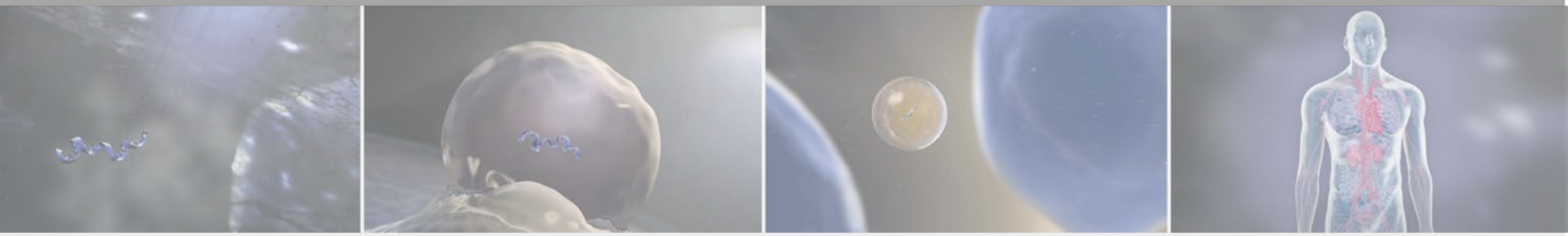


Extracellular RNA Communication

NIH Common Fund Program

DEPARTMENT OF HEALTH & HUMAN SERVICES · USA

NIH National Institutes of Health
Office of Strategic Coordination - The Common Fund

Extracellular RNA Communication Program

New Paradigm: RNAs are released from cells and (may) go on to influence cells that receive them.
RNAs are found in the environment: food, bacteria (think microbiome).

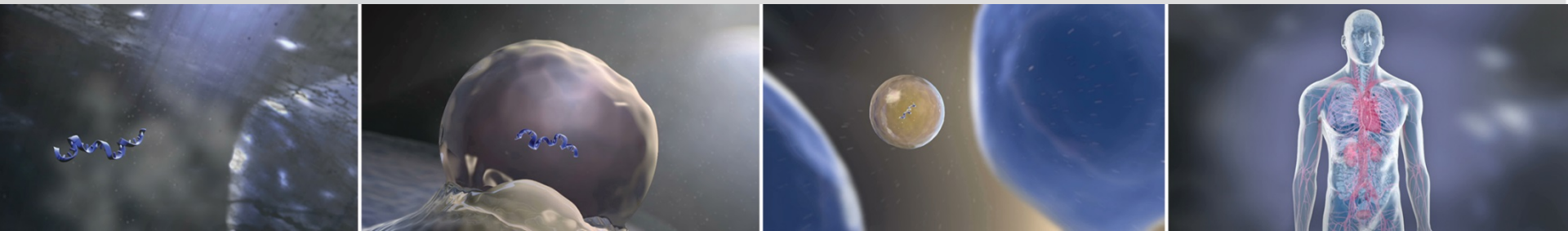
NATURE CELL BIOLOGY VOLUME 9 | NUMBER 6 | JUNE 2007

nature cell biology

Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells

Hadi Valadi[1,3], Karin Ekström[1,3], Apostolos Bossios[1], Margareta Sjöstrand[1], James J. Lee[2] and Jan O. Lötvall[1,4]

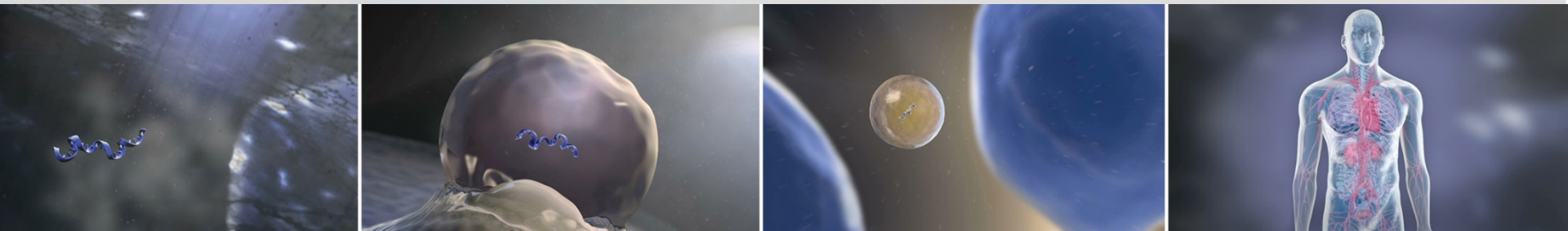
An opportunity to explore new paradigms of cell-to-cell communication based on release, transport, uptake, and regulatory role of exRNAs



Extracellular RNA Communication Program

Focus of this program:

- How do cells use exRNAs to send messages to cells in distant organs?
- Do diseased cells produce different exRNAs than healthy cells, and what is the impact of these exRNAs? Can diseases like cancer spread through release of exRNAs?
- Can researchers harness the communication powers of exRNAs to turn a diseased cell into a healthy cell? (Therapeutic potential.)
- Can exRNAs be used as biomarkers to diagnose disease, monitor progression, and measure response to therapy?



The ExRNA Communication Program is a Trans-NIH Effort

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Jessica Tucker, Ph.D., (NIBIB)
Sundar Venkatachalam, Ph.D. (NIDCR)

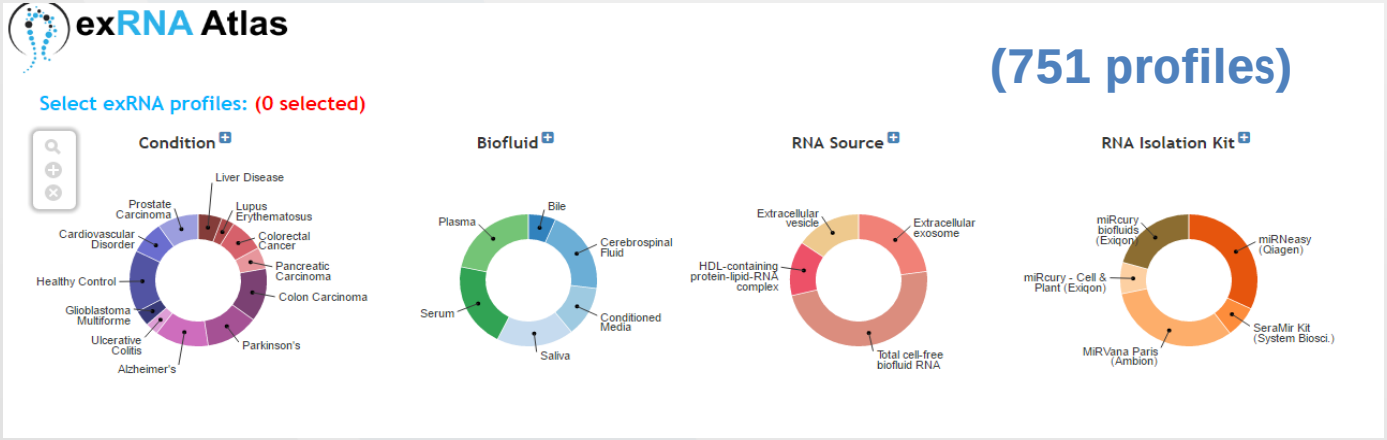
Program Initiatives

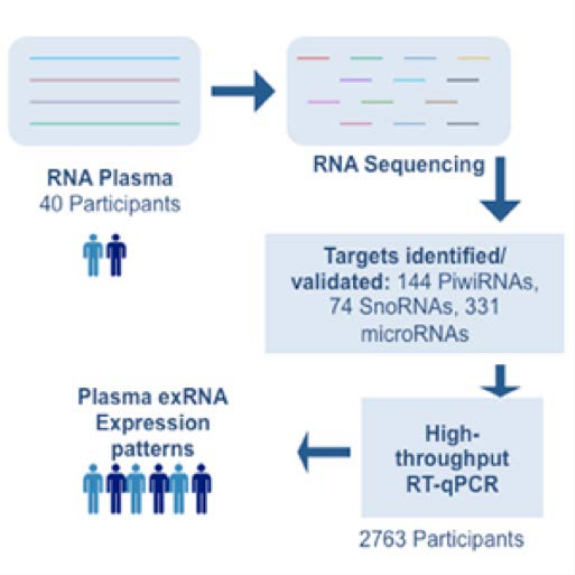
- **Defining a Comprehensive *Reference Profile* of Circulating Human Extracellular RNAs (U01)**
  - Goal: To develop reference profiles for non-coding regulatory exRNAs from healthy human body fluids.
- **Extracellular *RNA Biogenesis, Biodistribution, Uptake, and Effector Function* (U19)**
  - Goal: To determine the biological principles guiding exRNA biogenesis, biodistribution, uptake, and effector function.
- **Clinical Utility of Extracellular RNA for *Biomarker Development* (UH2/UH3)**
  - Goal: To identify and quantify exRNA-based biomarkers derived from human body fluids in order to diagnose diseases, monitor disease progression, and measure response to therapy.
- **Clinical Utility of Extracellular RNA for *Therapy Development* (UH2/UH3)**
  - Goal: To develop and demonstrate the potential for clinical utility of exRNAs as therapeutic agents.
- ***Data Management and Resource Repository* (DMRR)**
  - Goal: To integrate the efforts of all ExRNA Communications program components and serve as a community-wide resource for exRNA standards, protocols, and data.

ABOUT PROJECTS PUBLICATIONS RESOURCES EVENTS BLOG

Exrna.org – a portal for broad dissemination of resources and data

Defining a Comprehensive Reference Profile of Circulating Human Extracellular RNAs (U01)

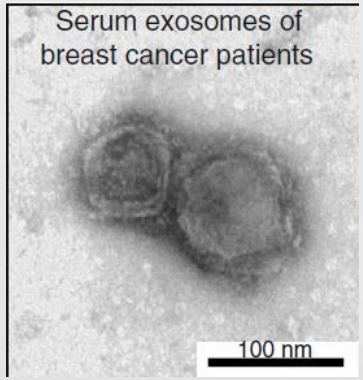




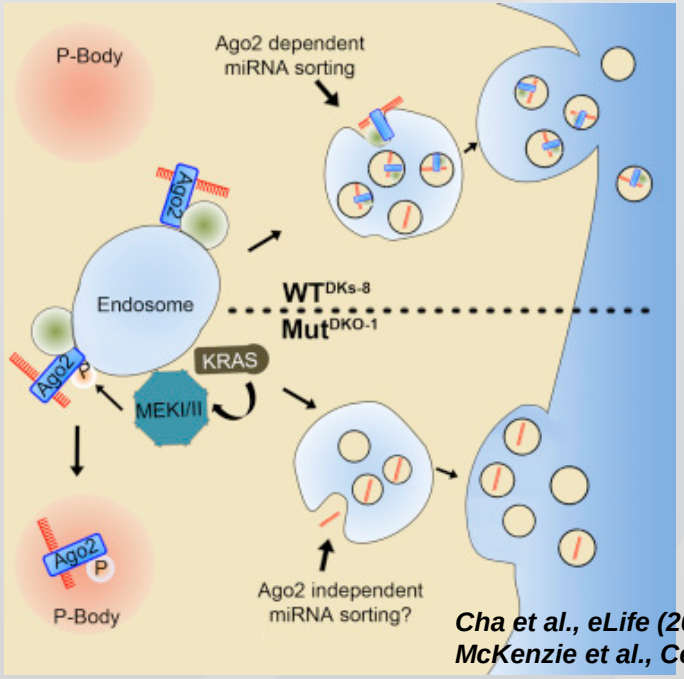
- Defining RNA profiles in Bile, CFS, Serum, Plasma, etc (751 profiles available)
- This represents “normal” individuals for comparison to experimental/disease/affected cohort.
- Appreciation for complexity and diversity in all sources, including plasma[1]:
  - Extracellular small RNAs are widely detected in the circulation in large populations
  - identified over a thousand human extracellular RNAs including microRNAs, piwi-interacting RNA (piRNA), and small nucleolar RNAs
  - Non-human RNAs detected

[1]Freedman, Jane E., et al. "Diverse human extracellular RNAs are widely detected in human plasma." *Nature Communications* 7 (2016).

Extracellular RNA Biogenesis, Biodistribution, Uptake, and Effector Function (U19)



Cancer Cell 26(5): 707-721



Cha et al., eLife (2015)
***McKenzie et al., Cell Reports* (2016)**

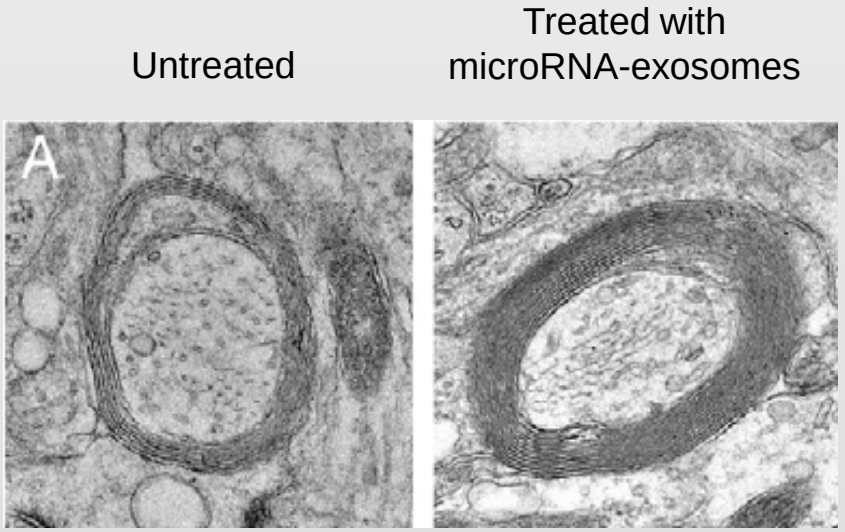
- Cancer exosomes use Dicer, TRBP, and AGO2 to process **pre-miRNAs to generate mature tumor inducing miRNAs**.
- Cancer exosomes mediate efficient and rapid silencing of mRNAs to reprogram a target cell transcriptome.
- **KRAS-MEK-ERK** signaling promotes Ago2 phosphorylation which inhibits Ago2-endosome association and sorting to exosomes
- **Ago2** levels and phosphorylation status control secretion of candidate miRNAs in exosomes
- Indication that RNPs (like Ago2) likely very involved in chaperoning RNAs to final destinations.
- First demonstration that **cell surface receptor signaling can determine loading of a specific miRNA into vesicles**

Calin (University of Texas)
Coffey, Weaver, Patton (Vanderbilt)

Clinical Utility of Extracellular RNA for Therapy Development (UH2/UH3):Neurological Diseases

Dr. Richard Kraig (University of Chicago) has shown **microRNA-containing exosomes promote the formation of myelin** in animal models of multiple sclerosis.

- Exosomes promoting myelin formation are released from immune cells stimulated with IFNγ, and are also produced by young animals and animals experiencing environmental enrichment.
- These exosomes contain microRNAs that promote oligodendrocyte differentiation and protect against inflammation.
- MicroRNA-containing exosomes promote myelin formation in the brain when administered nasally



Pusic et al. (2014) J. Immunology, 266(1-2); Pusic et al. (2014) Glia, 62(2)

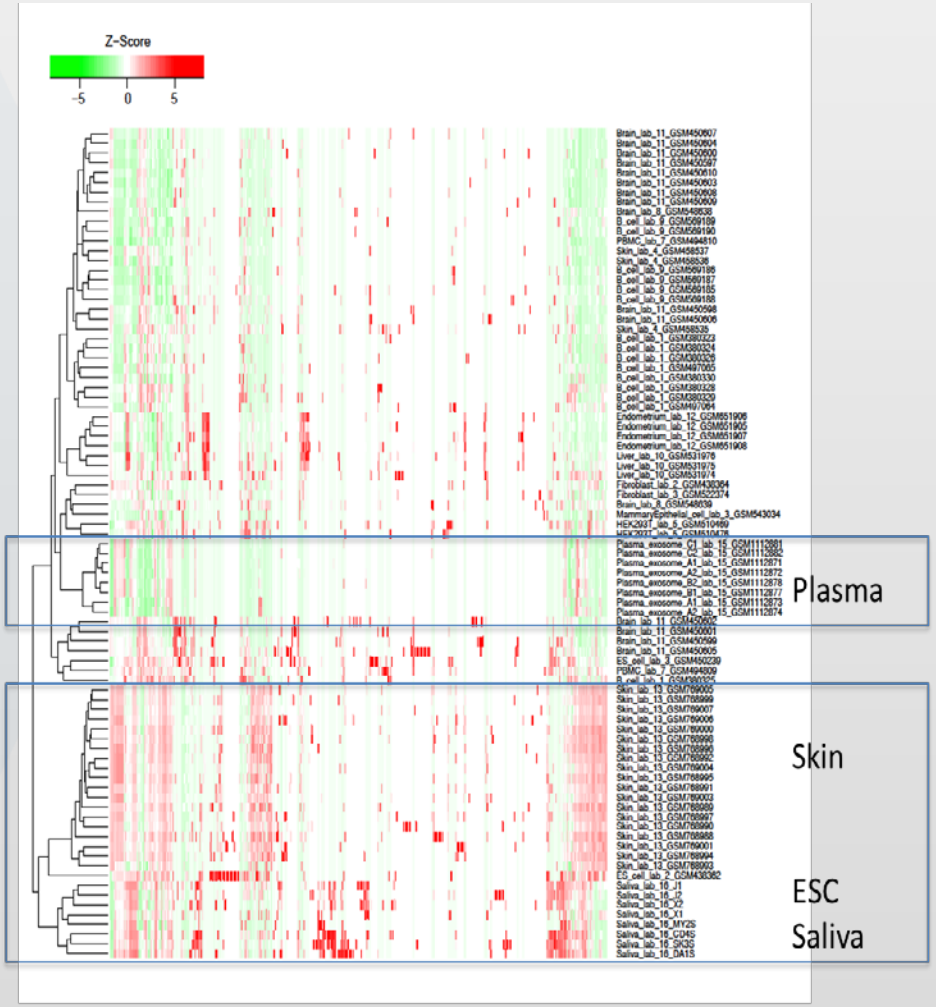
Clinical Utility of Extracellular RNA for Biomarker Development (UH2/UH3): Gastric Cancer

Verified biomarkers for validation: 9 Long RNAs and 7 small RNAs (100 GC and 100 non-GC).

Final cohort: Saliva from 750 GC patients and 750 non-GC controls Individual biomarker validation (500 GC/ 500 non-GC)

Salivary exRNA biomarker panel configuration

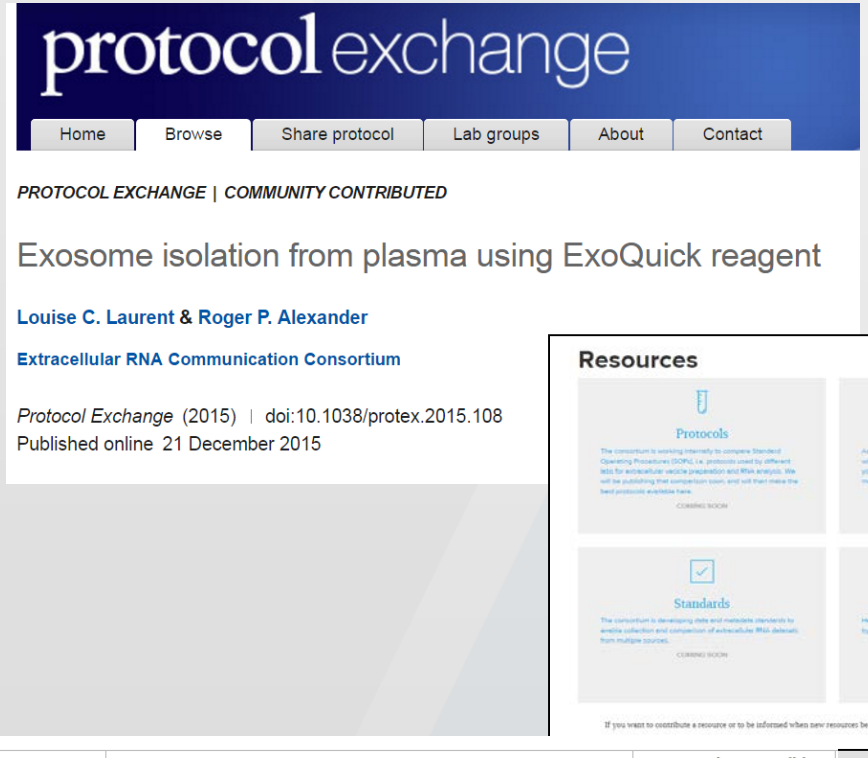
Next: Identification of **bacterial exRNA** biomarkers for gastric cancer in salivary



Bahn JH, Zhang Q, Li F, Chan TM, Lin X, Kim Y, Wong DT, Xiao X. The landscape of microRNA, Piwi-interacting RNA, and circular RNA in human saliva. Clin Chem. 2015;61(1): 221-30. PMCID: 4332885.

David Wong (UCLA)

Data Management and Resource Repository (DMRR)

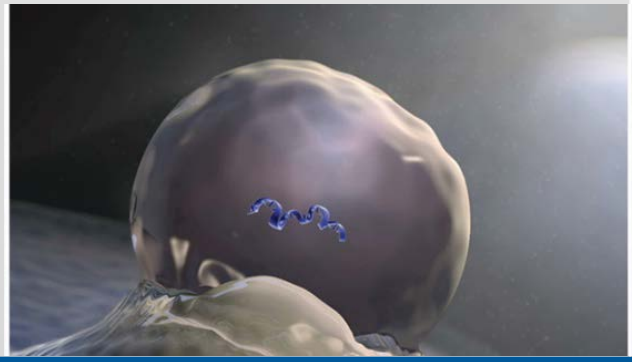


Schema	Description	Doc Template For Editing in Excel
Biosamples TABBED Model	Detailed information about the sequenced sample, biofluid source, etc. Samples can be used in any number of experiments.	Biosample Template ; Multi-tabbed Format
Donors TABBED Model	Information about each individual donor who contributed biosamples.	Donor Template
Studies TABBED Model	A study groups together experiments or analyses for public data release purposes.	Study Template
Experiments TABBED Model	An experiment contains instrument and library preparation information and groups together one or more runs.	Experiment Template
Analyses TABBED Model	An analysis contains secondary analysis results.	Analysis Template
Submissions TABBED Model	Information about PI / submitter associated with submission.	Submission Template
Runs TABBED Model	A run contains sequencing reads submitted in data files.	Run Template

- Genboree Workbench Long RNA-Seq analysis pipeline
- exceRpt - Genboree Workbench Small RNA-seq analysis pipeline

Some Consortium Wide Accomplishments

- 197 total publications in 50 different scientific journals, demonstrating broad reach of the program into various biomedical specialties.
- Generation of highly standardized protocols for vesicle isolation and RNA purification from body fluids, body fluid collection and processing, etc.
- Revision of Gene Ontology terms (GO terms) relevant for exRNA-containing particles.
- Developed standards and standard formats, tools and applications, for RNA-Seq data and metadata standards, which is essential to data harmonization
- PIs are leading a new Gordon Conference and a Keystone Meeting.
- Developing imaging tools to visualize and track vesicles.
- Developing a set of cell lines with mutations in all the known components of vesicle biogenesis.



Remaining Challenges

- ExRNA communication mechanisms are in early discovery stage
- The consortium has gelled and is working together to address some of the cross-cutting fundamental discovery goals of the program.
- All therapy (8) and biomarker (10) projects are moving forward
  - oligodendrocyte differentiation
  - siRNA targeting of mutant Huntingtin mRNA
  - edible plant-derived nano-vector delivery
  - biomarkers for various cancers, cardiovascular risk, placental dysfunction, CNS disease
- However, successful therapeutics should have a foundation of defined biological principles behind them. Some therapeutic goals may be dependent on determining underlying biology – which takes more time.
- Much of this foundational work is hypothesis-generating and broadly enabling, but are there are opportunities for additional focused investments to reveal new paradigms within another 4-5 years?