The Target ALS Multicenter Postmortem Tissue Core

Facilitating Open and Collaborative Access to Data and Resources to Accelerate ALS Research

Lyle W. Ostrow MD PhD
Johns Hopkins University School of Medicine
Lostrow1@jhmi.edu

Johns Hopkins University
- Lyle Ostrow
- Kathleen Wilsbach
- Kathy Gallo
- Kwang-Kai Sauer

Barrow Neurological Institute
- Robert Bowser
- Tina Kovalik
- Lizzi Neylon
- Jessie Duncan

Columbia University
- Matt Harms
- Neil Shneider
- Jessica Singleton
- Benjamin Hoover
- Marie-France Likanje
- Simone Norris

Georgetown University
- Brent Harris
- Galam Khan
- Ashwini Galaday

UCSD
- John Ravits
- Maria Rodriguez
- Gilbert Gutierrez

Washington University St. Louis
- Cindy Ly
- Tim Miller
- Maggie Ireland

NYGC
- Hemali Phatnani
- Duyang Kim
- Nadia Propp
- Delphine Fagegaltier
- Samantha Fennessey

Neurobank / CIB
- Katie Jentoft
- Jason Walker
- Hong Yu
- Prasha Vigneswaran
- Lucia Alvarado-Balderrama

What did we set out to accomplish?

- Provide high-quality, well characterized post-mortem tissue for academic and industry researchers throughout the world.
  - Maximize use of every case.
  - Ensure responsible use of the tissues.
  - Accelerate ALS research.
  - Foster collaboration.
  - Promote open science and the rapid sharing of data
- The sites are linked by a web-based database including de-identified clinical and demographic data, bar-coded tissue inventories, neuropathological data, and QC measures.
- Standard operating procedures (SOPs) for tissue dissection, processing, QC analysis, clinical data elements, and neuropathological characterization are specifically optimized for ALS research.

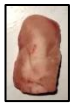
A Federated Model with Centralized Data Curation and Genetics

- Autopsies and data collection are performed at six geographically distributed academic centers (**Core Sites**).
- Postmortem tissues and slides are stored at (and disbursed from) each Core Site.
- Frozen tissue samples, FFPE slides, and associated de-identified data are provided to non-profit academic researchers free of charge.
- Industry labs pay a "transmittal fee" per sample/slide (to defray costs for procurement, curation, and distribution), which is standardized across sites.
- Local site inventories and the corresponding clinical and pathological metadata are linked using platforms developed by the Center for Innovation & Bioinformatics (**CIB**) at MGH, funded by a separate grant by the **ALS Association**.
- Whole Genome Sequencing (**WGS**) and bulk tissue RNA-Seq for multiple CNS regions are performed centrally at the New York Genome Center (**NYGC**), funded separately by grants from the **ALS Association** and **Tow Foundation**. A separate grant from **Target ALS** supports data curation and disbursement from NYGC.
  - The WGS and RNA-Seq raw data are made immediately available without embargo or intellectual property concerns, as soon as the data passes QC.

Researchers using samples and data retain full ownership of their ideas and results, without authorship/IP requirements.

Tissue Dissection

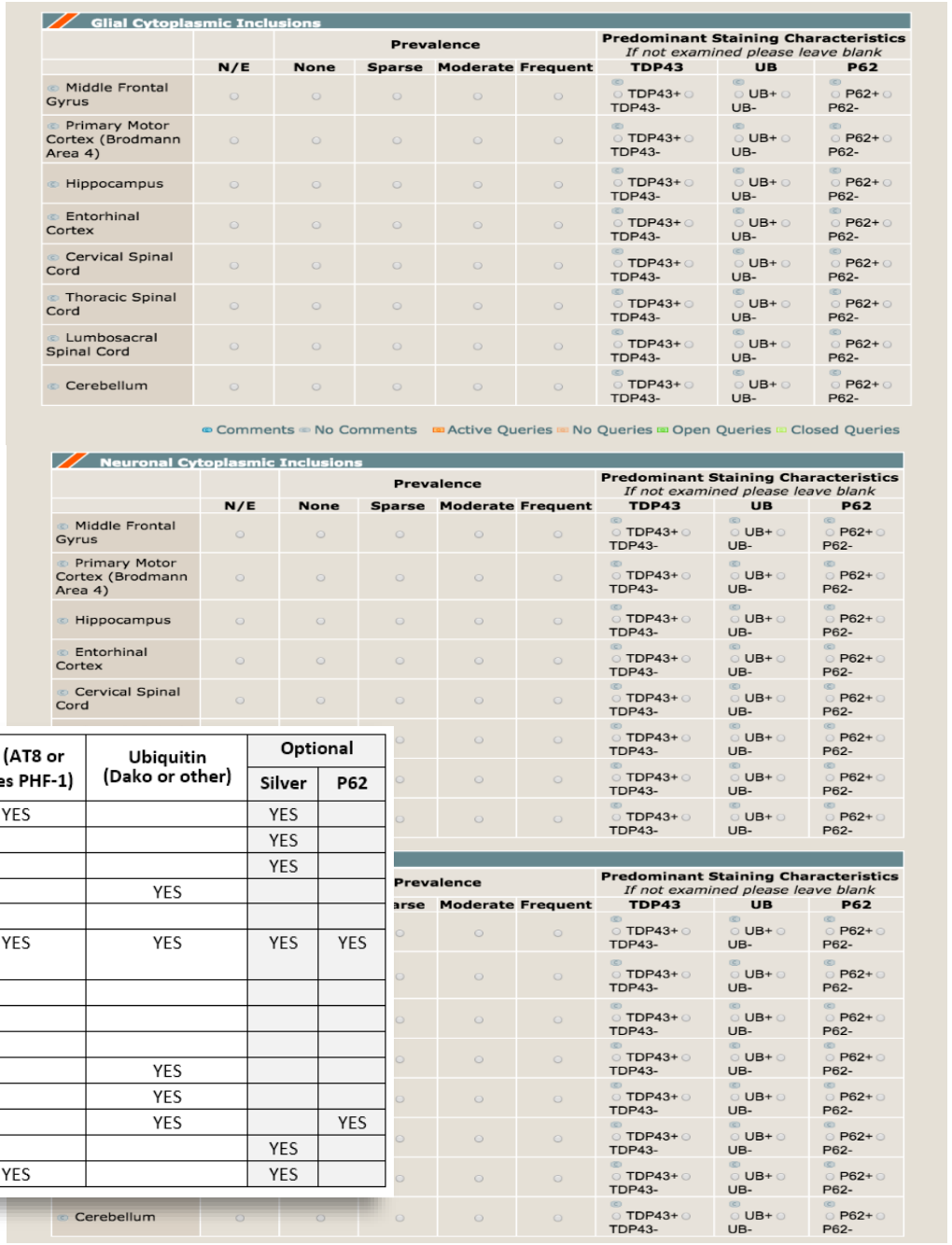
- We collect **frozen and fixed CNS tissues**, **liver**, and **muscle** from ALS/MND and non-neurologic control autopsies.
- Tissue dissection, processing, QC analysis, clinical data elements, and neuropathological characterization are standardized, <u>but also readily modifiable to meet the evolving needs of our researchers</u>.
- Goal is to produce the **maximum number of individual, optimally sized tissue samples from each ALS-relevant region** while preserving the architecture of the tissue.
  - Minimizes subsequent freeze-thaw and labor that otherwise is necessary when re-dissecting frozen slabs or regions to send samples to collaborators.
- **Standardized region nomenclature** enables bar-coding and cataloging of **hierarchical** tissue inventories in a centralized core database.
- Tissue inventories and all data are linked using **online searchable platforms**.
- **Sites are free to do more dissection**, other regions, etc., as long as their method enables banking of these standard regions from **<u>at least one hemisphere, brainstem, and majority of the spinal cord</u>**.



GUID Number:	Enter GUID in Worksheet Name																			
Site Id #																				
TISSUE REGION (Regions in bold are required)	**Label-Visible Abbreviation**	**Frozen Barcode Labels**												**Paraffin Barcode Labels**						
		LEFT				**RIGHT**				**UNSPECIFIED**				**LEFT**		**RIGHT**		**UNSPEC**		
BRAIN REGIONS																				
Cortical Regions		A	B	C	D	A	B	C	D	A	B	C	D	E	F	E	F	E	F	
Primary Motor Cortex: (precentral gyrus)	MotCtx																			
Medial	**MotMed**																			
Middle	**MotMid**																			
Lateral	**MotLat**																			
Primary Sensory Cortex (postcentral gyrus)	**SenCtx**																			
Frontal Pole Cortex	**FrCTX**																			
Temporal Lobe Cortex	**TempCTX**																			
Occipital Pole (Visual) Cortex	**OccCTX**																			
Insular Cortex	InsCTX																			
Parietal Lobe Cortex	ParCTX																			
Middle Frontal Gyrus Cortex	MidFrCTX																			
Other Cortex	CTX-Other																			
Hippocampus	**HP**																			
Amygdala	AmyG																			
Basal ganglia	BG																			
Globus pallidus	GP																			
Striatum	STR																			
Caudate	Cau																			
Putamen	Put																			
Brainstem en bloc	Brstm																			
Midbrain/Substantia nigra	**MB-SN**																			
Pons	**Pons**																			
Medulla	**Medulla**																			
Cerebellum	**Cbl**																			
Cerebellar Hemisphere	Cbl Hem																			
Cerebellar Vermis	Cbl Vrm																			
Choroid Plexus	ChorPlx																			
Cingulate Gyrus	CinG																			
Thalamus	Thal																			
SPINAL CORD																				
Cervical Spinal Cord	**CervSC**																			
Thoracic Spinal Cord	**ThorSC**																			
Lumbo-sacral Spinal Cord	**LumSC**																			
Cauda equina	CaudaSC																			
Unspecified Spinal Cord	SC-Unsp																			
Liver	**Liver**																			
Skin	**Skin**																			
Muscle - ***Collect at least 1 from list below; multiple per case preferred.***																				
Biceps	Mus-Bcps																			
Deltoid	Mus-Delt																			
Diaphragm	Mus-Dphrm																			
Intercostal	Mus-Intrcos																			
Psoas	Mus-Psoas																			
Other Muscle	Mus-Other																			
Nerve																				
Dorsal root ganglion	DRG																			
Nerve Root	NRt																			
Motor (Ventral) Nerve Root	Mot-NRt																			
Cervical Motor Nerve Root	C-M-Nrt																			
Thoracic Motor Nerve Root	T-M-Nrt																			
Lumbo-sacral Motor Nerve Root	L-M-Nrt																			
Sensory (dorsal) Nerve Root	Sen-NRt																			
Cervical Sensory Nerve Root	C-S-NRT																			
Thoracic Sensory Nerve Root	T-S-NRT																			
Lumbo-sacral Sensory Nerve Root	L-S-NRT																			
Peripheral Nerve	PN																			

Neuropathology

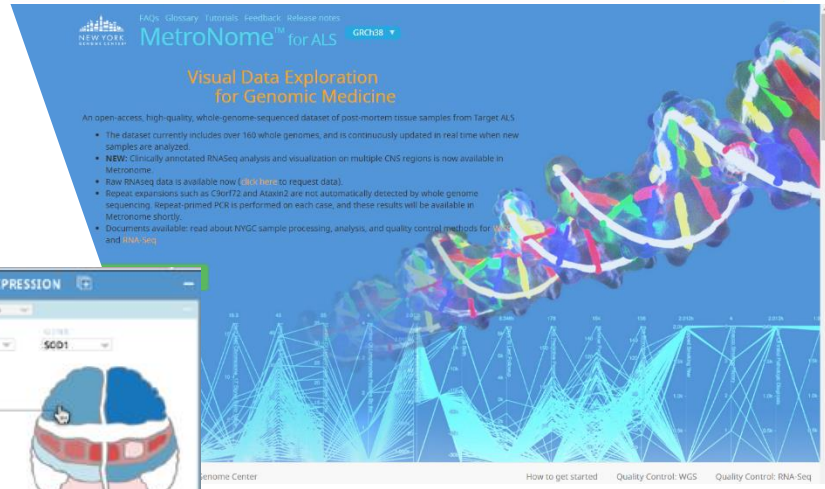
- Neuropathological data elements are customizable and entered into online database.
- The neuropathology is linked to the de-identified clinical data, genetics, and the available frozen and fixed tissue inventories.
- A standardized minimum diagnostic slide set is required for each case (modifiable).

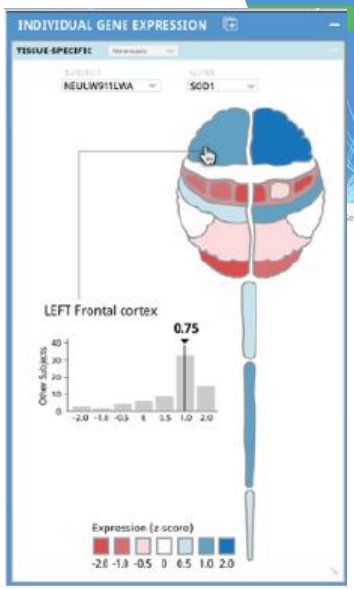


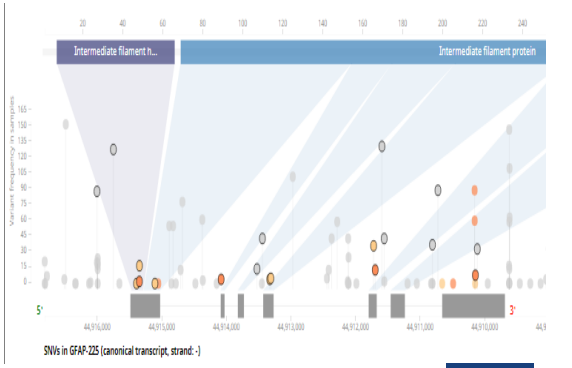
Block	H&E	H&E + Luxol FB (or Nissl + LFB)	TDP43 and/or pTDP43	Tau (AT8 or Davies PHF-1)	Ubiquitin (Dako or other)	Optional	
						Silver	P62
Midfrontal gyrus	YES		YES	YES		YES	
Superior & Middle temporal	YES					YES	
Occipital Cortex	YES					YES	
Motor Cortex	YES		YES		YES		
Basal ganglia/basal forebrain	YES						
Hippocampus + Entorhinal cortex (medial temporal lobe)	YES		YES	YES	YES	YES	YES
Midbrain	YES						
Pons	YES						
Medulla		YES					
Spinal Cord, cervical + thoracic	YES	YES	YES		YES		
Spinal Cord, lumbosacral	YES	YES	YES		YES		
Cerebellum	YES		YES		YES		YES
Inferior parietal lobule	YES					YES	
Amygdala	YES			YES		YES	

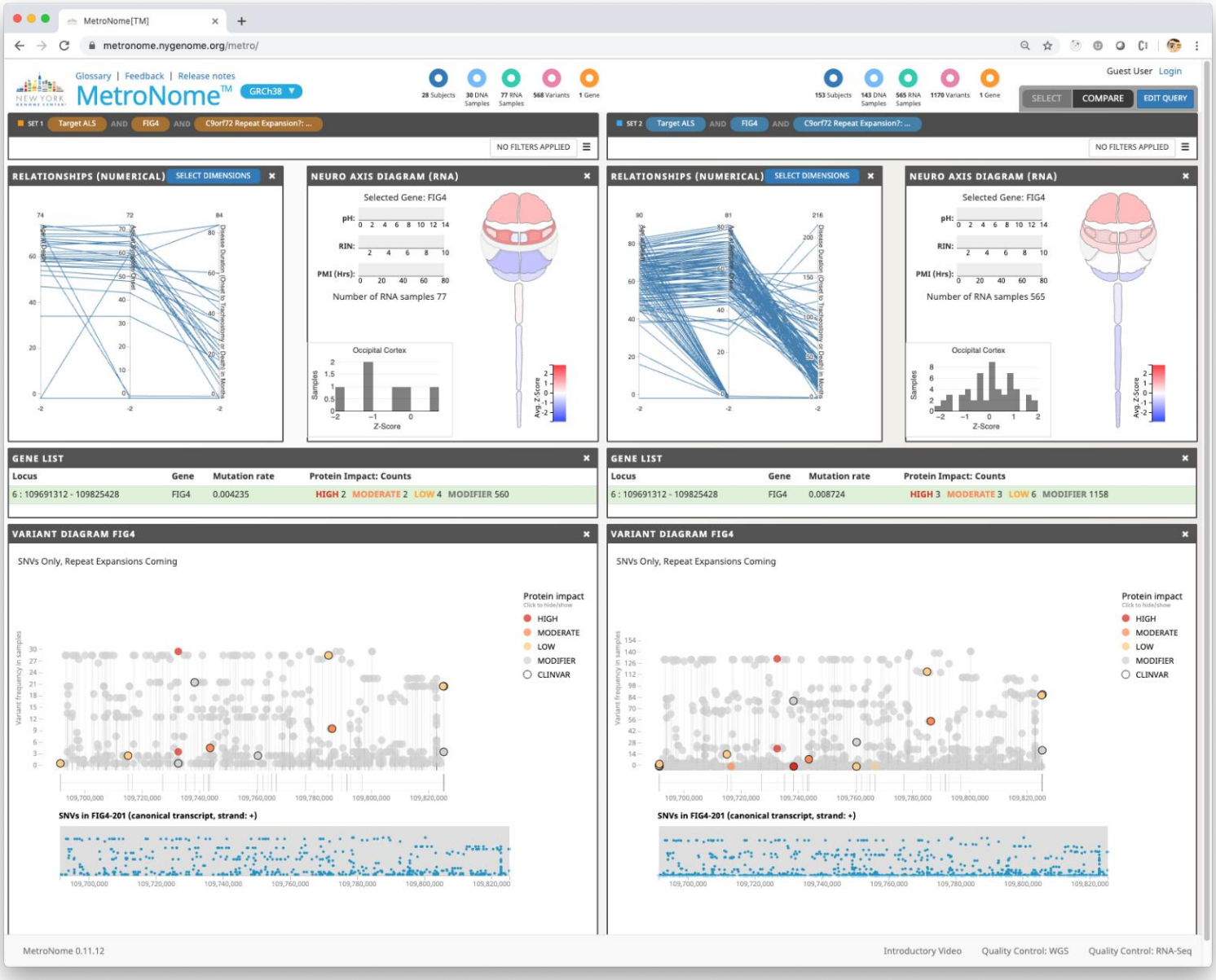
Genetics

- Whole Genome Sequencing (**WGS**), and multiple CNS region **RNA-seq** are performed on every autopsy.
- After passing QC, new genetic data is made immediately and freely available, linked to the tissue samples and de-identified metadata, with no embargo or IP concerns.
- WGS and RNA-seq raw data files in multiple formats can be requested via an **online form** and established data transfer workflow.
- **C9orf72** and **Ataxin2** are separately tested by rpPCR at Columbia University (Matt Harms Lab).
  - Also perform **ExpansionHunter** on PCR-free DNA and comparing the results.
- The clinically annotated genomic and RNAseq data can be visualized and explored online using the **MetroNome Visual Data Exploration Platform**.
- Exploring ways to integrate additional data analysis and visualization tools into our workflow – drop-seq/nuc-seq, spatial proteomics/MS, deep sequencing for somatic mosaicism, St. Jude Cloud analysis tools, etc.

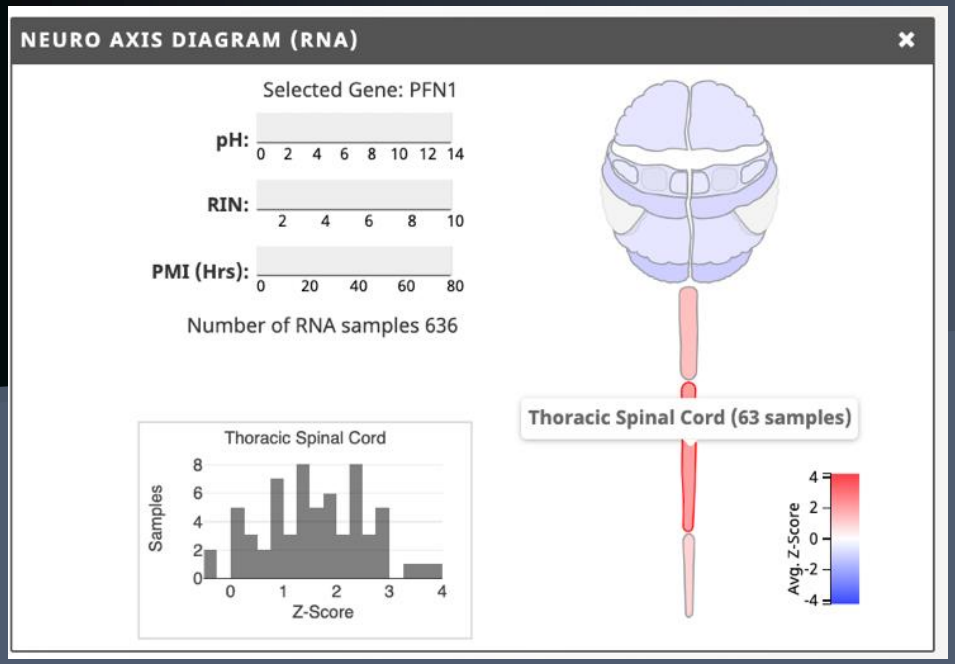








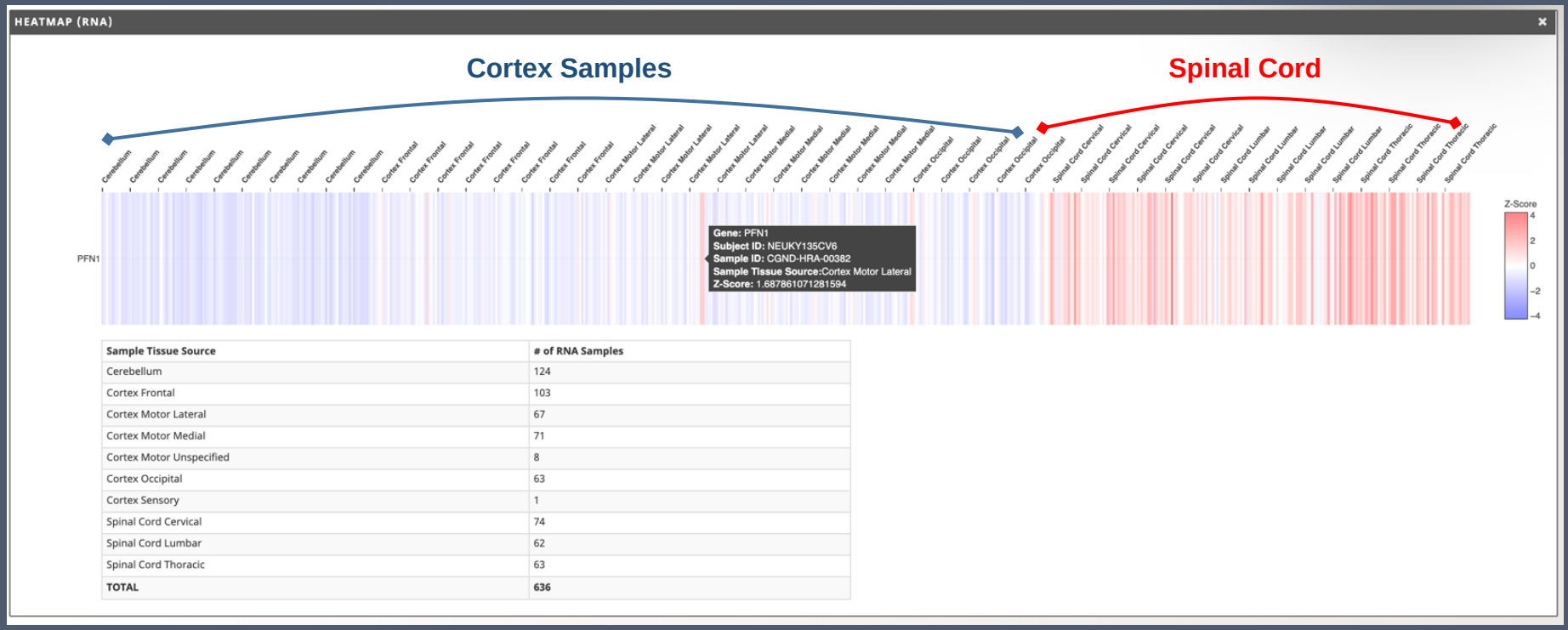
Comparison of cohorts with C9orf72 repeat expansions (left) and without (right), showing gene expression patterns for FIG4 in an anatogram (top) and variants (bottom).



Metronome query for the gene PFN1

The neuro axis diagram (left) clearly shows higher expression in spinal cord, and particularly in thoracic spinal cord.

The RNA heatmap (bottom) confirms generally higher expression in spinal cord samples, but also reveals a couple samples with very high cortical levels.



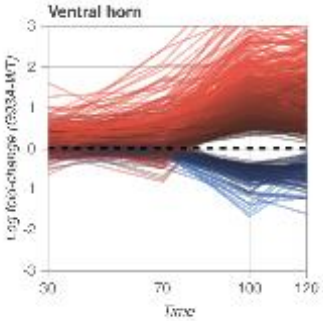
Sample Tissue Source	# of RNA Samples
Cerebellum	124
Cortex Frontal	103
Cortex Motor Lateral	67
Cortex Motor Medial	71
Cortex Motor Unspecified	8
Cortex Occipital	63
Cortex Sensory	1
Spinal Cord Cervical	74
Spinal Cord Lumbar	62
Spinal Cord Thoracic	63
TOTAL	**636**

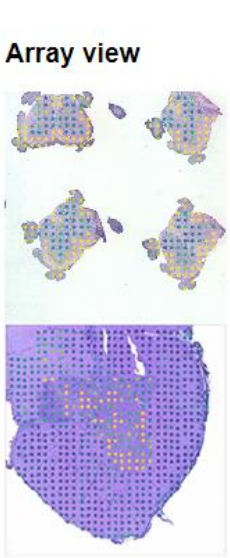
How do we use the genetic data?

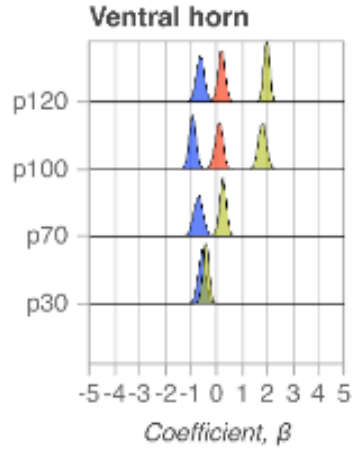
- Identify relevant tissue samples and slides for requests.
  - Variants or expression changes in specific targets
  - “Clean controls”
  - Comparing spatial patterns with published imaging biomarker data
- Examining whether gene expression patterns are consistent with activation of pathways modulated by potential new drugs candidates.
- Identify whether specific subgroups display gene signatures enabling patient selection for clinical trials.
  - Segregating patients based on gene expression, splicing, spatial patterns…
  - Do these genetic patterns correspond to secreted biomarkers?

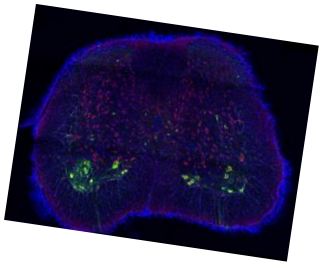
Spatial Transcriptomics (ST)

- Separately funded program at NYGC.
- ST can be correlated with standard neuropath slide images and data, detailed clinical phenotyping, WGS, and high-quality bulk RNAseq data from the same decedents.
- Online platform to visualize and explore ST data, including searching for specific genes in corresponding human and mouse datasets.
- Frozen and fixed samples and slides from the corresponding autopsy cases and regions can then be provided to researchers for further studies.

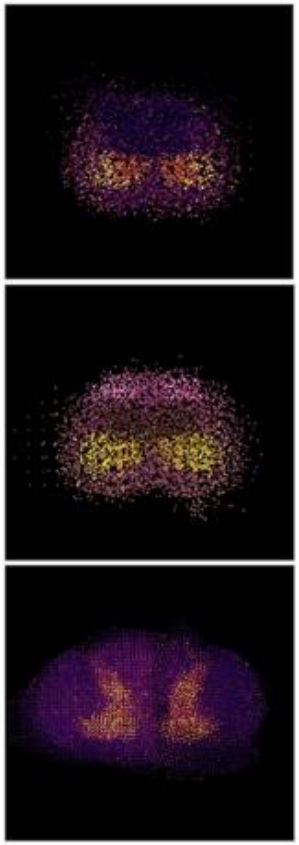


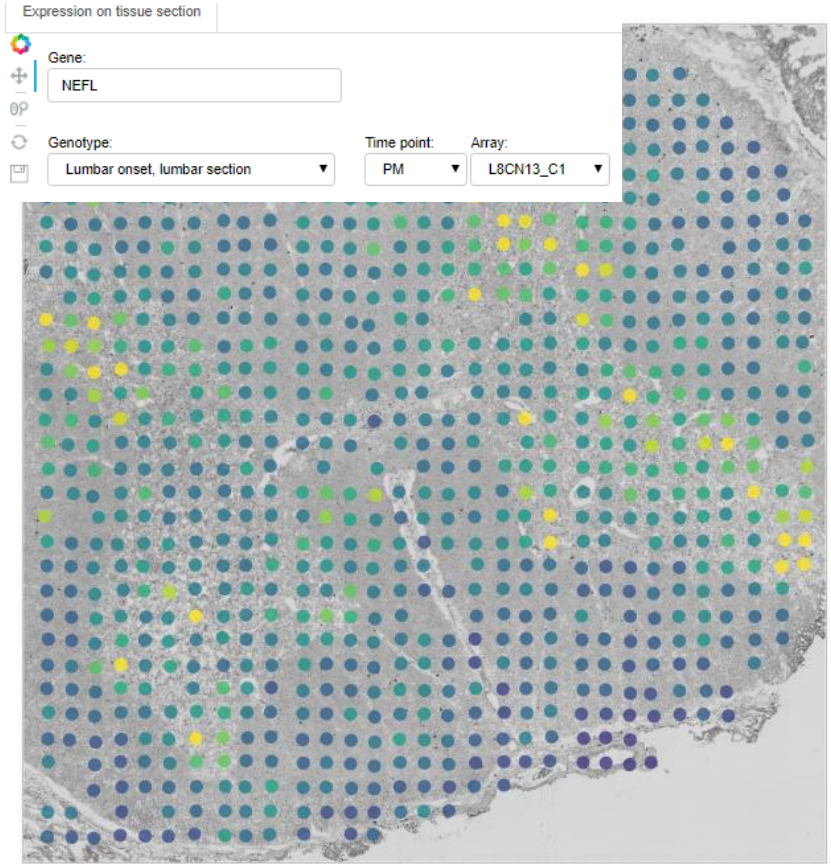






ALS-ST.NYGENOME.ORG

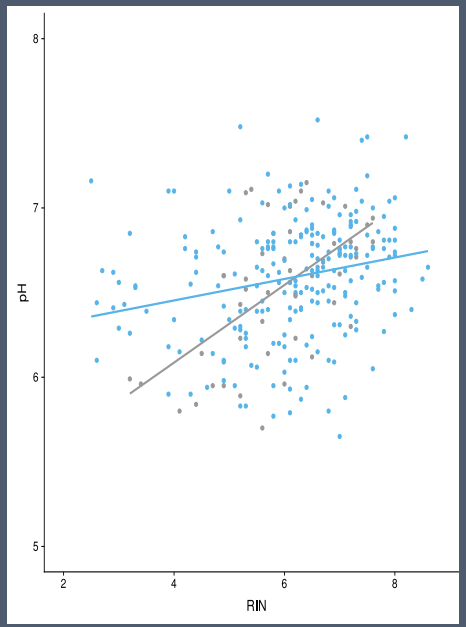


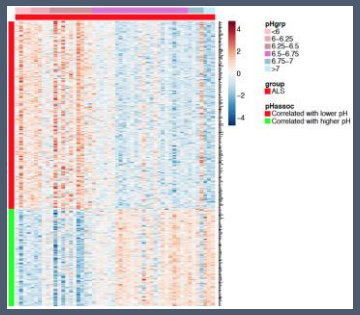


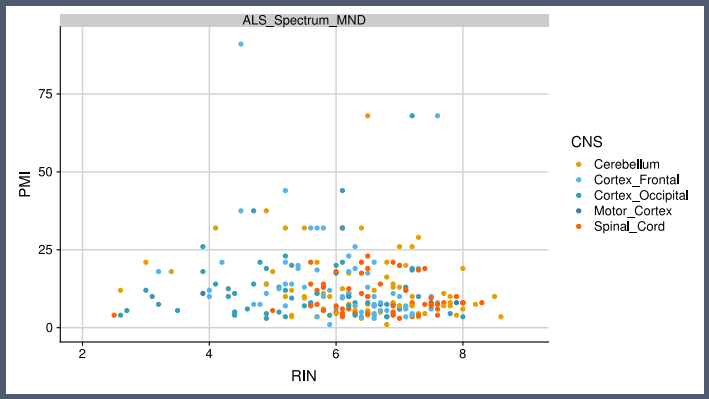
QC Measures on multiple CNS regions

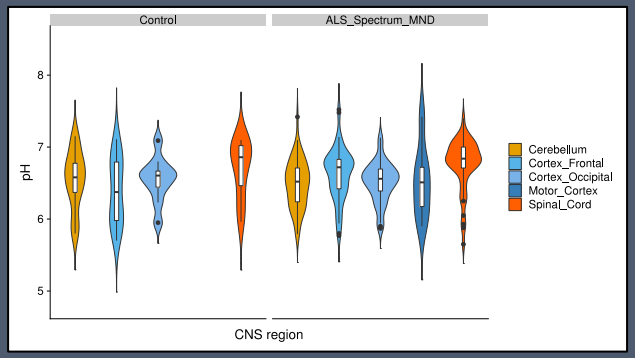
- Post-mortem Interval
- RNA Integrity Number (RIN)
- Tissue pH

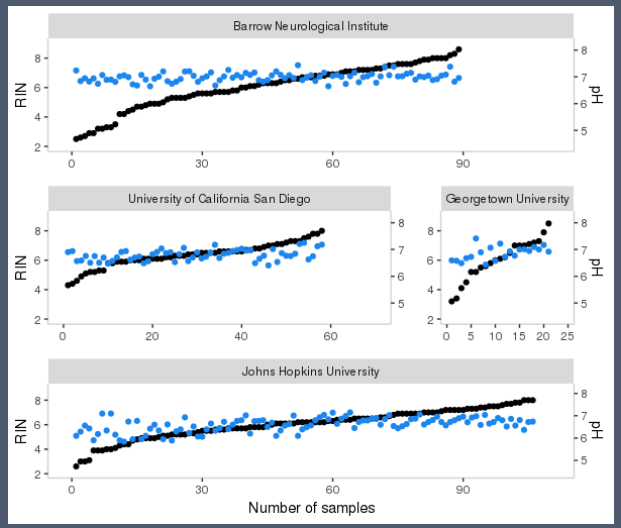
All QC data is linked to the samples and genetics.

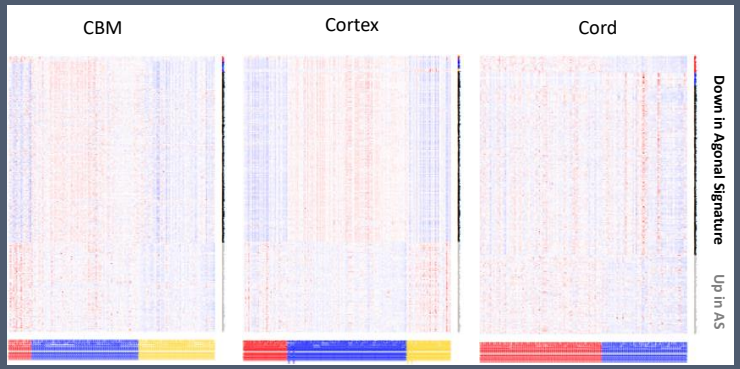












NEW YORK GENOME CENTER®

High Throughout Sequencing

FastQ

Alignment to GRCH38 (STAR)

BAM

Visualized report

Output data

- Total reads/samples
- % rRNA, **mitochondrial reads**
- **Mean GC content**
- % Duplicates/Library complexity
- Insert size
- % aligned reads
- % gene assignment, strandedness
- **Gene Body Coverage**
- **Xist vs. chrY transcripts expression**
- **Patient (WGS-RNA concordance)**
- Gene quantification
- **Unsupervised Clustering** (batch, swap)
- Normalized Gene expression
- **Expr. Tissue-enriched markers**
- Transcript quantification

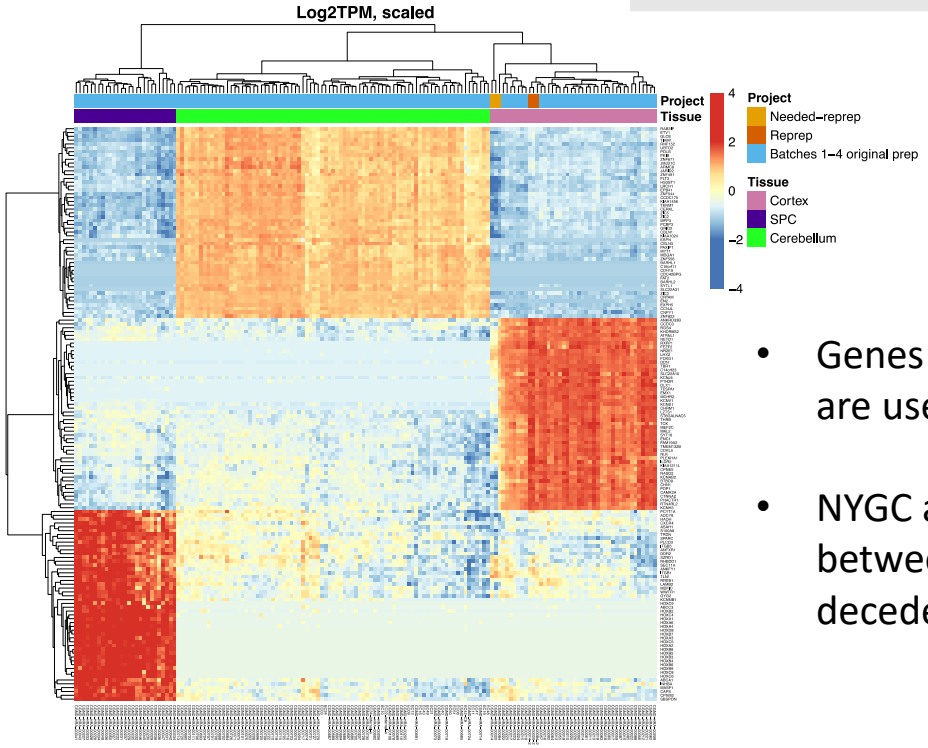
RSeQC, Picard Mark Duplicates

verifyBamID

FeatureCounts DESeq2

Kallisto

Review
Sample Passes QC -> delivered

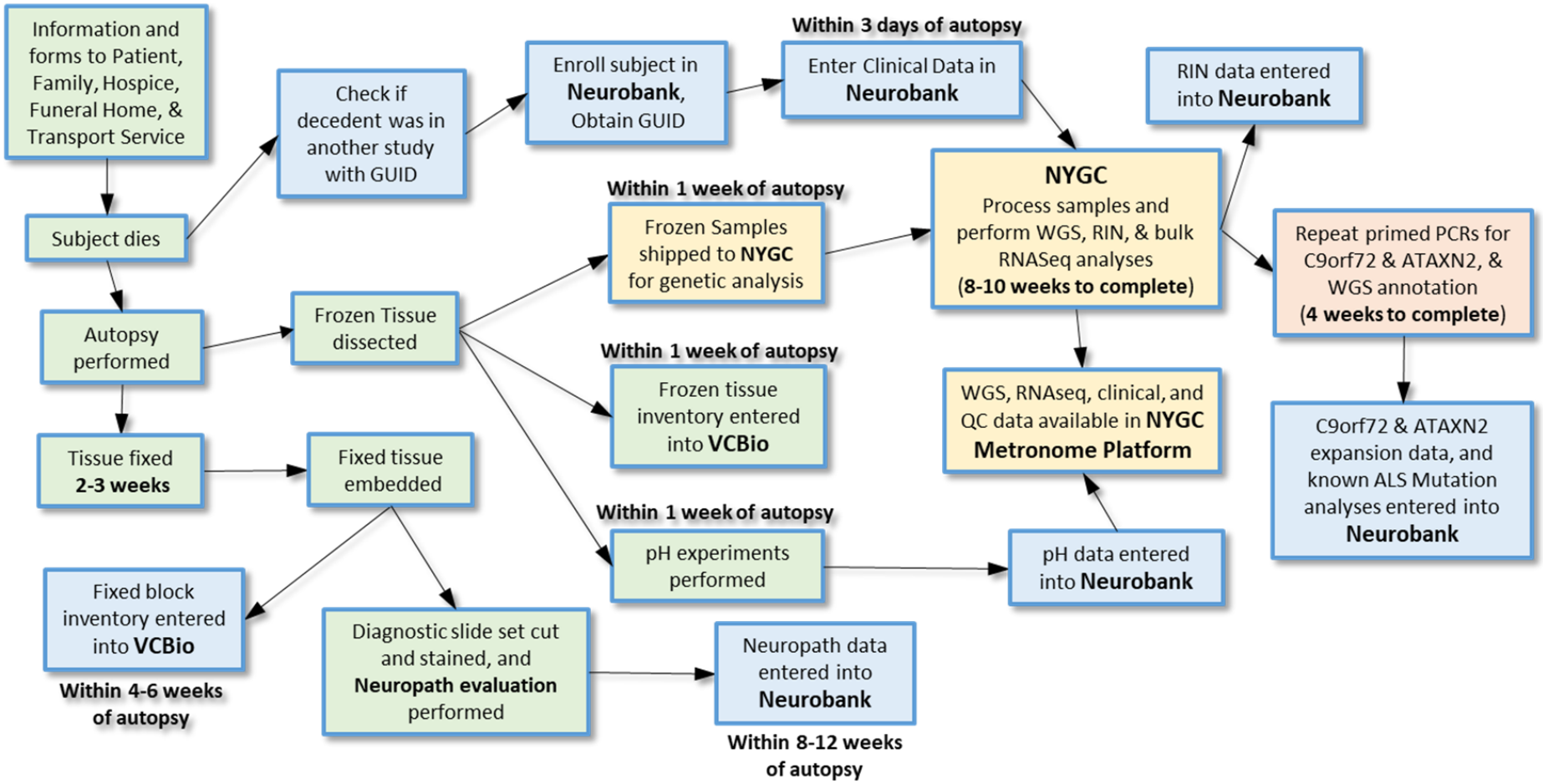


- Genes specific of the Cortex, Spinal Cord and Cerebellum are used to detect mislabeled samples.

- NYGC also systematically computes the concordance between the RNASeq samples and WGS from each decedent.

Symptom Onset → Disease Course → Autopsy

- Clinical data is collected pre-mortem.
  - Number and timing of visits differ.
  - Data such as clinical phenotype and EMG results may be collected multiple times at different points in the disease course.
- Gross pathological data, post-mortem interval (PMI), and other peri-mortem variables are collected at the time of autopsy.
- Frozen and fixed tissue inventories are entered after the autopsy dissection is completed.
- Samples for WGS and RNASeq need to be sent to NYGC for analysis.
- Harms Lab needs to obtain portions of the NYGC samples for C9orf72 determination.
- Genetic data needs to be analyzed and annotated (Harms Lab *and* NYGC), uploaded to Metrononome, and entered back into into Neurobank.
- Fixed tissues must remain in formalin/paraformaldehyde for a couple weeks before they can be embedded in paraffin, after which slides need to be cut and stained.
- pH determinations require further experiments on specific regions from each case, then this data then needs to be entered into the Neurobank database.
- A formal autopsy report is generated 30-60 days after death after which the neuropathological data is entered into the database.

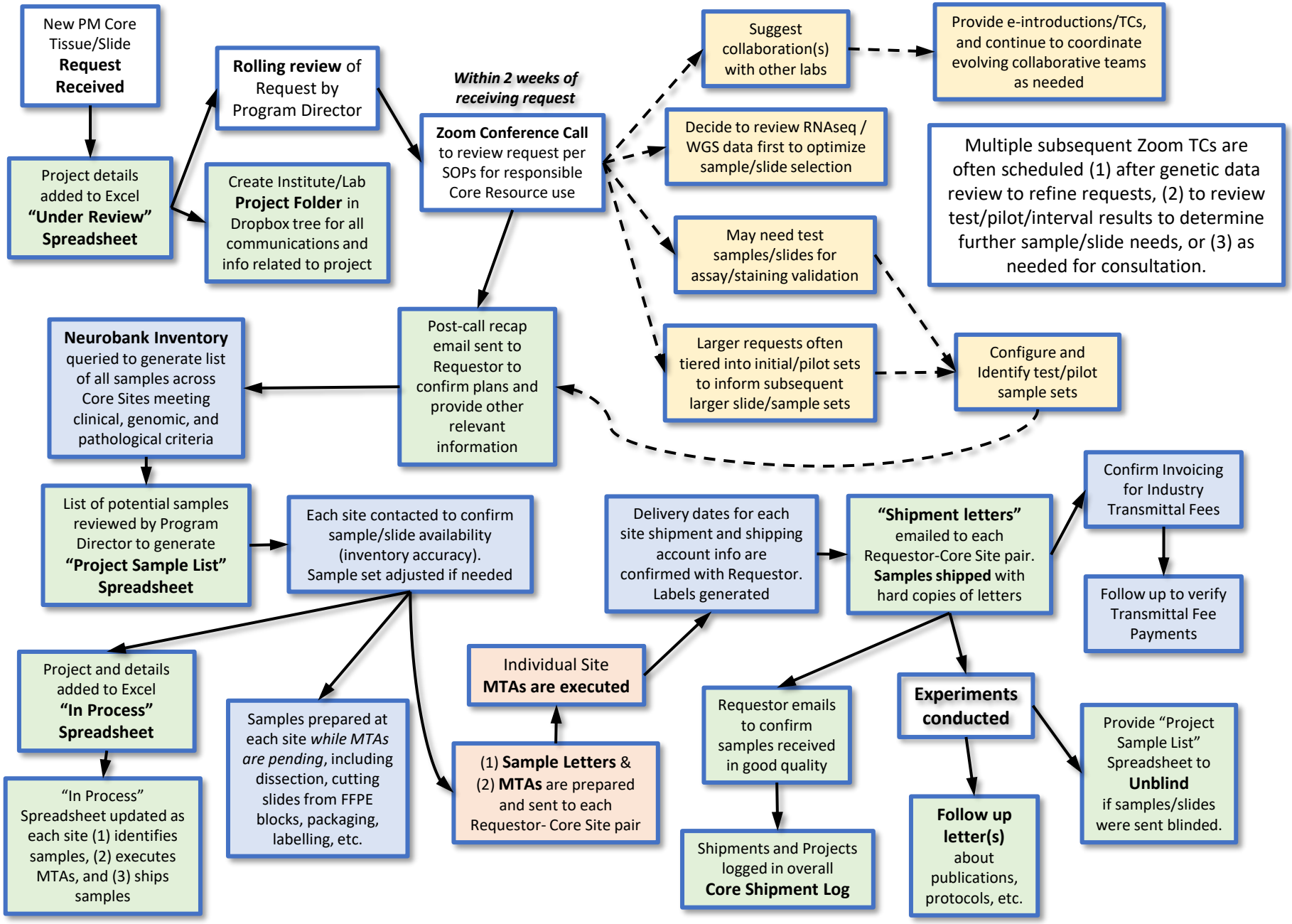


And then comes the really hard part that has actually been consuming all of our time!

In contrast to many biorepository efforts which focus primarily on sample procurement and analysis, most of our efforts are devoted to efficiently designing and supplying the optimal sets of samples, slides, and data resources to best accelerate each individual research project.

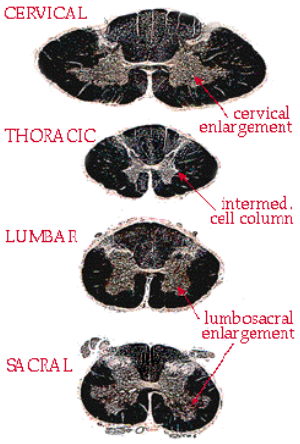
Accelerating research and fostering collaboration

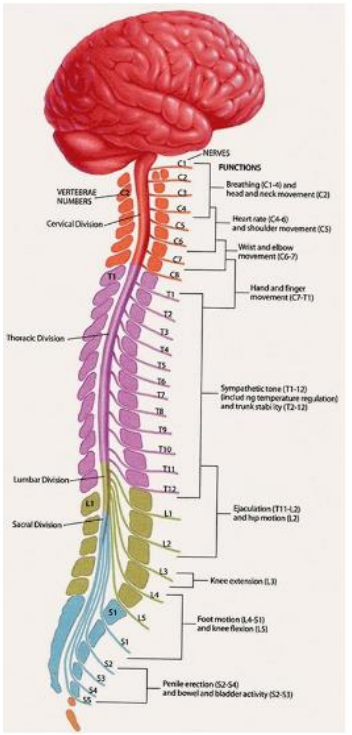
- All policies are designed to provide samples and data as quickly as possible, while ensuring responsible, open, and unbiased use of all Core resources.
- Requests for samples and data are reviewed **on a rolling basis**, using **established criteria that emphasize experimental feasibility and appropriateness of sample sizes and quantities**.
- We have formulated **standard MTAs for both academic and industry collaborators** for all sites with common language.
- **Our goal is to say "yes" to every request**, thus the Core Director works closely with each researcher to help optimize experimental plans, validate assays, and *ensure that researchers are always thinking about the next step – such as identifying potential biomarkers for eventual clinical trials*.
- For a post-doc, new investigator, or established scientist with an entirely new idea, we can rapidly provide the resources and data to obtain preliminary results ("discovery"), and then foster further development and real-time collaboration as the idea evolves.
- Often, we can **facilitate collaborations with established academic or industry labs already using our Cores**, or invoke other existing Core Resources to provide robust complimentary data and results.
- New ideas can be tested rigorously within weeks, rather than the months-to-years that would normally be needed to apply for grants, get funded, establish lab assays locally, and conduct preliminary experiments.

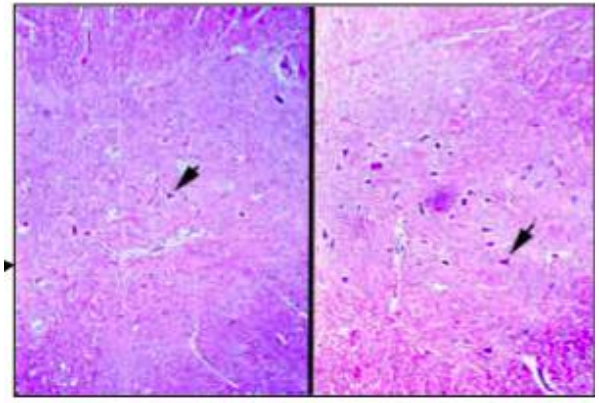

New PM Core Tissue/Slide **Request Received**
Rolling review of Request by Program Director
Project details added to Excel **"Under Review" Spreadsheet**
Create Institute/Lab **Project Folder** in Dropbox tree for all communications and info related to project
Within 2 weeks of receiving request
Zoom Conference Call to review request per SOPs for responsible Core Resource use
Suggest collaboration(s) with other labs
Provide e-introductions/TCs, and continue to coordinate evolving collaborative teams as needed
Decide to review RNAseq / WGS data first to optimize sample/slide selection
May need test samples/slides for assay/staining validation
Larger requests often tiered into initial/pilot sets to inform subsequent larger slide/sample sets
Multiple subsequent Zoom TCs are often scheduled (1) after genetic data review to refine requests, (2) to review test/pilot/interval results to determine further sample/slide needs, or (3) as needed for consultation.
Configure and Identify test/pilot sample sets
Post-call recap email sent to Requestor to confirm plans and provide other relevant information
Neurobank Inventory queried to generate list of all samples across Core Sites meeting clinical, genomic, and pathological criteria
List of potential samples reviewed by Program Director to generate **"Project Sample List" Spreadsheet**
Each site contacted to confirm sample/slide availability (inventory accuracy). Sample set adjusted if needed
Project and details added to Excel **"In Process" Spreadsheet**
"In Process" Spreadsheet updated as each site (1) identifies samples, (2) executes MTAs, and (3) ships samples
Samples prepared at each site *while MTAs are pending*, including dissection, cutting slides from FFPE blocks, packaging, labelling, etc.
Individual Site **MTAs are executed**
(1) **Sample Letters** & (2) **MTAs** are prepared and sent to each Requestor- Core Site pair
Delivery dates for each site shipment and shipping account info are confirmed with Requestor. Labels generated
"Shipment letters" emailed to each Requestor-Core Site pair. **Samples shipped** with hard copies of letters
Confirm Invoicing for Industry Transmittal Fees
Follow up to verify Transmittal Fee Payments
Requestor emails to confirm samples received in good quality
Shipments and Projects logged in overall **Core Shipment Log**
Experiments conducted
Follow up letter(s) about publications, protocols, etc.
Provide "Project Sample List" Spreadsheet to **Unblind** if samples/slides were sent blinded.

Examples of experimental design considerations with human postmortem tissues

- **Conserving the scarcest resources**
  - Non-neurological control tissues
  - Cervical and lumbosacral spinal cord
  - Decedents with specific genetic mutations
- **Bulk tissue assays on spinal cord**
  - Can be biased by sectioning axis and grey vs. white matter at different cord levels
  - Often useful especially for motor neuron-related targets/pathways
- **When is it appropriate to use thoracic instead of cervical or lumbosacral spinal cord?**
- **Using Cortical regions vs. spinal cord.**
  - Motor neuron cell bodies
  - UMN vs LMN pathology
- **Pathological variability vs assay variability – meaning of "end stage" tissue**
  - Pathology can be highly variable through the neuroaxis within a given decedent
  - Importance of having pathological data to correlate with assays
- **Considerations for proper controls**
  - Using non-neurological control decedent tissues vs. comparing involved to uninvolved cortical regions.
  - May depend on whether measurement is MN specific
  - Genetic ALS vs sporadic ALS
- **"Please be sure that all of the ALS samples include pathological TDP-43 inclusions."**
- **Normalizing ELISA/WB data to cell-type specific markers.**
- **Human CNS Tissues have substantial Autofluorescence**
- **"Slide arithmetic"**
  - 5 slides each from 3 CNS regions, 10 sporadic ALS, 10 C9 ALS, 10 non-neuro controls = 450 slides







How are we doing?

- We have provided many thousand tissue samples and slides to over 120 academic and industry labs, facilitating more than 200 different ALS research projects.
  - In the 34 months between March 2017 and December 2019, we disbursed ~6,200 slides and over 1,200 frozen tissue specimens for 125 different research projects, and usage has continued increasing since then.
  - Slides and tissue samples are usually provided in several batches, as the projects progress and we work together to optimize experiments.
- Over 150 data transfers comprising >700 terabytes of genomic data have been provided to 70 academic and industry research institutions in 15 different countries.
- We routinely provide letters of support for grants and fellowship applications to NIH, MDA, ALSA, Packard, DOD, and others.
- Several large collaborative projects involving industry, academia, and nonprofits have directly resulted from use of these resources.

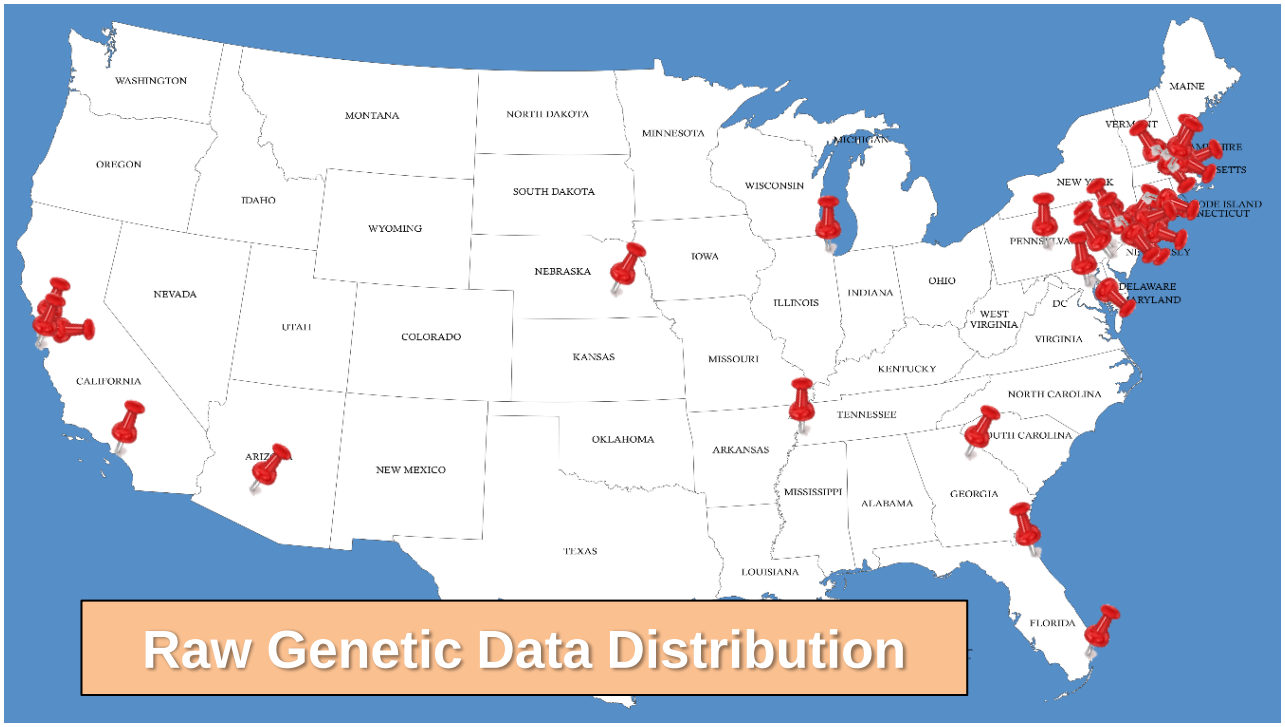
Examples of research using our PM Core tissues

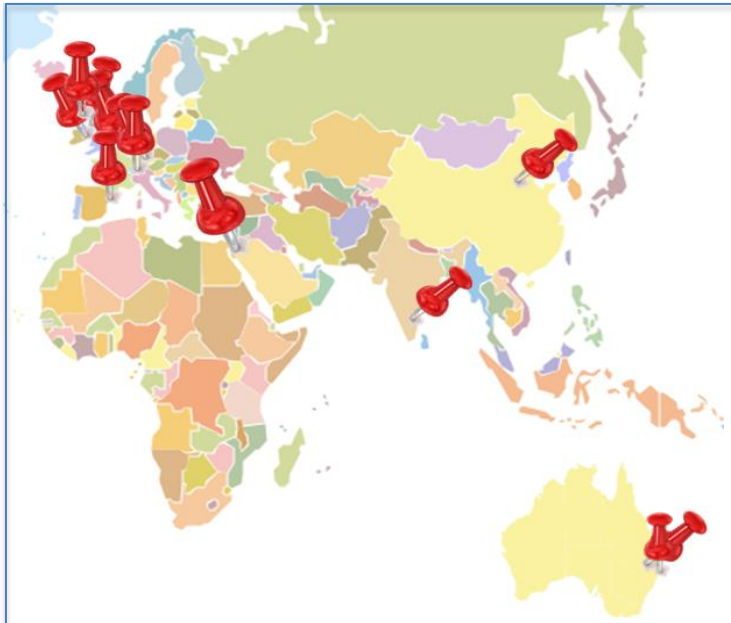
- Aberrant transient multiprotein assembly complexes
- ADAR2 assoc. RNA editing in C9orf72
- Antisense RAN dipeptides in C9orf72
- Autophagy-related protein 7 (Atg7) in sporadic ALS
- C9orf72 southern blots from different brain regions and organ tissues
- Comparing nuc-sec of postmortem spinal cord MNs and iPSC-MNs.
- Connexin dysfunction in ALS
- Correlating RNAseq with heavy metals
- CSF biomarkers in choroid plexus
- Developing a muscle protein multiplex assay for ALS diagnosis.
- Disrupted Blood-CSF barrier integrity in choroid plexus
- DPR localization and interaction with nuclear transport in C9orf72
- Ephrins in ALS
- Epigenetics of C9orf72
- ER-associated degradation (ERAD)
- Exosomes in SOD1 ALS
- GDE2 in SOD1 ALS
- Golgi impregnation to visualize dendritic trees
- HITS-CLIP for TDP-43
- Human endogenous retroviruses (HERV-K) in ALS
- IHC for senescence pathway targets
- Klotho as a novel therapeutic target in neurodegenerative disease
- LINE1 retrotransposons
- Long non-coding RNAs (lncRNAs) in SBMA (Kennedy's Disease)
- Lrp1 and lipoprotein metabolism in ALS
- Mass spec / proteomics in C9orf72
- Mechanisms of cortical hyperexcitability
- MG53 (TRIM72) and membrane repair
- Microglial dysfunction in C9orf72 FTD
- Novel antibody development and ELISAs for TDP43 aggregates
- Nuclear RNA transcriptomics in C9orf72
- Nucleolar stress, p52 and ribosomal subunits
- Nucleoporins in C9orf72
- OPTN and RIP1 kinase in necroptosis
- Palmitoylation in SOD1 ALS
- Pathobiology of ALS4 (SETX mutation)
- Pathobiology of C9orf72 neurodegeneration
- Post-translational modifications of TDP-43
- QC studies of human postmortem tissues and relationship to RNA expression profiles
- Rab-protein mediated endocytosis in C9orf72
- RAGE dependent microglial signaling
- RAN-Gap mislocalization in oligodendroglia and OPCs
- Retromer complex defects, TDP43, and protein aggregates
- RIP1K in microglial activation
- RNA editing enzyme levels and glutamate receptor subunit editing
- RNA Foci in C9orf72
- RNA helicase repression of RAN translation in C9orf72
- Sphingolipid metabolism in ALS
- Spinal cord and cortical single cell transcriptomics (nuc-sec)
- TDP-43 and mis-splicing of cryptic exons
- TDP-43 control of retrotransposon expression
- The endothelin system in ALS
- Tissue proteomics and analysis of extracellular matrix in ALS
- Toll-like receptor (LRR4) signaling
- Transcription factor EB (TFEB) mislocalization
- TREM2 and microglial-associated neuroinflammation
- UBQLN2 in FTD and ALS
- Unbiased RNA clustering of ALS, FTD, and matched controls
- Urate, NRF2, and antioxidant signaling

*Researchers using Tissues and Slides from the Target ALS Postmortem Core**

- **AbbVie**
- **AC Immune**
- **Amgen**
- Barrow Neurological Institute – 2 labs
- **Biogen – 4 labs**
- Boston University – 2 labs
- Casa Sollievo della Sofferenza, ITALY
- Cedars-Sinai Medical Center
- Children's Hospital of Philadelphia
- **Codiak Biosciences**
- Columbia University – 6 labs
- Cold Spring Harbor Labs
- Duke University
- **Denali Therapeutics**
- Center for Neurodegen Dis (DZNE), GERMANY
- Emory University
- Georgetown University
- **GlaxoSmithKline (GSK)**
- Harvard/MGH – 11 labs
- Houston Methodist Research Institute
- INSERM, Paris, FRANCE – 4 labs
- Institute of Molecular & Cell Biology, SINGAPORE
- Jefferson University – 2 labs
- Johns Hopkins University – 16 labs in 5 depts
- Kansas City University
- **Merck**
- MIT – 2 labs
- **Myotherapeutics**
- New York Genome Center
- NIH (both NINDS & NIA) – 4 labs
- **Novartis**
- NYU Langone/Rockefeller – 2 labs
- **Pfizer**
- **Prosetta Biosciences**
- **Regeneron**
- Rockefeller University
- **Sanofi Genzyme**
- San Raffaele Scientific Institute, ITALY
- Stanford University
- SUNY Stonybrook
- **Takeda Pharmaceutical**
- Uniformed Services University of the Health Sciences
- **United Neuroscience**
- University Hospital Leuven, BELGIUM
- University of Arizona
- University of British Columbia, CANADA
- UCSD – 3 labs
- UCSF – 2 labs
- University of Chicago
- University of Florida - 2 labs
- University of Lyon, FRANCE
- University of Maryland
- University of Massachusetts – 3 labs
- University of Miami
- University of Pittsburgh
- University of Texas at Arlington
- University of Utah
- University of Zurich, SWITZERLAND
- **Verge Genomics**
- Weizmann Institute, ISRAEL

list is not complete





Aarhus University, Denmark
Bar-Ilan University (Israel)
Benevolent AI – London (UK)
DZNE – Munich (Germany)
Iggy get out – Sydney (Australia)
Inst for Stem Cell Bio and Regen Med (India)
Leiden University Med Center (Netherlands)
Nebion – Zurich (Switzerland)
Novartis – Basel (Switzerland)
NRGene (Israel)
Sant Pau Biomed Res Inst – Barcelona (Spain)
Teva (Israel)
Tsinghua University (China)
Ulster University (N. Ireland)
University College London – London (UK)
University Med Ctr Groningen (Netherlands)
University Med Ctr Utrecht (Netherlands)
University of Queensland (Australia)
Vlaams Instituut v Biotechnologie (VIB) (Belgium)
Weizmann Institute of Science (Israel)

Abbvie – Chicago, IL
Alector – San Francisco, CA
Amgen R&D – Cambridge, MA
Barrow Neurological Institute – Phoenix, AZ
Biogen – Cambridge, MA
Blueprint Bio – Newport Beach, CA
Boston Children's Hospital – Boston, MA
CSHL – Cold Spring Harbor, NY
Columbia University - New York City, NY
Denali Therapeutics – San Francisco, CA
Emory University – Atlanta, GA
Genentech – San Francisco, CA
Genetic Intelligence – New York City, NY
GSK – Newark, NJ
Harvard, Boston, MA
Icahn School of Med at Mount Sinai – NYC, NY
Janssen R&D – Raritan NJ
Johns Hopkins University – Baltimore, MD
Lam Therapeutics – Guilford, CT
Mayo Clinic – Jacksonville, FL
MIT – Cambridge, MA
Mount Sinai – New, York City, NY
NIH (NINDS & NIA) – Bethesda, MD
Penn State University - University Park, PA
Pfizer – New York City, NY
Sangamo – Richmond, CA
St. Jude Children's Hospital – Memphis, TN
U Mass Medical School – Worcester, MA
UCSF – San Francisco, CA
University of Arizona – Tucson, AZ
University of Miami – Miami, FL
University of Massachusetts, Boston, MA
University of Nebraska – Lincoln, NE
University of Pennsylvania – Philadelphia, PA
University of Texas at Dallas, Dallas, TX
Variantyx – Framingham , MA
Verge Genomics – San Francisco, CA
WAVE Life Sciences – Cambridge, MA
Yale University – New Haven, CT

Example of how a collaborative biomarker-driven project evolves in our Ecosystem

(there are many others)

Human endogenous retrovirus-K contributes to motor neuron disease

Wenxue Li,[1]* Myoung-Hwa Lee,[1]* Lisa Henderson,[1] Richa Tyagi,[1] Muzna Bachani,[2] Joseph Steiner,[2] Emilie Campanac,[3] Dax A. Hoffman,[3] Gloria von Geldern,[1] Kory Johnson,[4] Dragan Maric,[1] H. Douglas Morris,[5] Margaret Lentz,[6] Katherine Pak,[7] Andrew Mammen,[7] Lyle Ostrow,[8] Jeffrey Rothstein,[8] Avindra Nath[1†]

Science Translational Medicine 30 Sep 2015: Vol. 7, Issue 307, pp. 307ra153

Human endogenous retroviruses (HERVs) constitute nearly 8% of the human genome and have been termed junk DNA (*1*). These retroviral sequences are remnants of infections that occurred over several million years, resulting in the integration of provirus genomes into the DNA of germline cells. Most HERV proviruses have accumulated numerous nonsense mutations that have rendered them defective (*1*). However, it is becoming increasingly apparent that endogenous retroviral sequences may get expressed under select pathological circumstances. Multiple complete sequences of the most recently acquired HERV-K are present in the human genome (*2*). HERV-K may be expressed in the brain of patients with amyotrophic lateral sclerosis (ALS) (*3*) and reverse transcriptase activity can be found in the blood and brain tissue of these patients (*4*–*8*), but the role of HERV-K in the pathophysiology of this disease remains unknown.

Human endogenous retrovirus-K contributes to motor neuron disease

Wenxue Li,[1]* Myoung-Hwa Lee,[1]* Lisa Henderson,[1] Richa Tyagi,[1] Muzna Bachani,[2] Joseph Steiner,[2] Emilie Campanac,[3] Dax A. Hoffman,[3] Gloria von Geldern,[1] Kory Johnson,[4] Dragan Maric,[1] H. Douglas Morris,[5] Margaret Lentz,[6] Katherine Pak,[7] Andrew Mammen,[7] Lyle Ostrow,[8] Jeffrey Rothstein,[8] Avindra Nath[1†]

Science Translational Medicine 30 Sep 2015: Vol. 7, Issue 307, pp. 307ra153

The ALS Association and Target ALS Partner to Uncover the Connection Between Ancient Retroviruses and ALS

Washington, D.C. (May 13, 2016)— In partnership with Target ALS, The ALS Association is using part of its original investment in the Center for Genomics of Neurodegenerative Disease (CGND) at the New York Genome Center (NYGC) to create a valuable resource of RNA sequence data generated from tissue samples and induced pluripotent stem cells (iPSCs). This resource will help advance a project examining how Human Endogenous Retrovirus (HERV) RNA sequences may play an important role in a proportion of ALS cases. Leading this project are Hemali Phatnani, Ph.D., Director of the CGND, Robert Darnell, M.D., Ph.D., Founding Director and CEO of NYGC, Avindra Nath, M.D., NIH/NINDS and Lyle Ostrow, M.D., Ph.D., Johns Hopkins University.

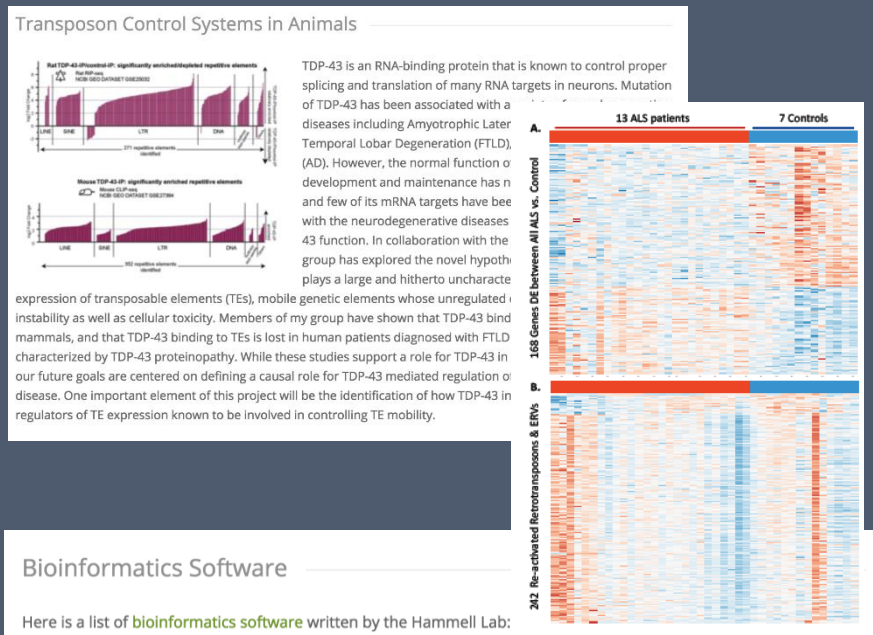
RNAseq and Retroelement Analysis

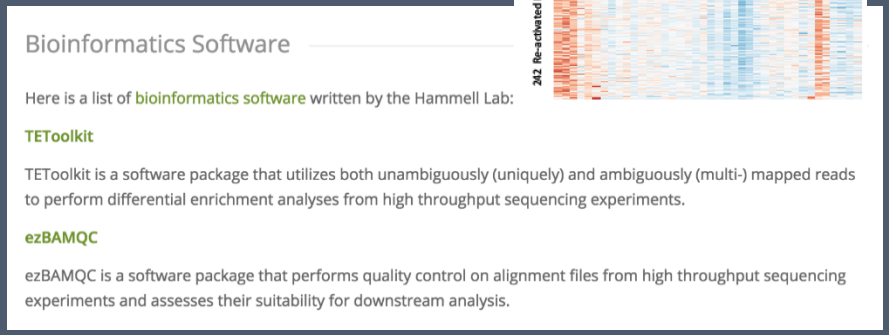
Human endogenous retrovirus-K contributes to motor neuron disease

Wenxue Li,[1]* Myoung-Hwa Lee,[1]* Lisa Henderson,[1] Richa Tyagi,[1] Muzna Bachani,[2] Joseph Steiner,[2] Emilie Campanac,[3] Dax A. Hoffman,[3] Gloria von Geldern,[1] Kory Johnson,[4] Dragan Maric,[1] H. Douglas Morris,[5] Margaret Lentz,[6] Katherine Pak,[7] Andrew Mammen,[7] Lyle Ostrow,[8] Jeffrey Rothstein,[8] Avindra Nath[1†]

Science Translational Medicine 30 Sep 2015: Vol. 7, Issue 307, pp. 307ra153

CSH Cold Spring Harbor Laboratory
HAMMELL LAB



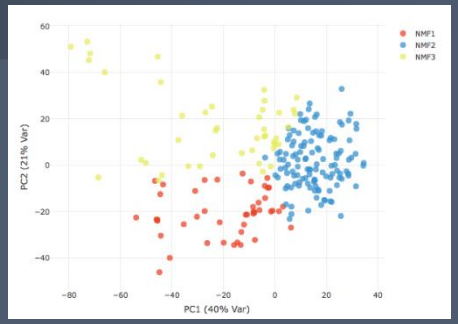


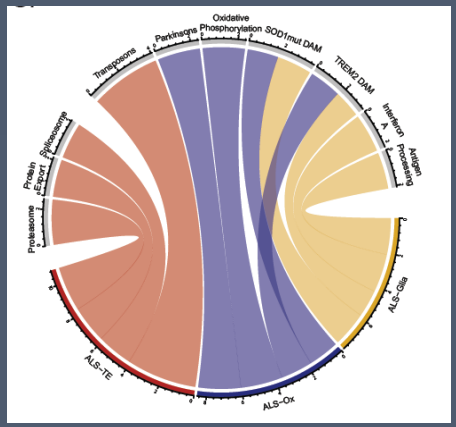
March 23rd, 2018 - "Lab Meeting" at NYGC with researchers from many centers.

Similar "meetings" about multiple different projects were my personal intro to regularly using Zoom for science a couple years before Covid-19!

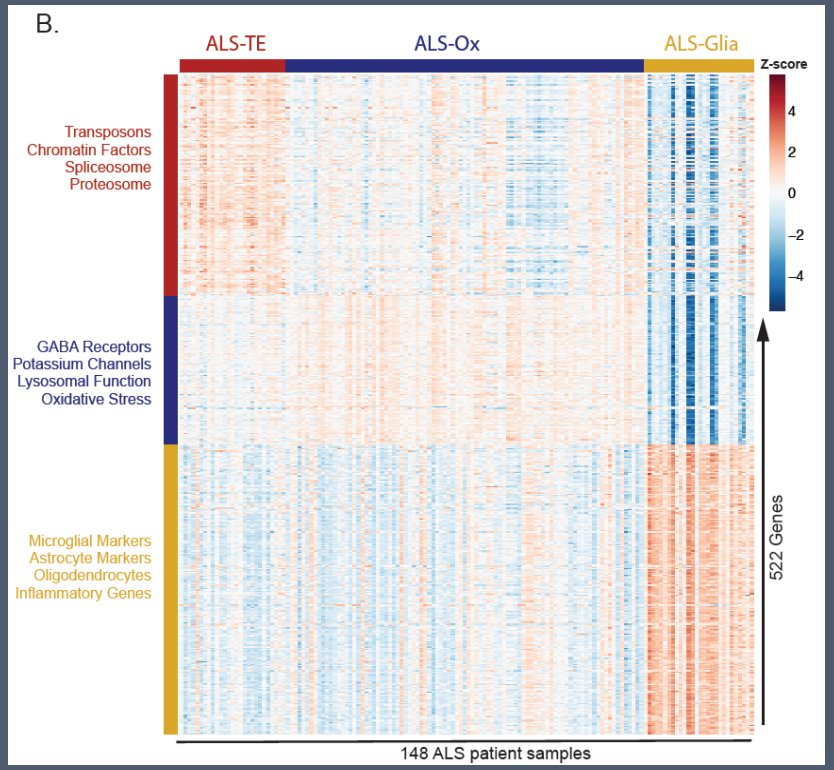
Cold Spring Harbor Laboratory
HAMMELL LAB

"Is it okay if I find things besides retroelements? Because we often find other things too."





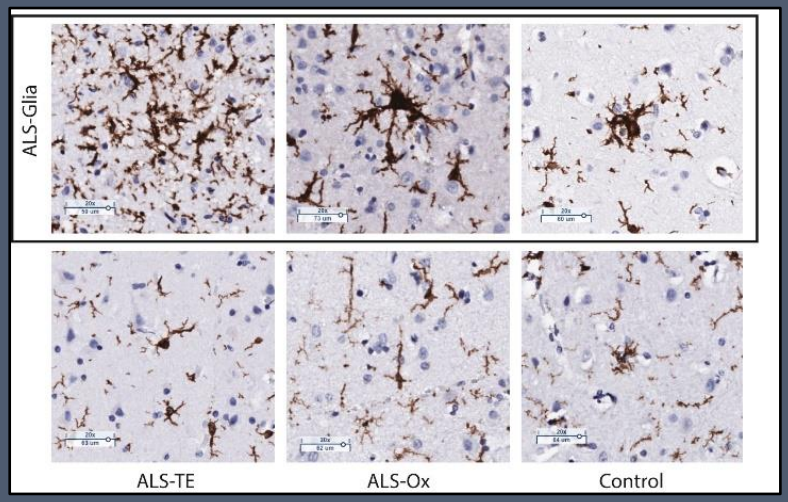
- Unbiased RNASeq analysis of postmortem cortex suggests distinct ALS patient clusters.
- Different groups show enrichment/depletion of markers for specific ALS pathogenic mechanisms.
- We have now annotated the decedents and samples by cluster identity.
- Can we find corresponding biofluid or muscle biomarker profiles?



CSHL Scientist Wins Chan Zuckerberg Award

December 7, 2018 Staff Leave a comment

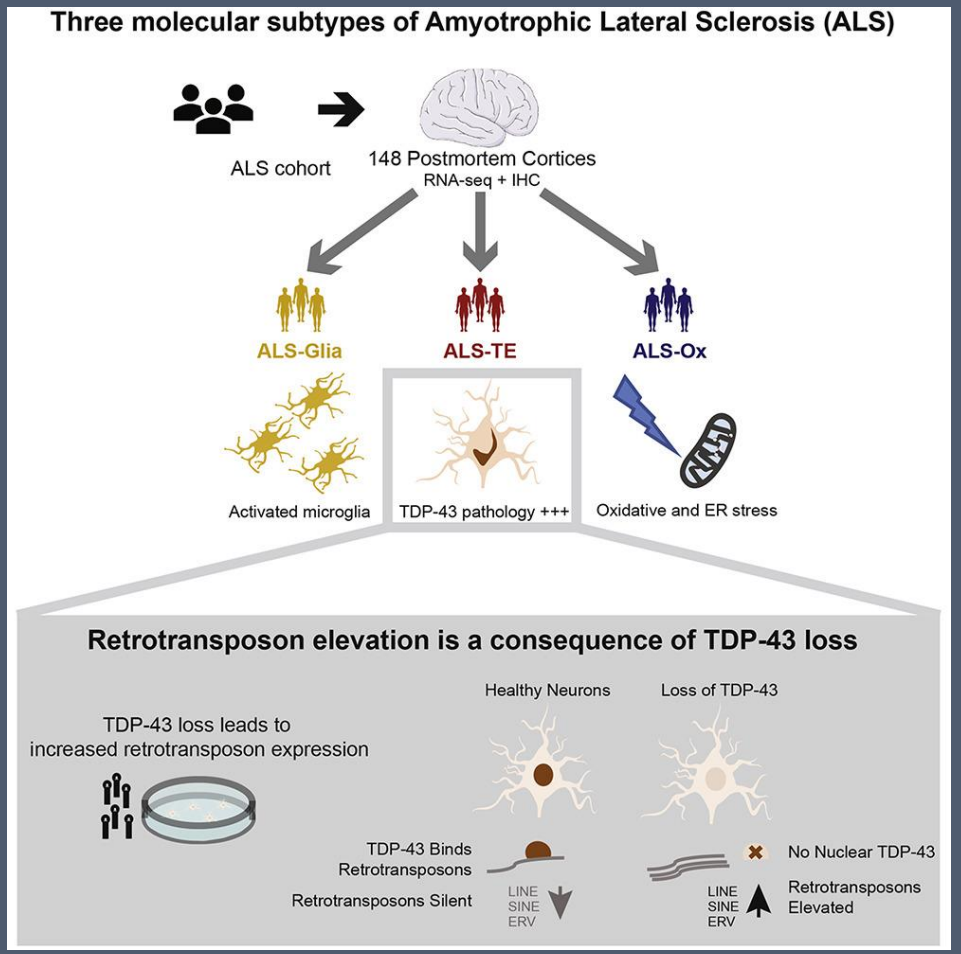
Cold Spring Harbor Laboratory Associate Professor Molly Hammell has been awarded the Chan Zuckerberg Initiative (CZI) Ben Barres Early Career Acceleration Award for her proposed work on amyotrophic lateral sclerosis, better know by the acronym ALS or Lou Gehrig's disease.



Article

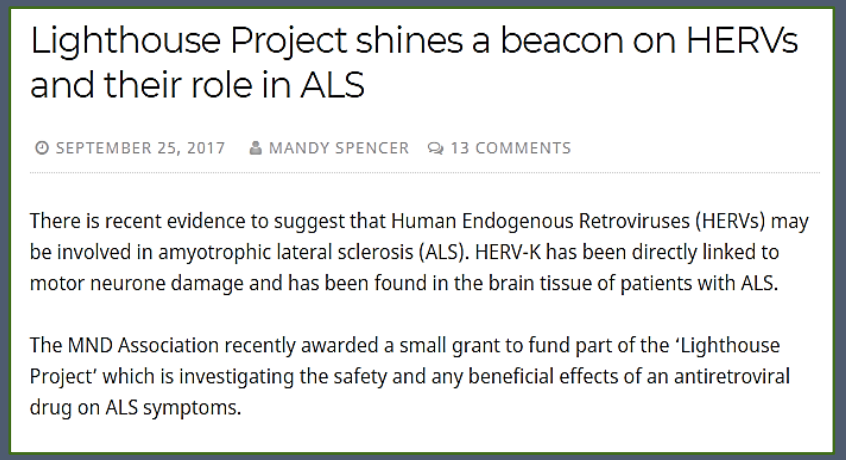
Cell Reports

Postmortem Cortex Samples Identify Distinct Molecular Subtypes of ALS: Retrotransposon Activation, Oxidative Stress, and Activated Glia



HERVs/TEs in ALS

- At least five ongoing projects involving labs at >10 institutions across four different countries are presently using Target ALS PM Core resources to explore the therapeutic potential of targeting HERVs (or other TEs) in ALS.
- This work has directly inspired **two early-phase clinical trials**.
- CZI invited Dr. Ostrow to represent the PM Core at their Neurodegeneration Challenge Network Kickoff Meeting.
  - As a result, several other CZI-funded research teams are now collaborating with our Target ALS and NYGC Core Efforts.
- Drs. Hammell, Ostrow, and Nath participated in an NIH workshop cohosted by NIA and NINDS to help define portfolio funding priorities and best practices for collaborative bioinformatics related to the growing interest in the roles of HERVs / other TEs in neurodegenerative disorders.

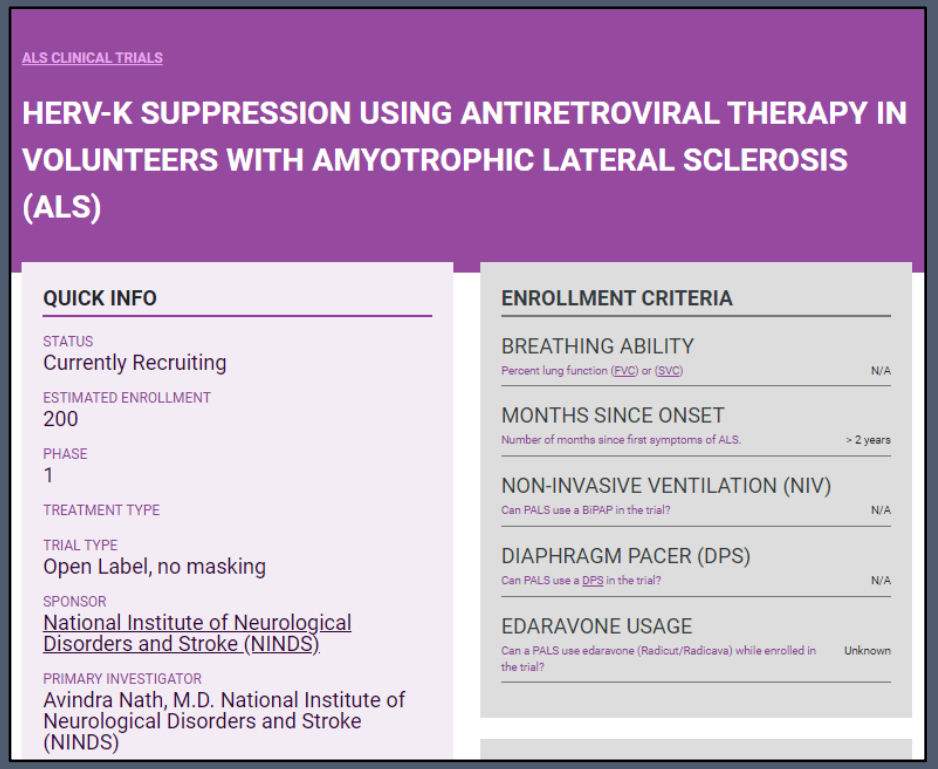


Lighthouse Project shines a beacon on HERVs and their role in ALS

SEPTEMBER 25, 2017 MANDY SPENCER 13 COMMENTS

There is recent evidence to suggest that Human Endogenous Retroviruses (HERVs) may be involved in amyotrophic lateral sclerosis (ALS). HERV-K has been directly linked to motor neurone damage and has been found in the brain tissue of patients with ALS.

The MND Association recently awarded a small grant to fund part of the 'Lighthouse Project' which is investigating the safety and any beneficial effects of an antiretroviral drug on ALS symptoms.



ALS CLINICAL TRIALS

HERV-K SUPPRESSION USING ANTIRETROVIRAL THERAPY IN VOLUNTEERS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS)

QUICK INFO

STATUS
Currently Recruiting

ESTIMATED ENROLLMENT
200

PHASE
1

TREATMENT TYPE

TRIAL TYPE
Open Label, no masking

SPONSOR
National Institute of Neurological Disorders and Stroke (NINDS)

PRIMARY INVESTIGATOR
Avindra Nath, M.D. National Institute of Neurological Disorders and Stroke (NINDS)

ENROLLMENT CRITERIA

BREATHING ABILITY
Percent lung function (FVC) or (SVC) N/A

MONTHS SINCE ONSET
Number of months since first symptoms of ALS. > 2 years

NON-INVASIVE VENTILATION (NIV)
Can PALS use a BiPAP in the trial? N/A

DIAPHRAGM PACER (DPS)
Can PALS use a DPS in the trial? N/A

EDARAVONE USAGE
Can a PALS use edaravone (Radicut/Radicava) while enrolled in the trial? Unknown

Excerpts from a few pharma/biotech raw genetic data requests…

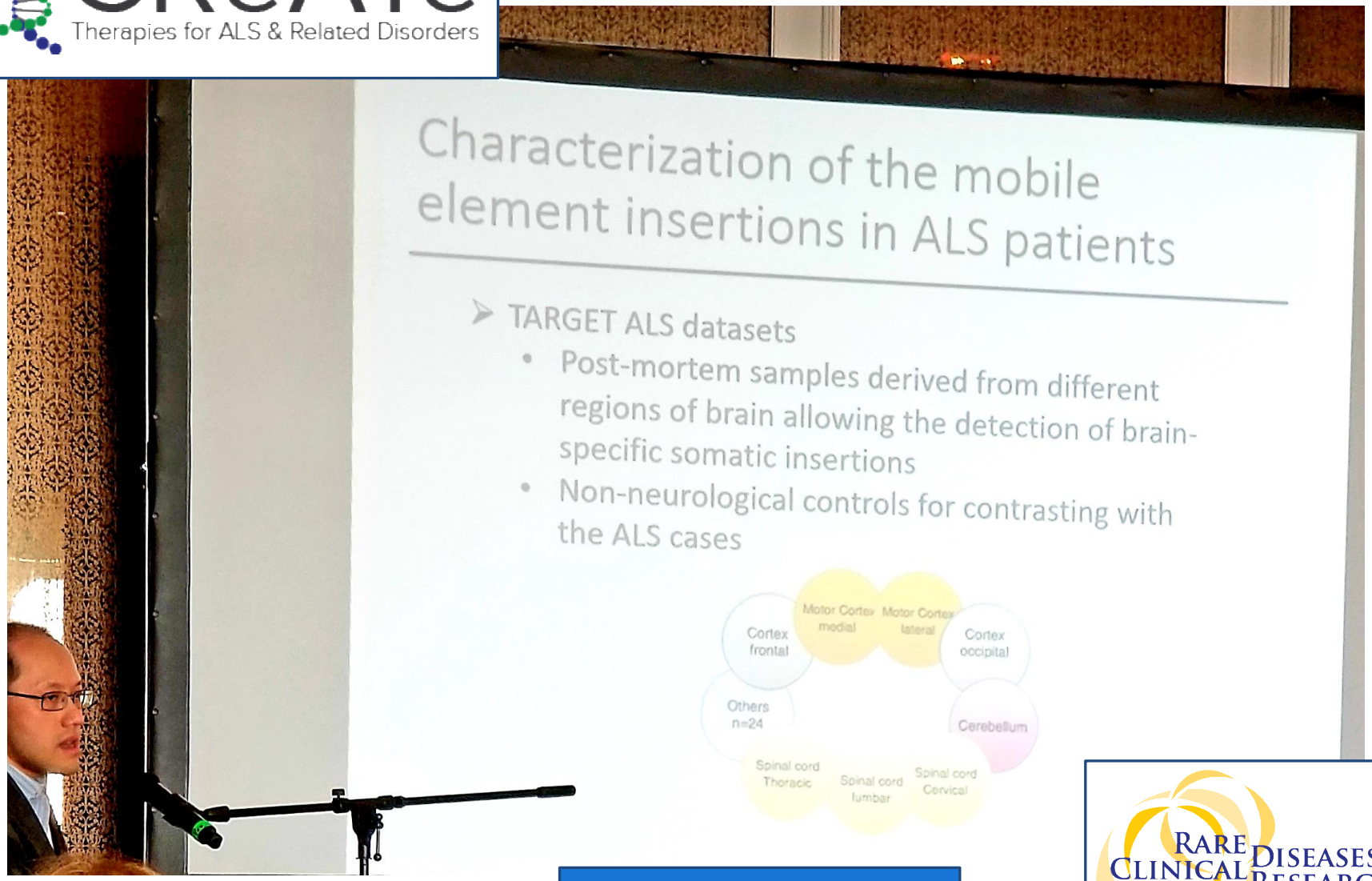
- We have developed generative models to uncover patterns in molecular and longitudinal clinical data that work by encoding hierarchical representations of the data and include the dependency structure codified in our platform. By applying our models to the Target ALS data we aim to identify molecular signatures that associate with the endotypes (such as survival) to provide insights into the molecular mechanisms driving clinical heterogeneity.

- We would like to reproduce results published by Tam et al, biorXiv, 2019, who described three distinct gene expression subgroups in Target ALS RNA-seq data from patient cortex samples.

- We are interested in identifying transcriptional start sites of both sense and antisense transcripts, and for understanding the transcriptome changes in patients with C9orf72 expansion mutation. This data will be used for the development of molecular biomarkers, that can be tracked for rescue with different treatments.

- We plan to do an eQTL analysis to relate SNP variation to expression levels in human brain. Using this data, we hope to identify genetic regulators of a set of genes implicated in ALS that we have identified using public and proprietary transcriptomic data. These genetic regulators in turn will become our focus for developing potential therapeutic strategies to treat ALS.

CReATe
Therapies for ALS & Related Disorders



ALS ASSOCIATION

TARGET ALS

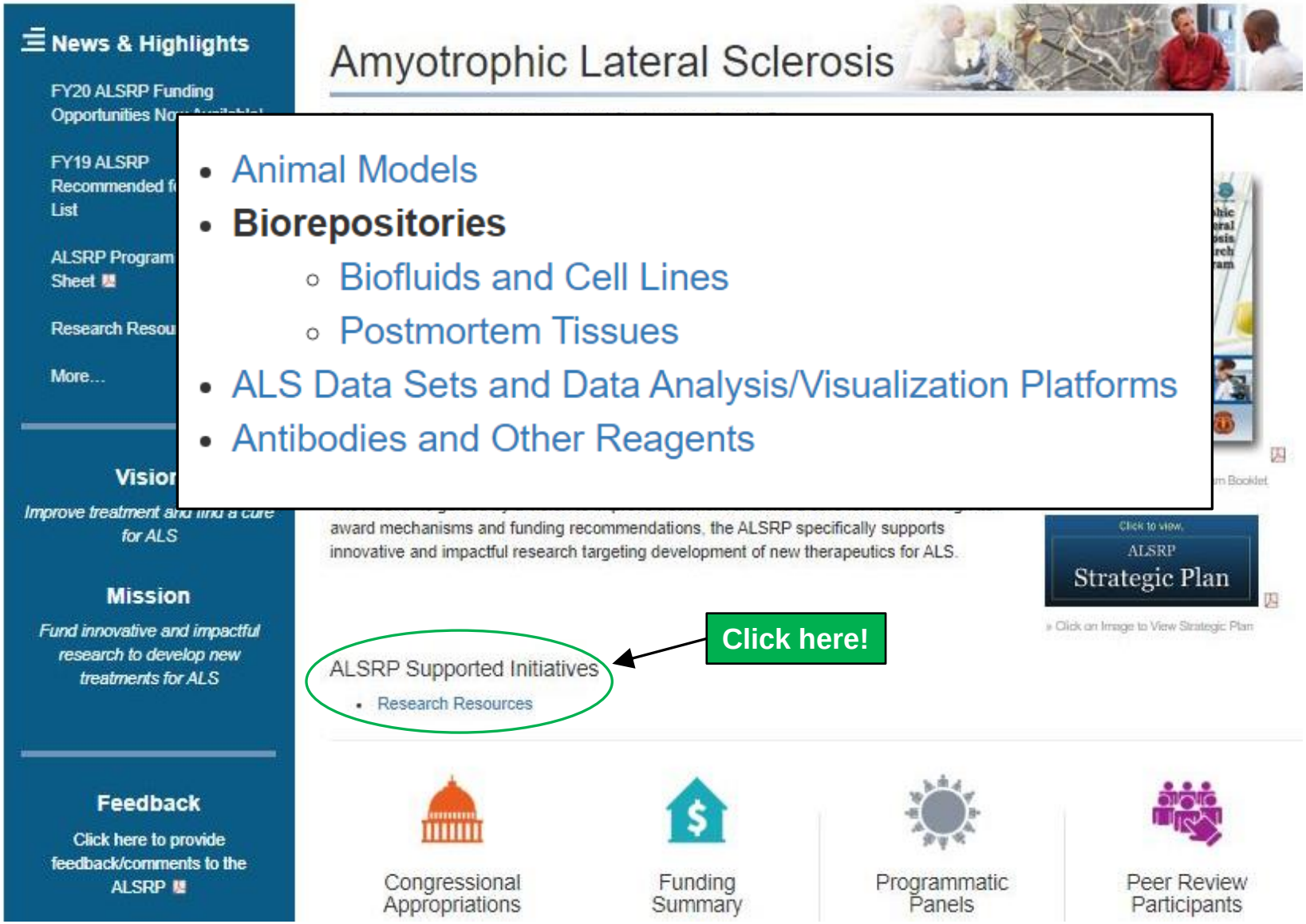
St. Jude Cloud

RARE CLINICAL DISEASES RESEARCH NETWORK
National Institutes of Health

CUTTING EDGE RESEARCH

More information on ALSRP Website https://cdmrp.army.mil/alsrp

CDMRP Department of Defense



”The World is our Lab”