
- 6 Normal

- 6 samples
- 2 samples
- 2 samples
- 2 samples
- 2 samples
- 4 samples
Circulating miR profiles differentiate APAP-induced liver injury
NGS Identified Known Liver Injury Associated miRs

miR122

miR192
miRs time course patterns cluster with conventional biomarkers

miRNAs with a similar pattern of response as GLDH

miRNAs with a similar pattern of response as Tbil

miRNAs with a similar pattern of response as ALT and AST

Biomarkers not associated with liver injury

miRNAs with different pattern

Hierarchical Clustering Based on Spearman Distance
Biological significance of observed miRs

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:

54 subjects

9 APAP (DILI)

9 Liver cirrhosis (LC)

7 Hepatitis (HBV)

7 Diabetes (T2DM)

22 healthy (Control)
miR profiles differentiate among variety of liver impairments

Individual impairments show distinct miR signatures
miRs associated with Hepatitis
miRs associated with Diabetes

miR-375
mir-146
mir-29
mir-21
mir-221
mir-27
mir-18
mir-22
mir-19
mir-16
mir-130
mir-20
mir-9
mir-103
mir-320
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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