Performance of miR-122 as a Serum Biomarker for Hepatotoxicity in Short Term Preclinical Studies

Tatiana Sharapova

Investigative Toxicology and Pathology

AbbVie Inc.

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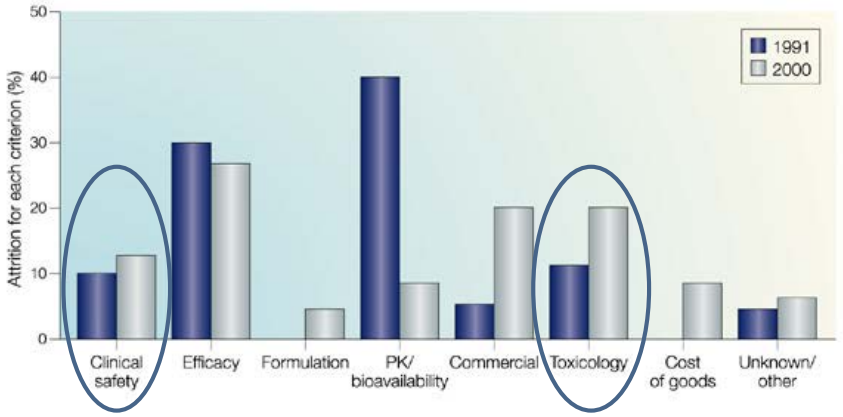
Biomarkers of Toxicity

What is The Need?

- High attrition rates in drug discovery and development due to toxicity or adverse events in preclinical animal studies and clinical trials
  - Current tools have limited ability to predict adverse response pre-clinically and in the clinic
    - Histology:
      - Invasive
      - Lacks sensitivity
      - Lacks longitudinal measurements
      - Lacks translation to clinic
    - Insufficient sensitivity or specificity of biomarkers for certain endpoints
    - For some toxicities no biomarkers available



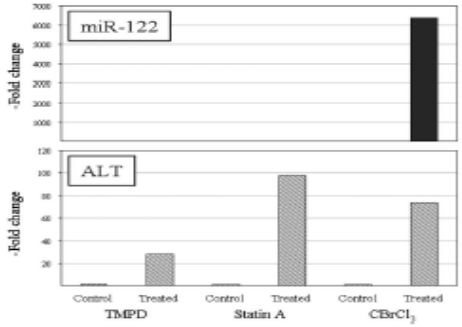
Kola and Landis, Nat Rev Drug Discov, 2004

The need to identify novel biomarkers of organ-specific toxicities with better sensitivity, specificity and cross-species translation

miR-122 as Serum Biomarker for Hepatotoxicity

miR-122:

- Liver specific expression
- Detected in serum upon liver injury



Laterza OF et al., Clin Chem, 2009

Objective:

- To evaluate the utility of miR-122 as a serum biomarker for liver injury in rats by comparing with traditional clinical chemistry liver enzymes (ALT, AST, GLDH)
  - Histopathology as the "gold standard"

Design:

- 263 rats (190 treated, 73 controls)
- 23 short-term toxicological studies
- 24 compounds (proprietary and reference)

Endpoints:

- Serum levels of miR-122
- Clinical chemistry (ALT, AST, GLDH)
- Liver histopathology

Methods for miRNA Quantification

Technical Details

Total RNA extraction:

- miRNeasy mini kit (Qiagen), protocol for biofluids
  - 200uL of biofluid
  - carrier RNA (E. coli or MS2 total RNA)
  - Spiked with synthetic miR-Cel-39, miR-Cel-54 and/or ath-159
  - RNA yield determined by NanoDrop

MicroRNA quantification: RT-qPCR

- SYBR Green-based assays (Qiagen, Exiqon)
- TaqMan assays (Applied BioSystems)

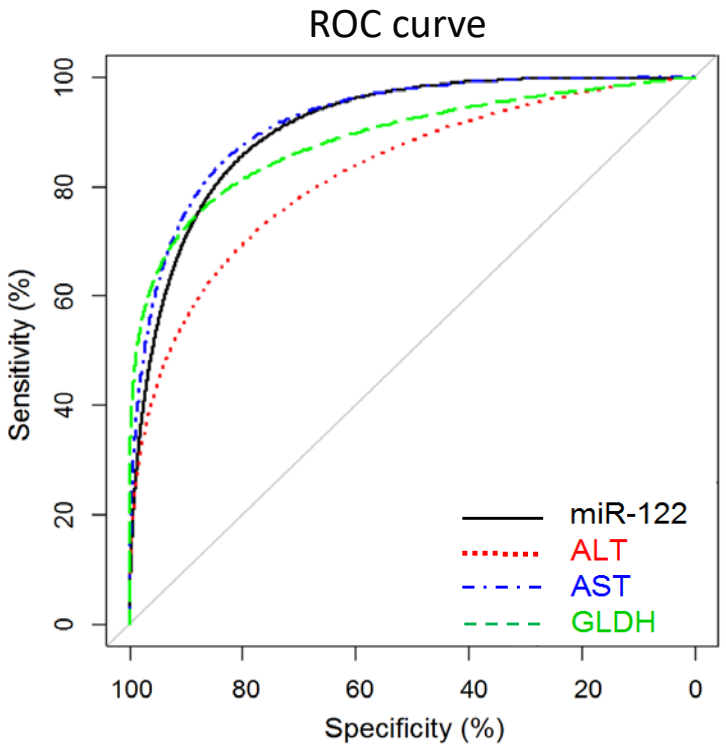
Data analysis

- Absolute quantification or ΔΔCt
- Data normalized to volume and spiked synthetic C. elegans miR-39 and miR-54

Performance of miR-122 in Assessment of General Liver Toxicity

- Each biomarker was assessed by ability to differentiate negative and positive liver response
- Positive or negative liver response established based on histopathological severity

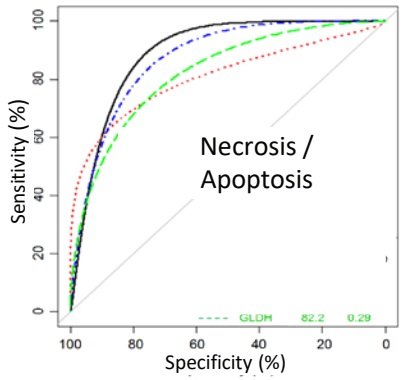
Marker	Sensitivity %	Specificity %	AUC	P-value
miR-122	56	95	90.9	
ALT	56	88	82.3	0.03
AST	62	92	91.9	0.76
GLDH	53	97	88.5	0.52



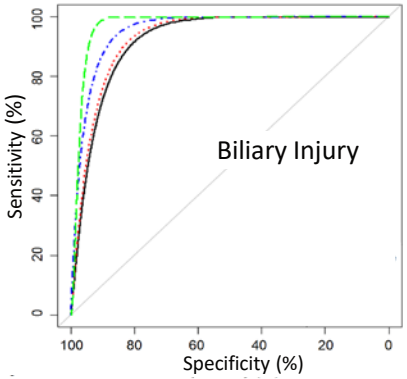
Summary:

- miR-122 outperformed ALT as a biomarker for liver toxicity
- miR-122 was similar in overall performance to AST and GLDH
- The addition of miR-122 to ALT, AST and GLDH improves diagnostic accuracy by 4%

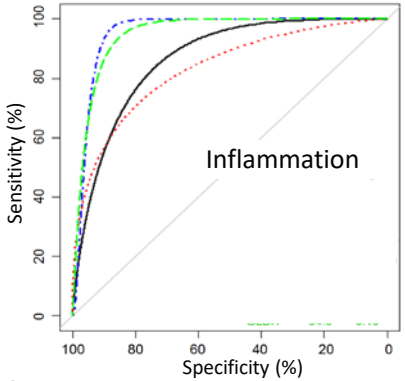
Performance of miR-122 in Assessing Subtypes of Hepatic Injury



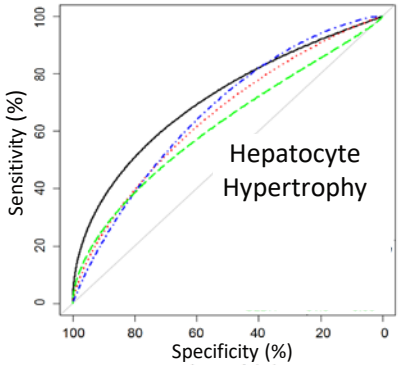
	AUC	P-value
miR-122	89.1	
ALT	80.8	0.24
AST	87.2	0.69
GLDH	82.2	0.29



	AUC	P-value
miR-122	91.9	
ALT	92.8	0.85
AST	95.2	0.44
GLDH	97.0	0.28



	AUC	P-value
miR-122	86.4	
ALT	83.0	0.68
AST	95.4	0.10
GLDH	94.9	0.10



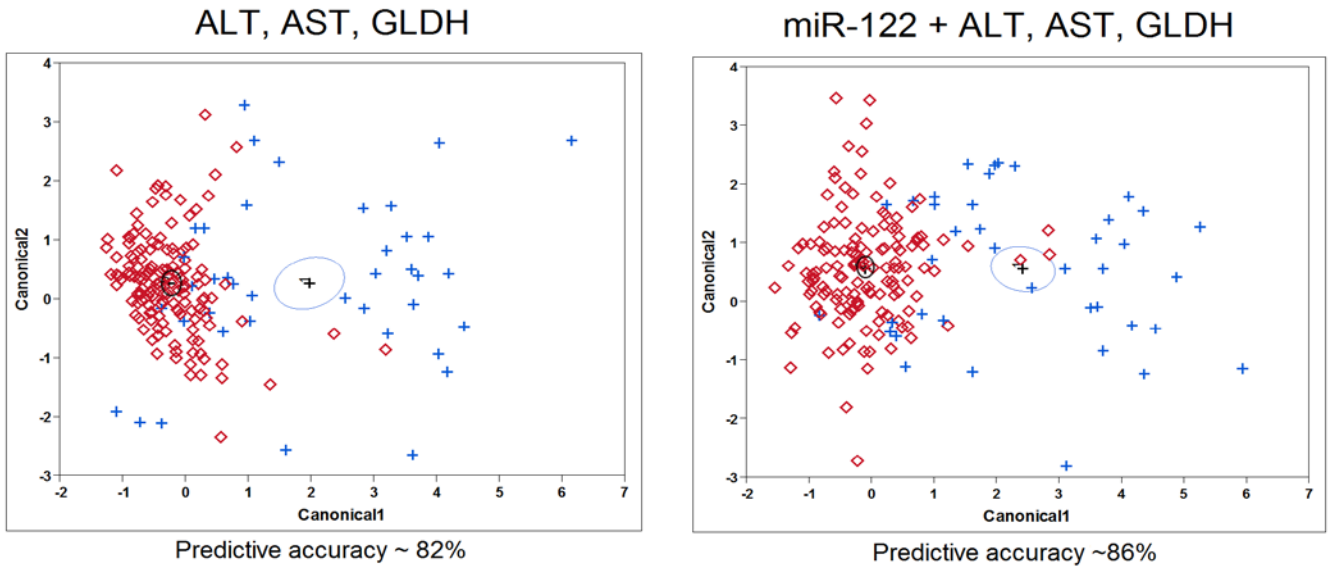
	AUC	P-value
miR-122	70.9	
ALT	65.1	0.19
AST	66.6	0.29
GLDH	61.8	0.06

Summary:

- miR-122 is the most sensitive biomarker to detect hepatocellular necrosis/apoptosis and hepatocellular hypertrophy
- miR-122 is inferior to AST and GLDH in predicting biliary injury and inflammation

miRNAs may perform differently depending on the type of toxicity in the same tissue

Additive Benefit of Serum miR-122 to a Clinical Chemistry Panel



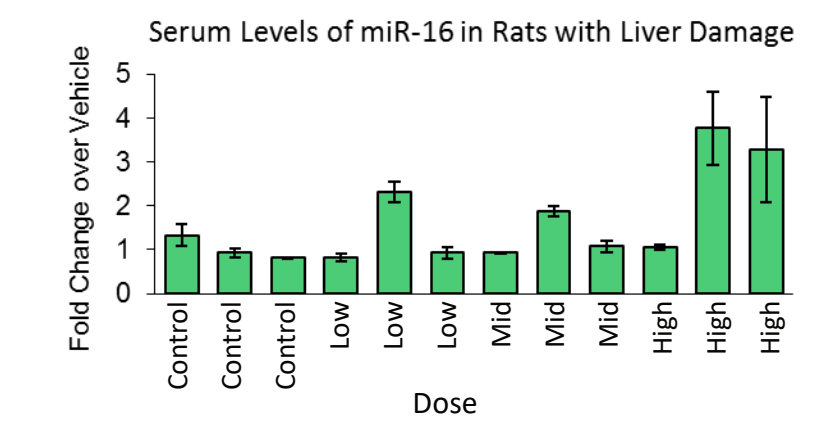
+ positive liver response; ◊ negative liver response

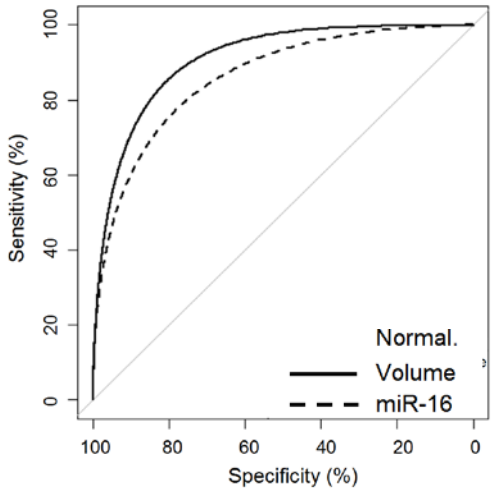
Summary:

- The addition of miR-122 to ALT, AST and GLDH improves diagnostic accuracy by 4% (Linear Discrimination analysis)
- The gain in predictive value is statistically significant (logistic regression; $p<0.001$), but of probably limited practical benefit for short-term rat studies

Performance of miR-122 with or without Normalization to an Housekeeping miRNA (miR-16)

- miR-16 has been reported to be ubiquitously expressed by all tissues and used as reference control gene in qPCR data analysis





Normalization	AUC	P-value
Volume + Spiked miRs	90.6	
miR-16	86.0	0.007

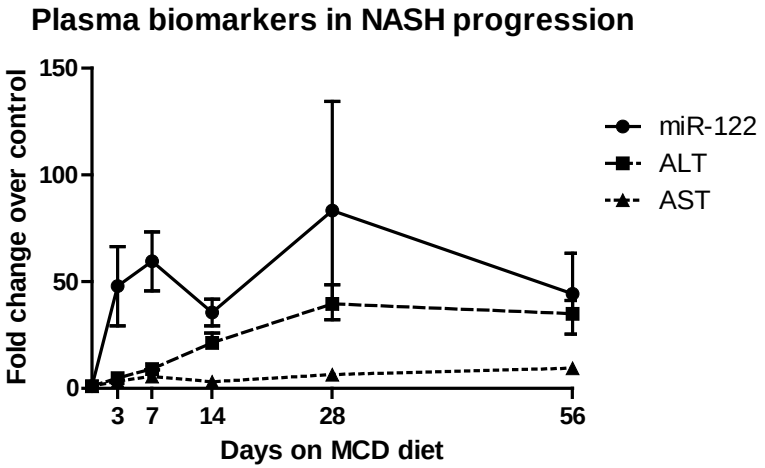
Summary:

- Normalization to miR-16 resulted in lower sensitivity and specificity of miR-122 compared to normalization to volume and spiked *C. elegans* miRs

Normalization of serum miRNA levels to housekeeping reference miRNA is not recommended

Circulating miR-122 as a Biomarker of Non-Alcoholic Steatohepatitis (NASH)

- Design: Mice receiving methionine-choline deficient (MCD) diet for 0, 3, 7, 14, 28 and 56 days
- Endpoints: Histopathology, clinical chemistry and serum miR-122



Clark JD., Sharapova T. et al., J Appl Toxicol., 2014

Summary:

- Serum miR-122 levels were elevated across all time points
- ALT/AST increases were less robust but better correlated with the progression of NASH

miR-122 has a potential as a biomarker for early detection of NASH and for monitoring the extent of liver injury in mouse efficacy models

Summary

- Serum miRNA-122 performs as well as the current biomarkers of hepatotoxicity in rats or of NASH in mice (i.e. ALT, AST, GLDH)
  - Evidence suggests that this translates to other species (i.e. humans)
  - Is there added value or clinical utility?
- Additional efforts are needed to improve analytical methods and to further characterize the performance characteristics of miRNAs as biomarkers
  - Samples collected are valuable in evaluation due to the large amount of data already collected in parallel
  - Development of Best Practices across organizations (precompetitive consortia like ILSI-HESI)

Acknowledgment

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Thank you

Q & A