HESI eSTAR
miRNA Biomarkers Workgroup
2019 Update

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Leadership

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Syril Pettit	HESI
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Connie Chen	HESI

Participants

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Victor Martinez	BC Cancer
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Philippe Couttet	Novartis
Peter Yuen	NIH
Connie Chen	HESI
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Ernie Harper	Newcastle University
Frank Simutis	Bristol-Myers Squibb
Graeme Doran	Abcam
Conor Rafferty	Abcam
Katie O'Shaughnessy	US EPA

Outline

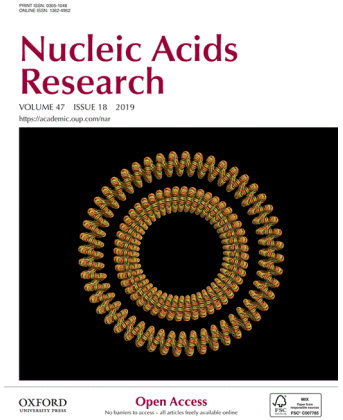
HESI eSTAR microRNA Workgroup

- Update on microRNA “gap analysis” review *10 minutes*
- Discussion of future directions of the workgroup *10 minutes*
- Update on nephrotoxicity microRNA localization study *15 minutes*
- Questions *5 minutes*

HESI microRNA biomarker gap analysis review

- **Problem statement:** *Despite our technical capability to measure miRNAs, they are not routinely employed as biomarkers which is in part due to the lack of standard approaches for sample preparation and measurements resulting in uninterpretable variability in quantitation, and overall uncertainty in the biological meaning of the alterations observed.*

- **Overall goal:** *Explore those areas that appear to be major impediments to routine acceptance and use of miRNA biomarkers give case examples of success and deficiencies in development. Suggestions on meeting the challenges associated with adoption of miRNAs for scientific, clinical, and regulatory uses.*



Evolution of content

Target journal: Nucleic Acids Research as a "Survey and Summary"

<u>50 pages, 1 graph, 3 tables</u>

- Major Challenge #1: Sampling
- Major Challenge #2: Measurement
- Major Challenge #3: Interpretation

- Case examples:

  miR-122

  Nephrotoxicity miRNA biomarkers

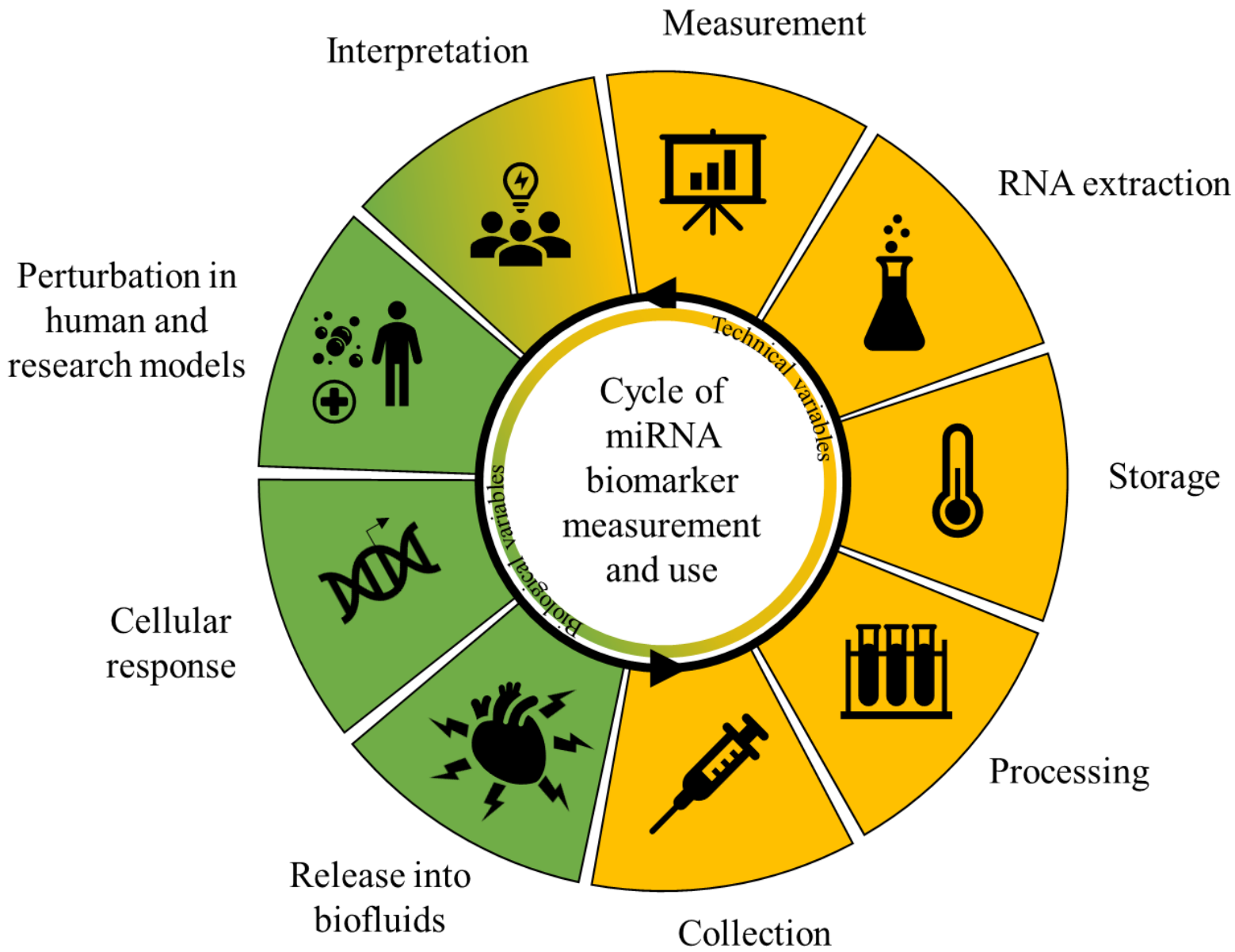
<u>21 pages, 3 graphs, 3 tables</u>

- Major Challenges:

  Technical Variables

  Biological interpretation

  Integrated case examples


Measurement
RNA extraction
Storage
Processing
Collection
Release into biofluids
Cellular response
Perturbation in human and research models
Interpretation
Technical variables
Biological variables
Cycle of miRNA biomarker measurement and use

Timeline

☑ Results from workgroup survey; identify priorities

☑ Organize overall outline; assign section workgroup leads and/or members; section leads coordinate work by section workgroup; propose timeline

☑ Section workgroups formed and initial literature scan completed; section update on gap focus and content

☑ First draft of sections of sections due; update by section leads; send drafts to entire group for comment

☑ Draft section reviewed and returned and sent to section leads

☑ Second draft of sections due; section updates; discuss integrating sections into unified document

☑ Single document due to workgroup; identify any gaps/transition edits; section leads coordinate writing Discussion/Future Aims

☑ Complete document draft due; entire workgroup discussion; elicit comments

☐ Written comments due from entire workgroup

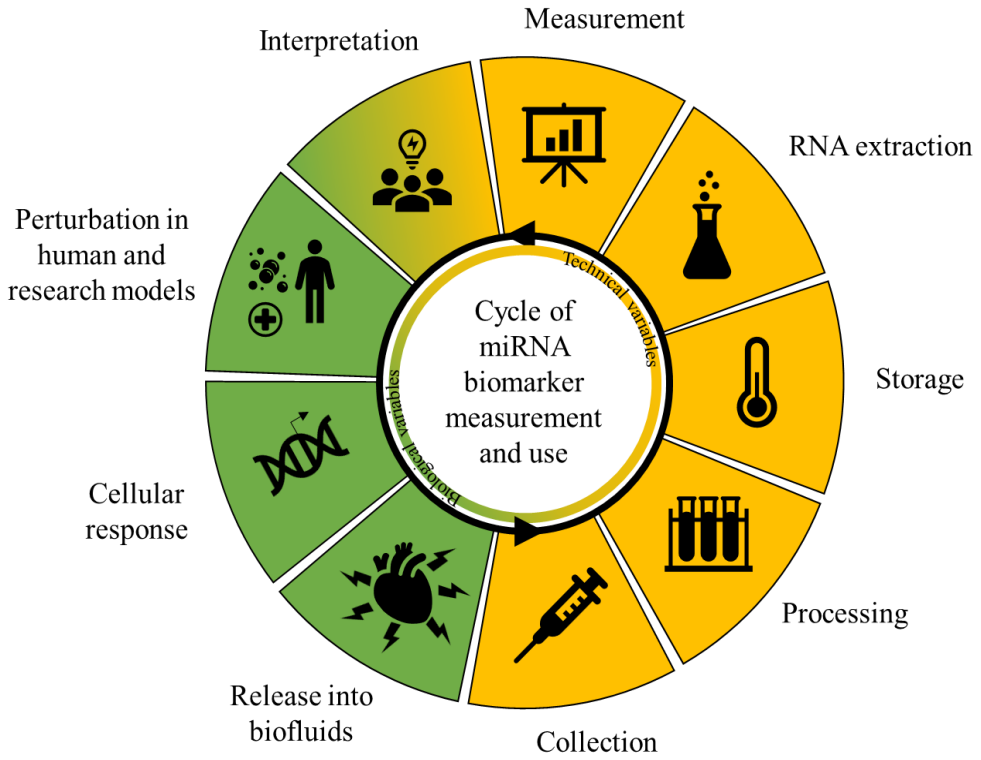
☐ All comments addressed from workgroup. Final draft due. Begin clearance processes.

☐ November 2019: Submission to NAR

Purposes of gap analysis

- Highlight HESI case examples and work in this field
  - Update on Nephrotox microRNA biomarker coming up!
- Provide scientific community synthesis of information, *opinion*, and guidance
- Define future directions for our workgroup

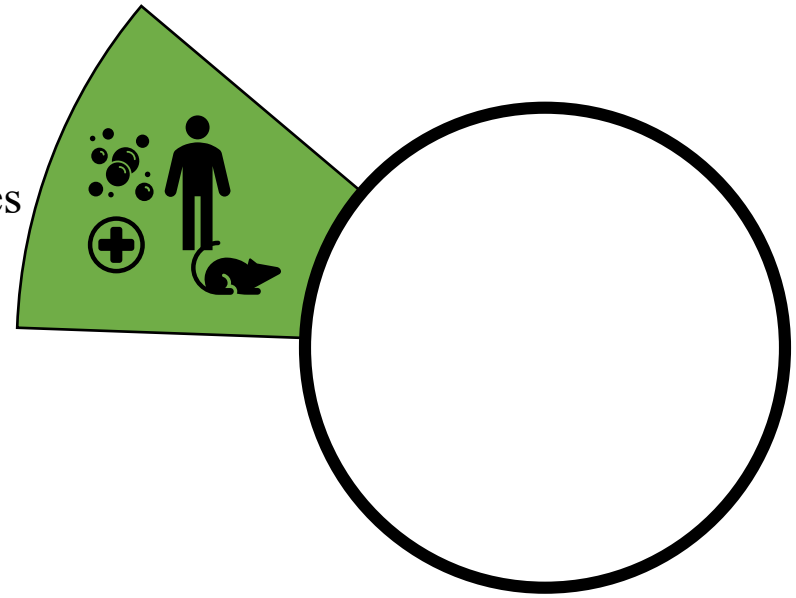
From gap analysis to future directions



- Translational case studies
- Single cell transcriptomics
- Microvesicles
- Combined biomarkers and panels
- Standard operational procedures
- Guidance on normalization

Translational case studies

Perturbation in human and preclinical species

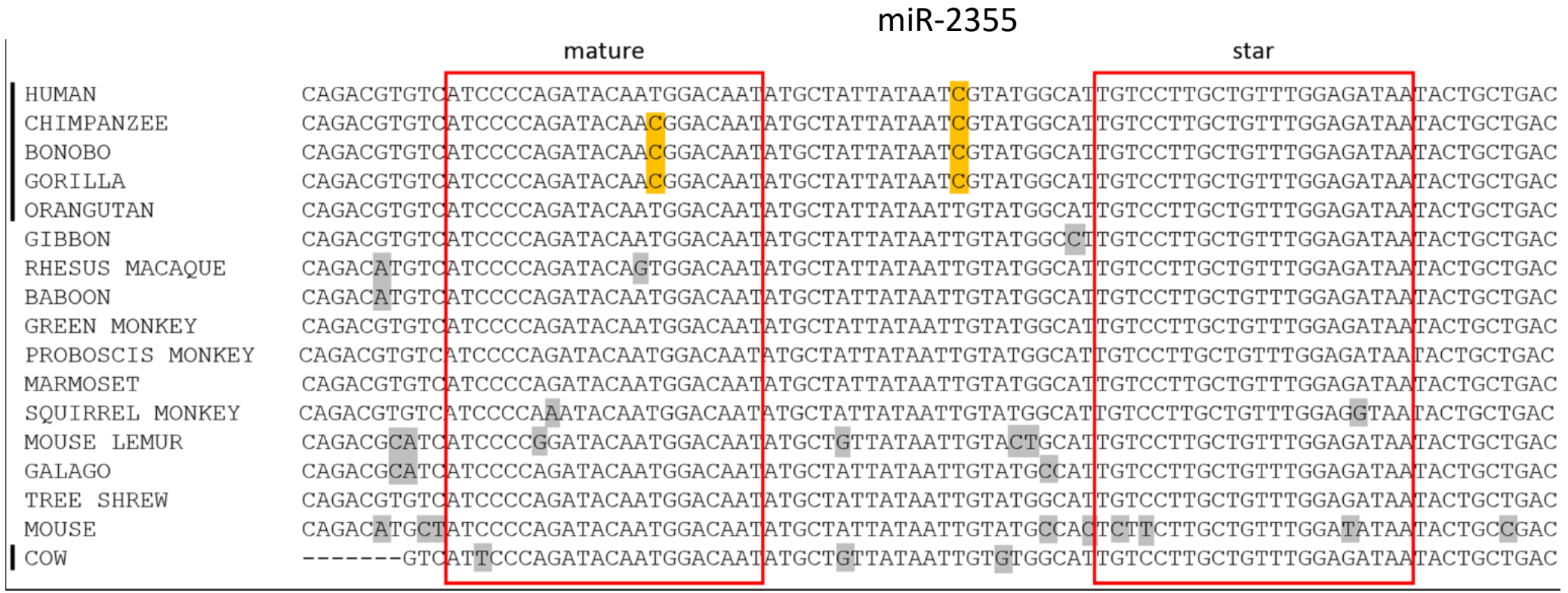


Problem statement: There are gaps in our understanding in the similarity and uniqueness of miRNA signatures noted in in vitro, non-clinical species (rat, dog, pig, NHP), and in humans.

Goal: Propose a specific case study to measure and compare signatures in different models of disease characteristics or exposures.

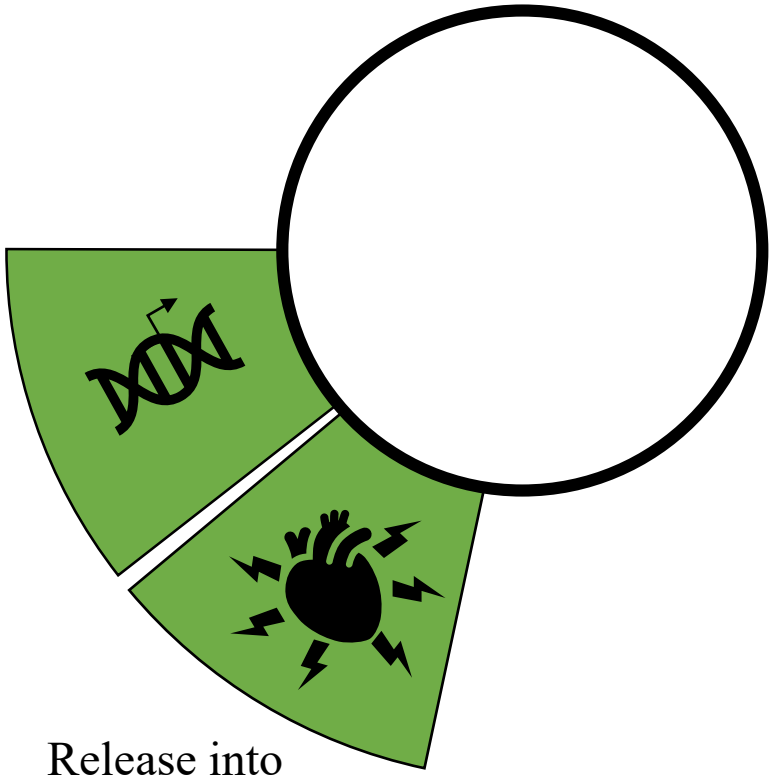
Sub-focus: isomiRs

Species-specific microRNAs



McCreight et al. PLOS ONE June 2017

Mechanisms of microRNA release



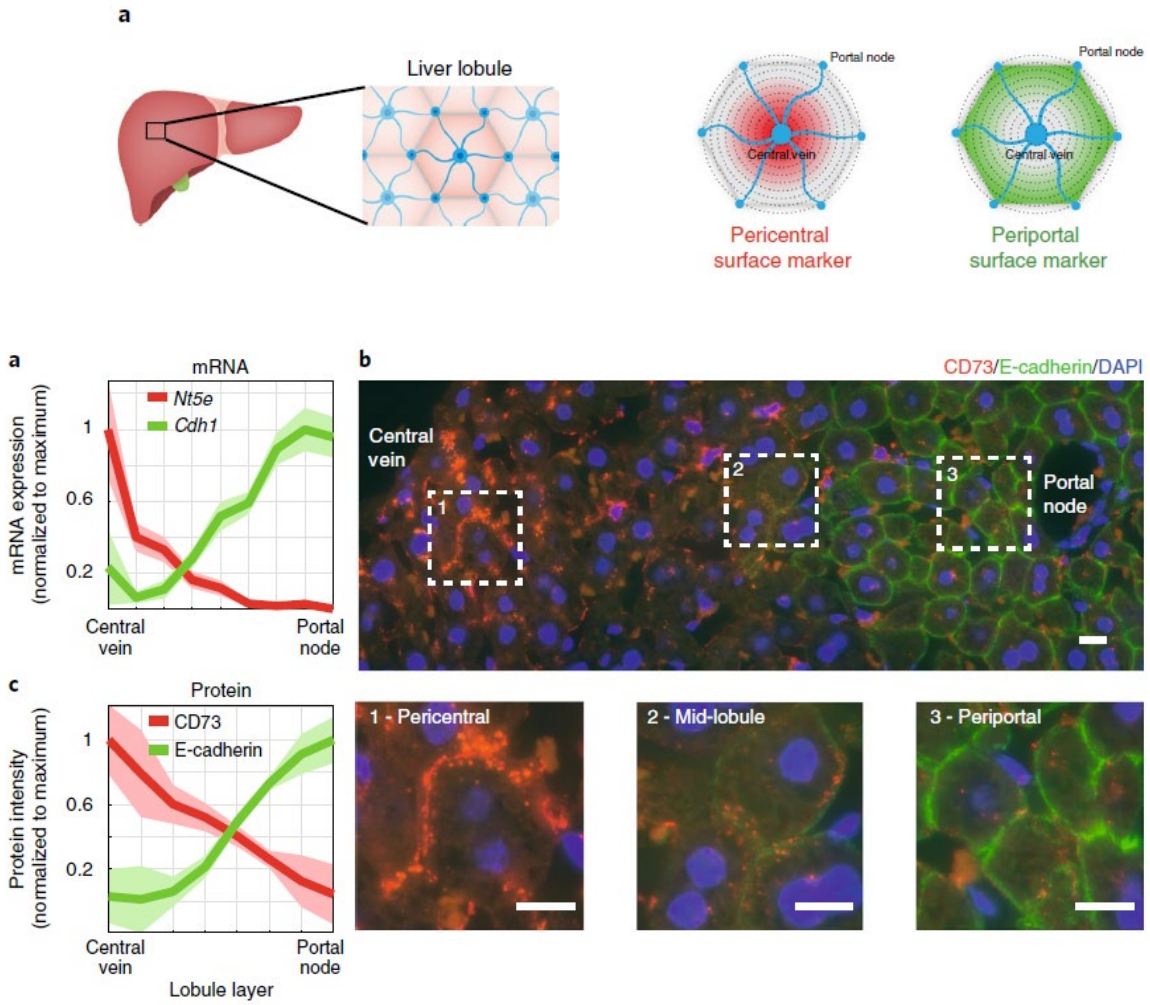
Problem statement: There is overall uncertainty as to the specificity, source, and mechanism of release of microRNA during tissue injury or a disease process.

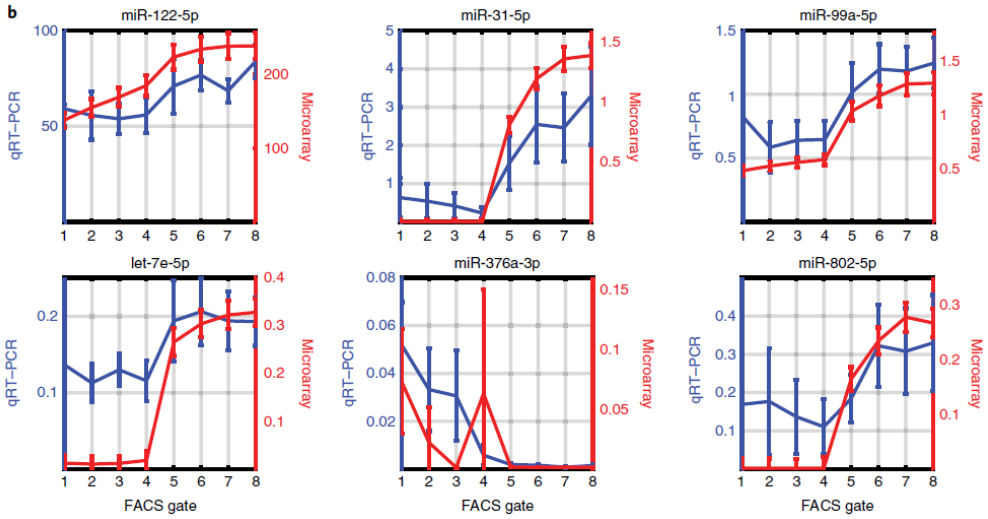
Goal: Add to growing body of knowledge to determine cell-specific origin and/or biological mechanisms that mediate microRNA release into biofluids.

Method #1: Utilize single cell transcriptomics to localize origin.

Method #2: Examine microvesicle microRNA fractions to determine enriched signatures of adverse outcome.

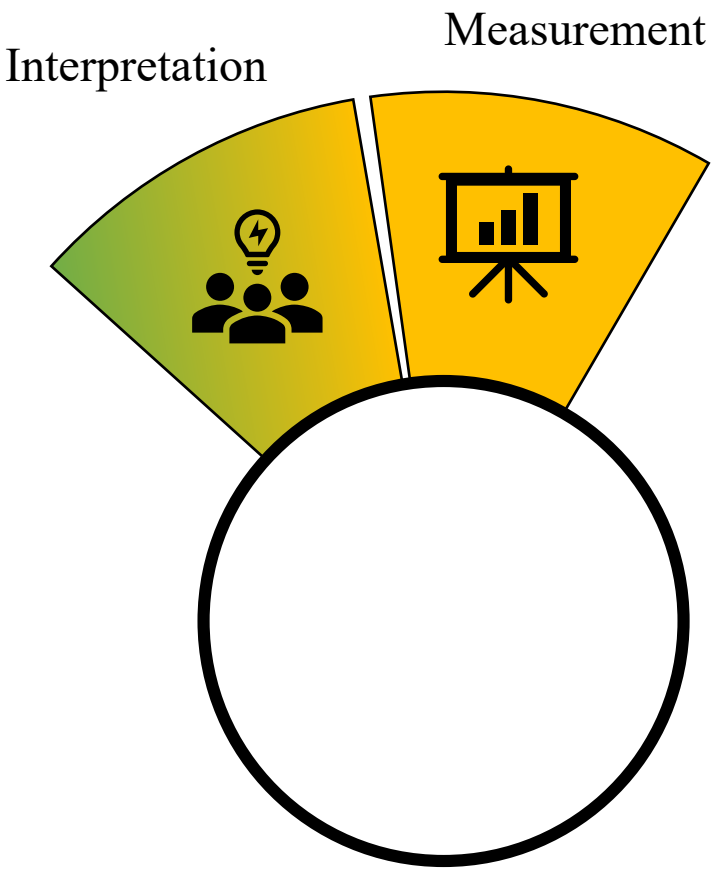
Spatial expression of liver microRNAs





Ben-Moshe et al. Nature Metabolism Sept. 2019

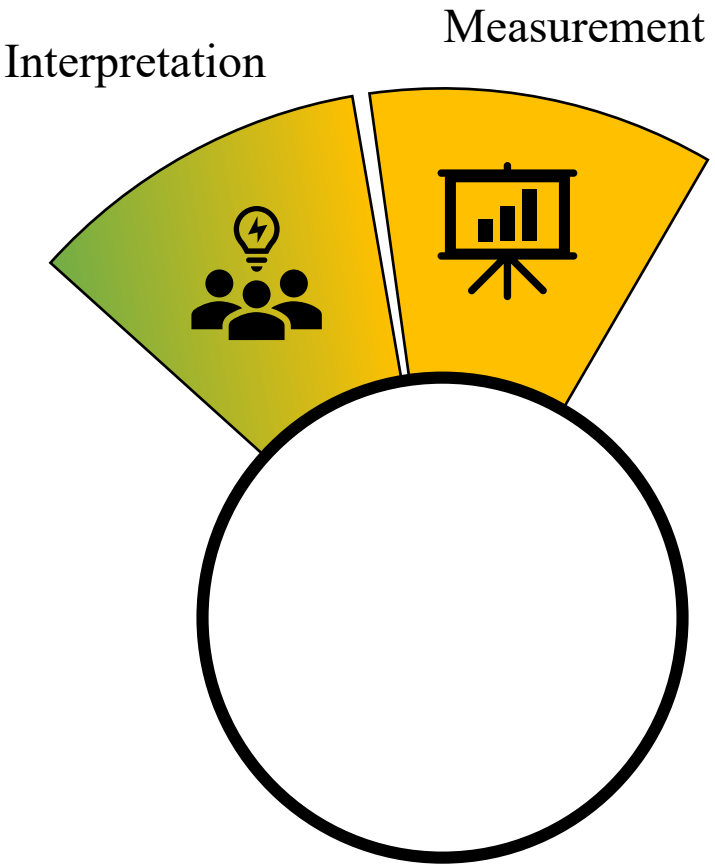
Increased prognostication



Problem statement: The added benefit of microRNA biomarkers compared to existing biomarkers is unclear.

Goal: Demonstrate quantitatively the added power or predictivity of microRNAs to an existing biomarker panel in a preclinical model or human cohort.

Standard operational procedures



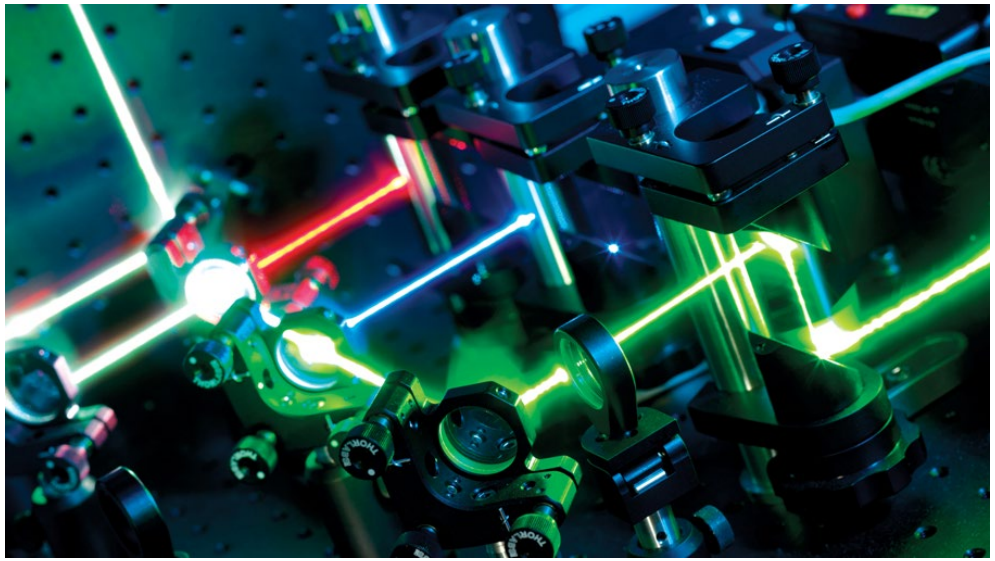
Problem statement: There is a lack of guidance for measurement and analytical methods for specific decision-making purposes.

Goal: Provide metrics, lab-to-lab comparisons, cost and efficiency estimates, and SOPs for microRNA measurements and analyses fit-for-purpose.

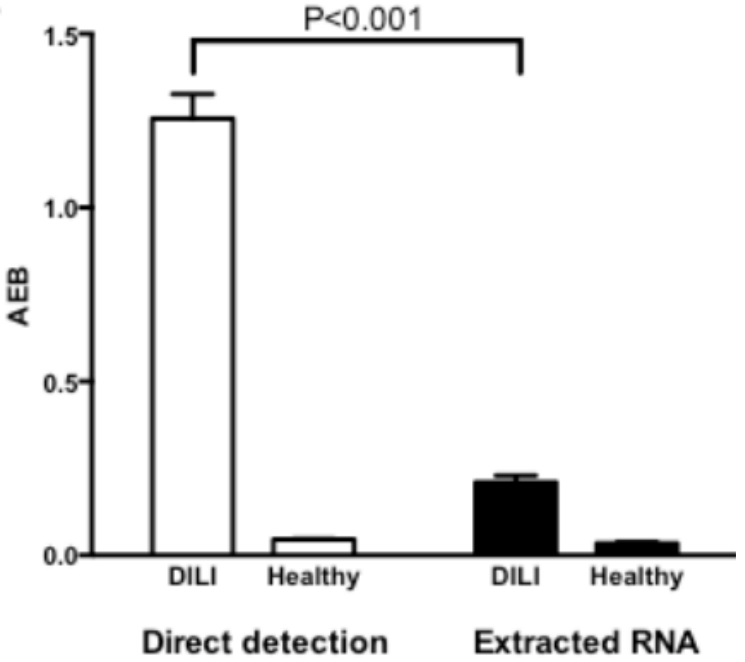
Focus #1: Clinical measurements

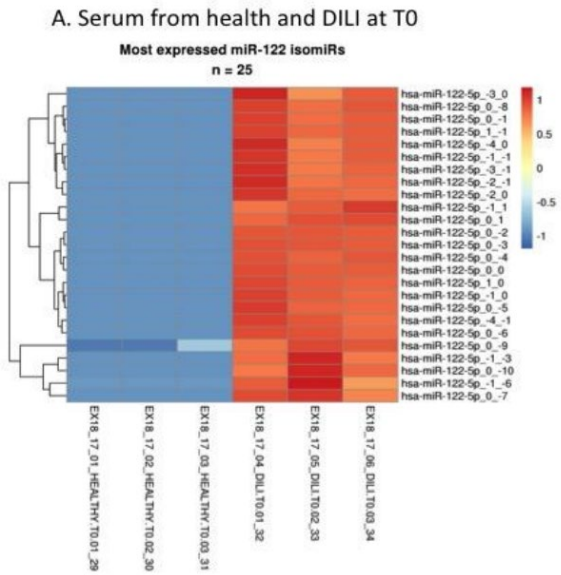
Focus #2: Normalization strategies

Performance of direct vs. extracted measurements



single molecule dynamic chemical labelling





Lopez-Longarela et al bioRxiv September 20, 2019.

Decision for future directions

For consideration:

- Expertise within the group and eSTAR
- Interest of participants and linked organizations
- Feasibility and time frame
- Costs
- Impact

Discussions begin this afternoon, please join us if you have interest