HEALEY ALS Platform Trial

Weekly Q&A – March 23, 2023

Guest Speaker

Jeffrey Rothstein, MD PhD
Johns Hopkins University

Professor of Neurology and Neuroscience
Director, Brain Science Institute
Founder and Director, Robert Packard Center for ALS Research
Founder, Director Answer ALS Research Program
Medical Director, Johns Hopkins MDA ALS Clinic

Focussed Mission

PACKARD CENTER
ALS Research at Johns Hopkins

- Discover what causes ALS (genetics/epigenetics)
- Develop research animal models to understand ALS and screen for drug and cellular therapies
- Discover therapies that could substantially slow, halt and ultimately cure ALS

- Based on the notion that aggressively pursued collaborative academic research can achieve goals quicker and with more focus.
- Mandatory open discussion of ongoing research to a scientific body of wide expertise with neurodegeneration, cell biology, animal models and pre-clinical investigations.
- Targeted, selected research projects open to monthly/annual review.
- Rapid funding of projects with “minimized” grant applications

Packard Center:
Largest Dedicated Academic Consortium in ALS

PACKARD CENTER
ALS Research at Johns Hopkins

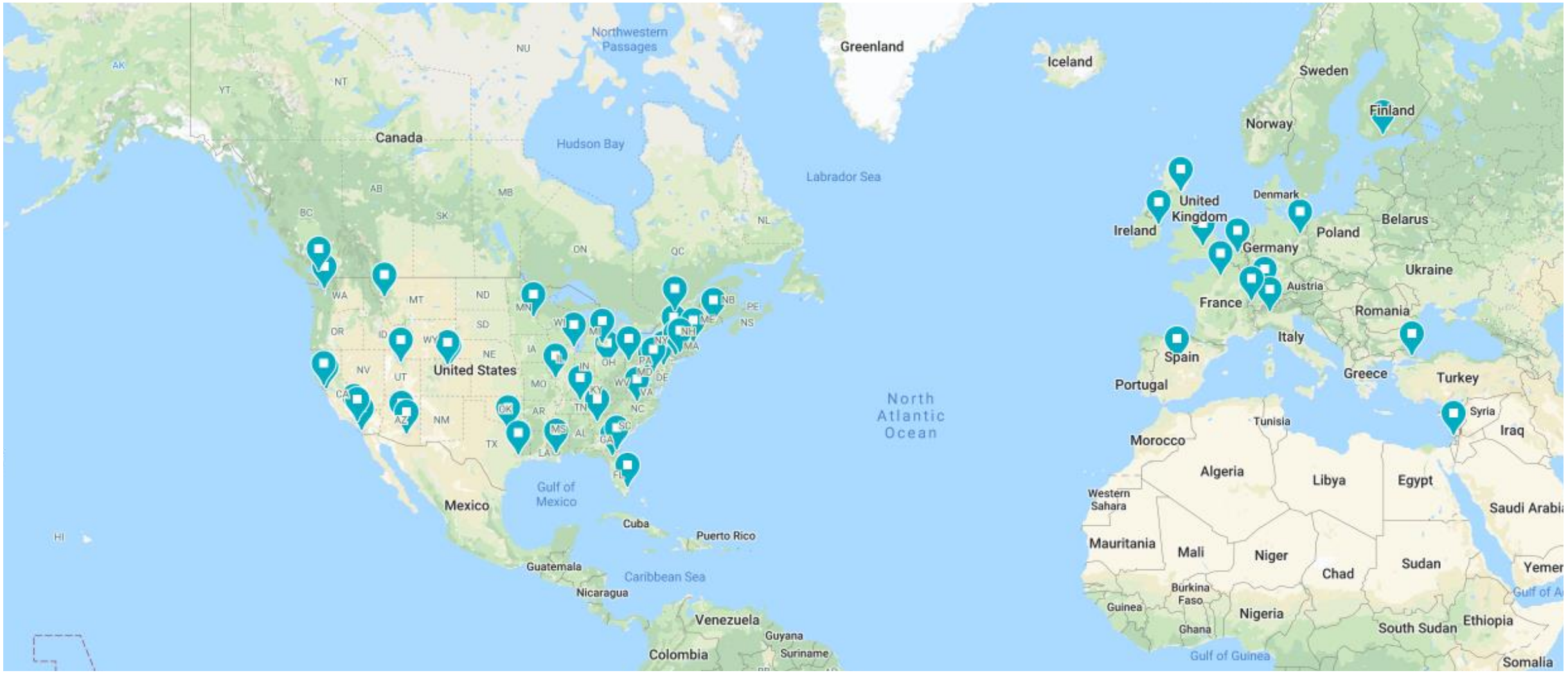
- >160 investigators collaborating over time and geography
  - ~20 years working together
  - Three continents
  - 8 countries
  - 18 states
  - 16 companies/organization
- >1300 Total research team members:
  - Basic and clinical researchers, post docs, graduate students, technicians, volunteers

Largest and distributed layout:

- >200,000 sq ft research labs

- Most valuable collection of researcher tools for understanding ALS and finding a therapy:
  - >50 mouse/Rat models (SOD1, C9orf72, ALsin, CCS, p150 ALS4, ubiqulin, etc)
  - >10 fly models
  - >10 fish models
  - >200 +ALS fibroblast cell lines
  - >1000 ALS iPS cell lines
  - >50,000 biological specimens

Family of Packard Researchers and Advisors



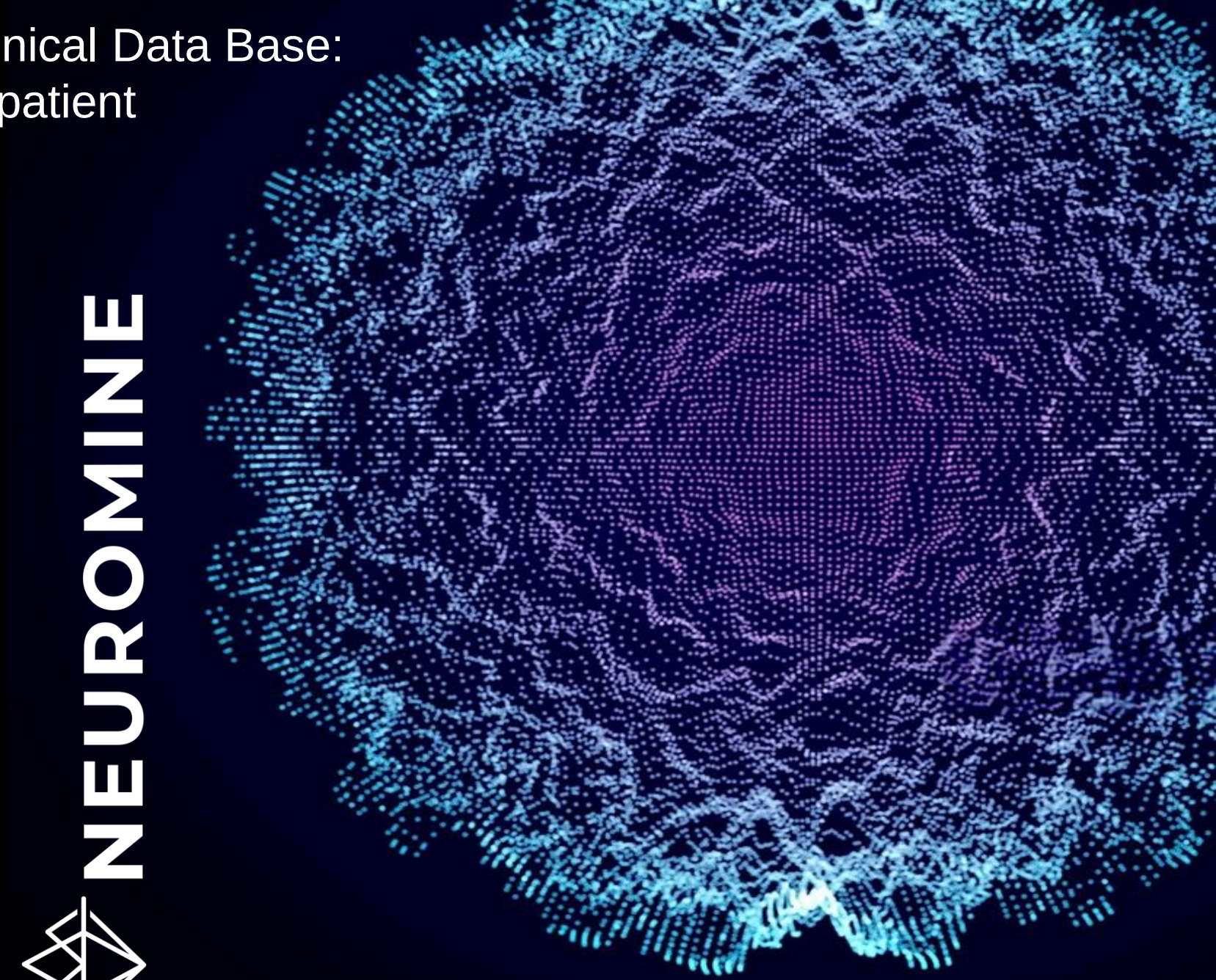
Largest ALS Biological and Clinical Data Base: 6 billion data points/patient

ANSWER ALS

And

PACKARD CENTER

NEUROMINE



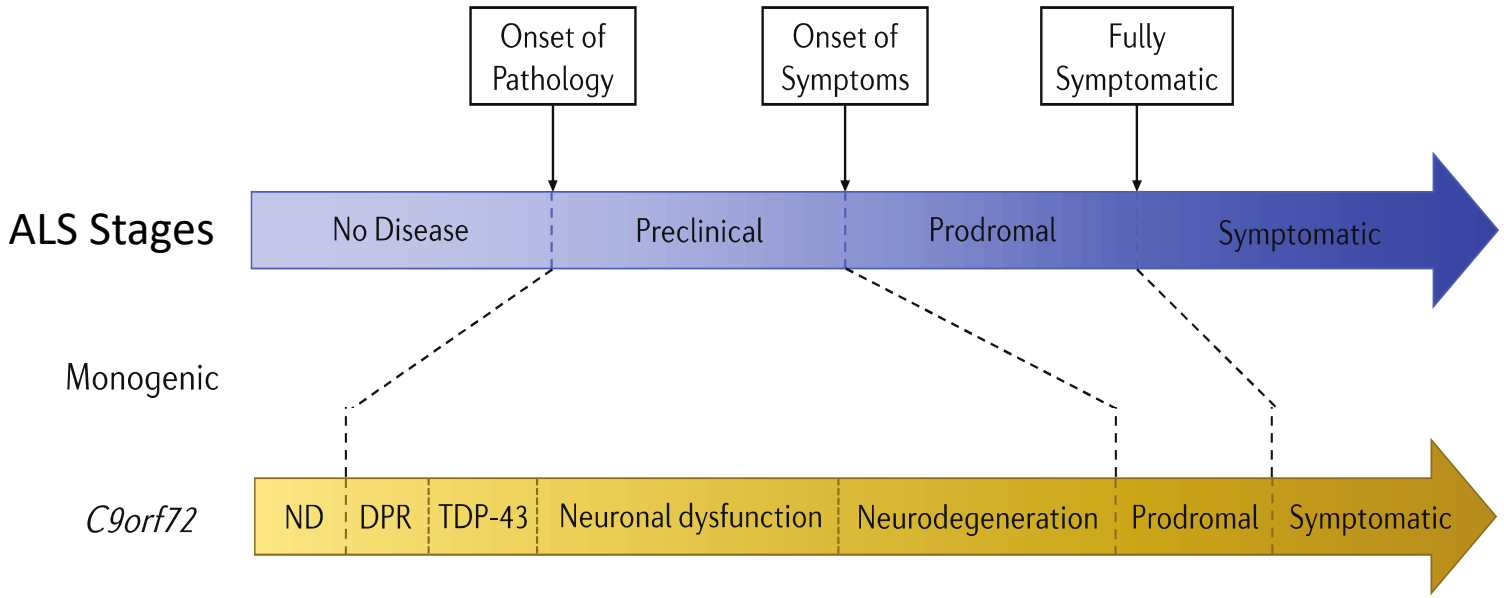
BioMarkers for ALS: Current status

Stratification vs prognostic vs target engagement vs outcome measures

Type of Assessment	MRI Imaging	Blood/CSF/Urine ("Biofluids")	PET Imaging
Molecular pathology or loss of function		**DPRs in C9 – poly(GP)** T-TDP-43/p-TDP-43 **Cryptic exon-encoded peptides**	TDP-43
Neurodegeneration	**"Shrinkage" of the brain (T1 MRI)** **Decreased brain connections (white matter on DTI)**	**NfL… NfH** p75	**Surrogate e.g. FDG**
Inflammation	MRI (Free water)	**GFAP, chitinases, complement proteins etc.**	**TSPO** (or other novel inflammatory tracers)
Synaptic function		**Neuronal markers (e.g. pentraxins)**	**Synaptic PET e.g. UCB-J**

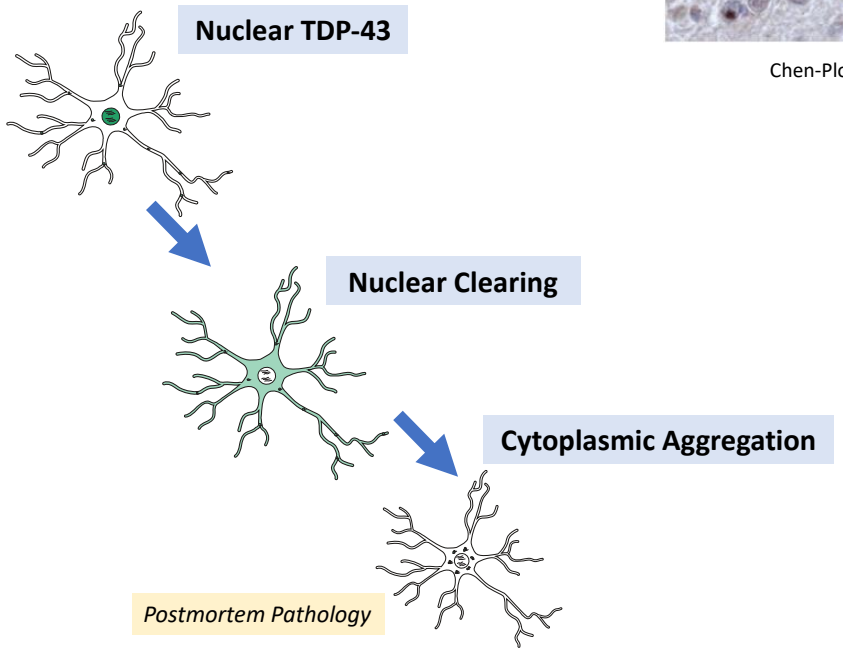
+ **digital** measures for cognition, speech, neuromuscular dysfunction

Priorities for biomarker research

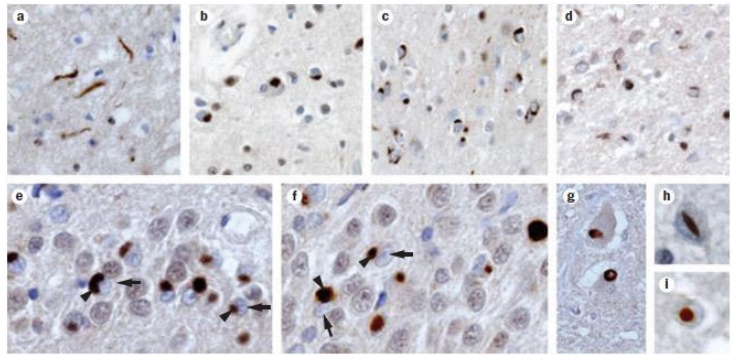


1. Expanding the panel of biomarkers that predict both phenoconversion (clinical disease onset) and being in proximity to phenoconversion (e.g. 1-5 years before clinical disease?).
2. May need to collect CSF from patients for better/more accurate detection

TDP-43 nuclear clearing is a pathological hallmark of most sALS: Loss of TDP-43 nuclear function leads to mis-regulation of hundreds of RNA species

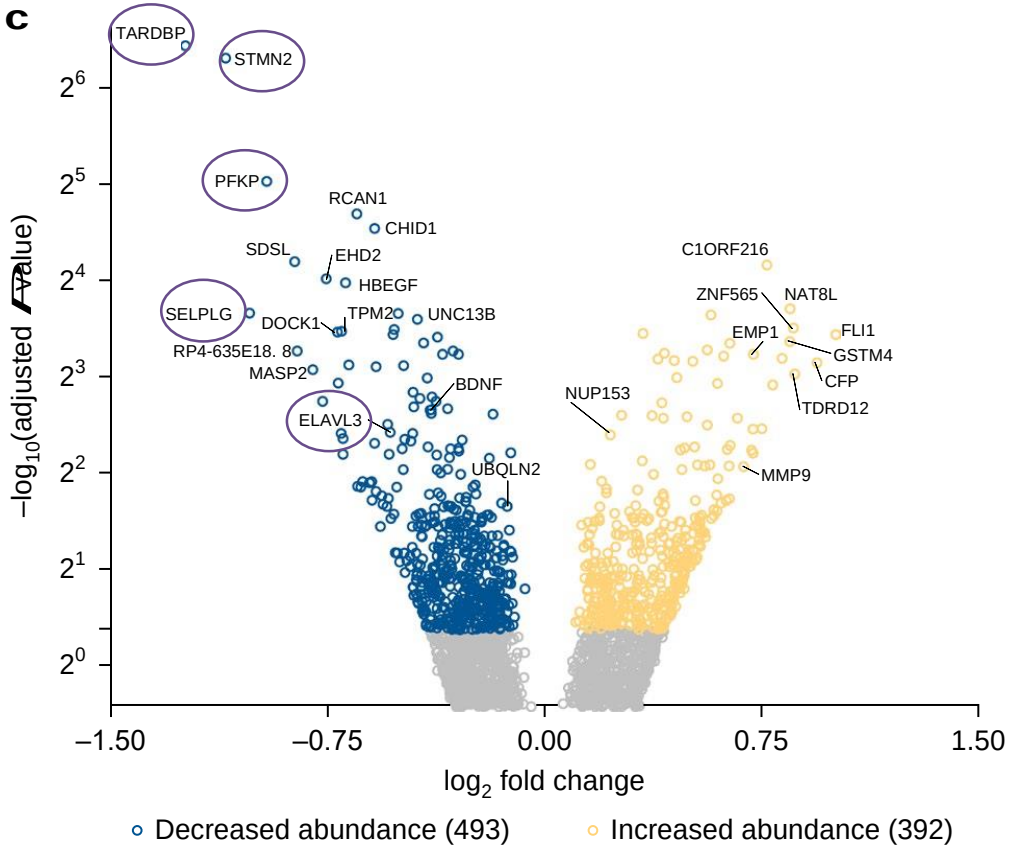


TDP-43 pathology in end-stage neurodegenerative disease



Chen-Plotkin et al (2010), *Nature Reviews Neurology*

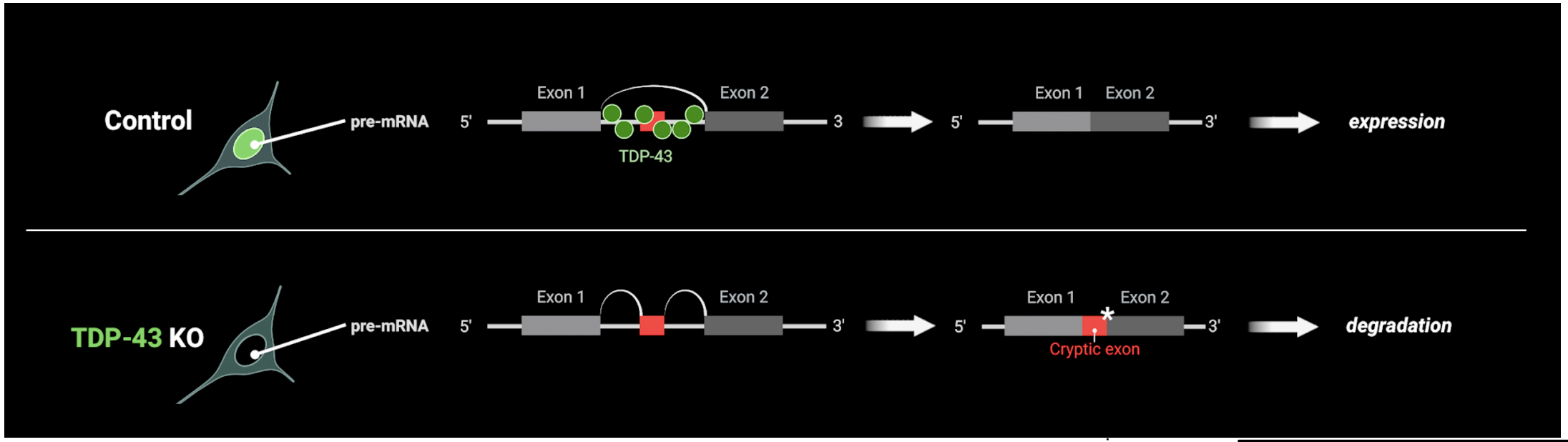
Defined set of altered RNAs with artificial TDP43 KD in human iPS MN

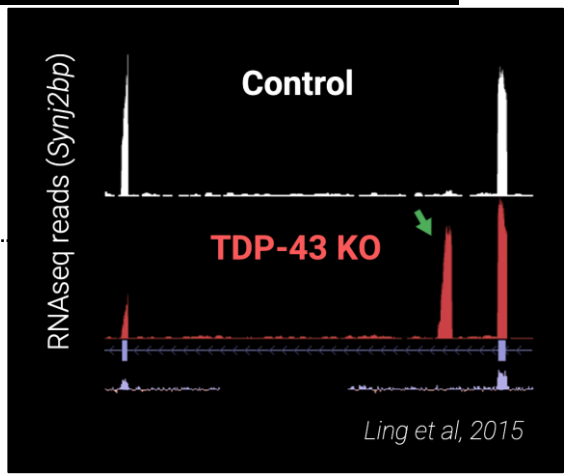


Klim et al, Nat. Neuro2019

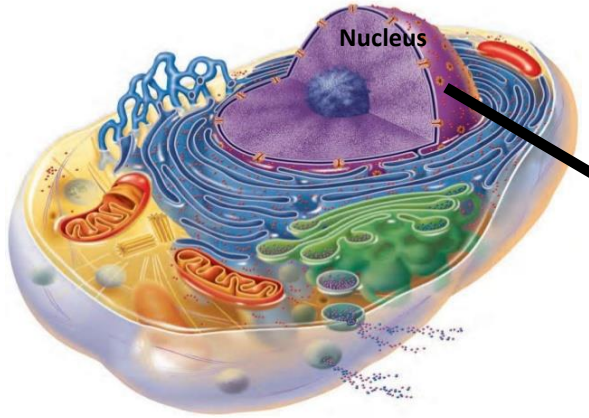
Loss of TDP-43 function causes “cryptic exon” inclusion in RNAs

These can cause disease – but also maybe be detected in patients cerebrospinal fluid (CSF)



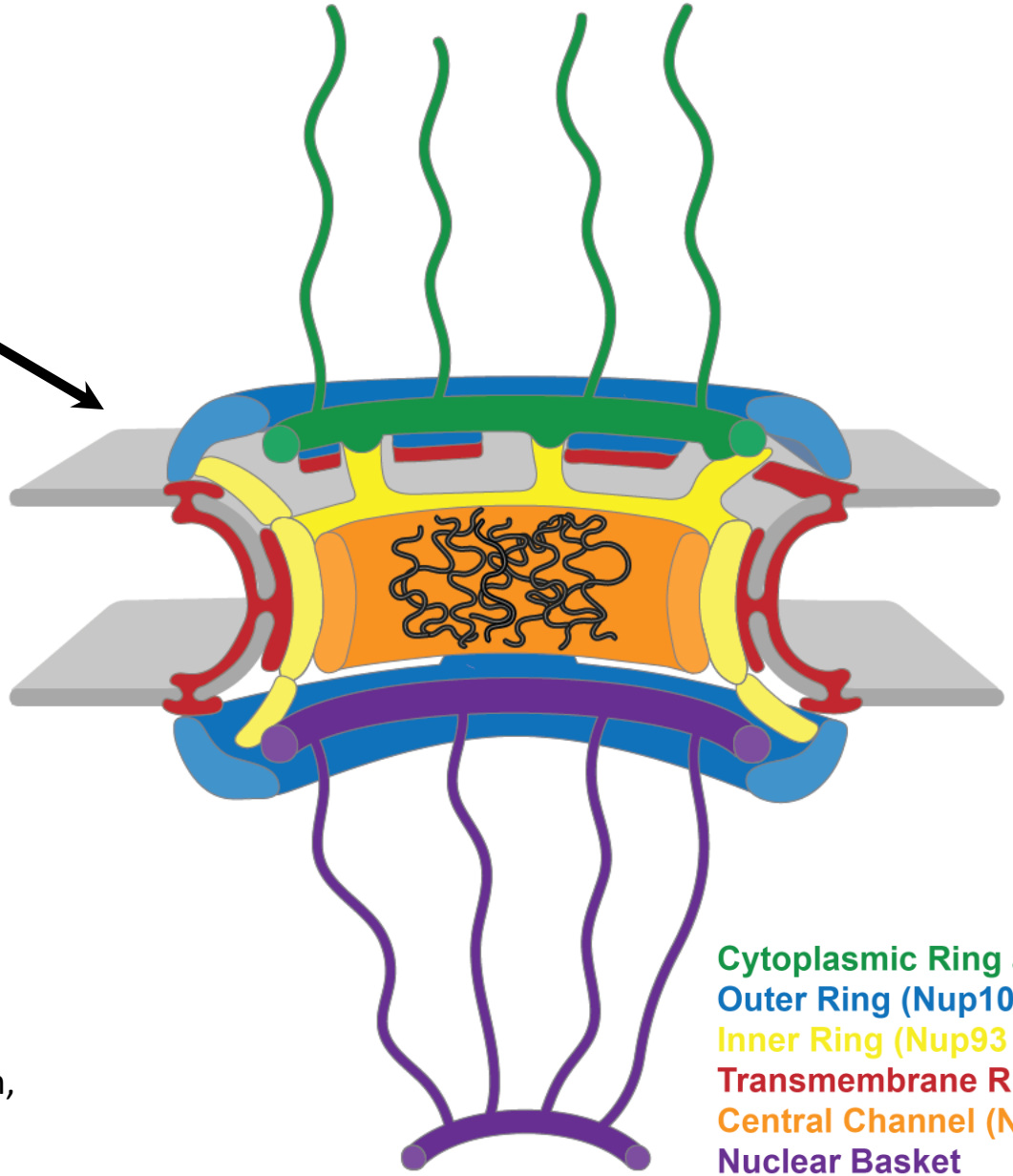


The nuclear pore complex coordinates fundamental cellular processes



Pearson Education

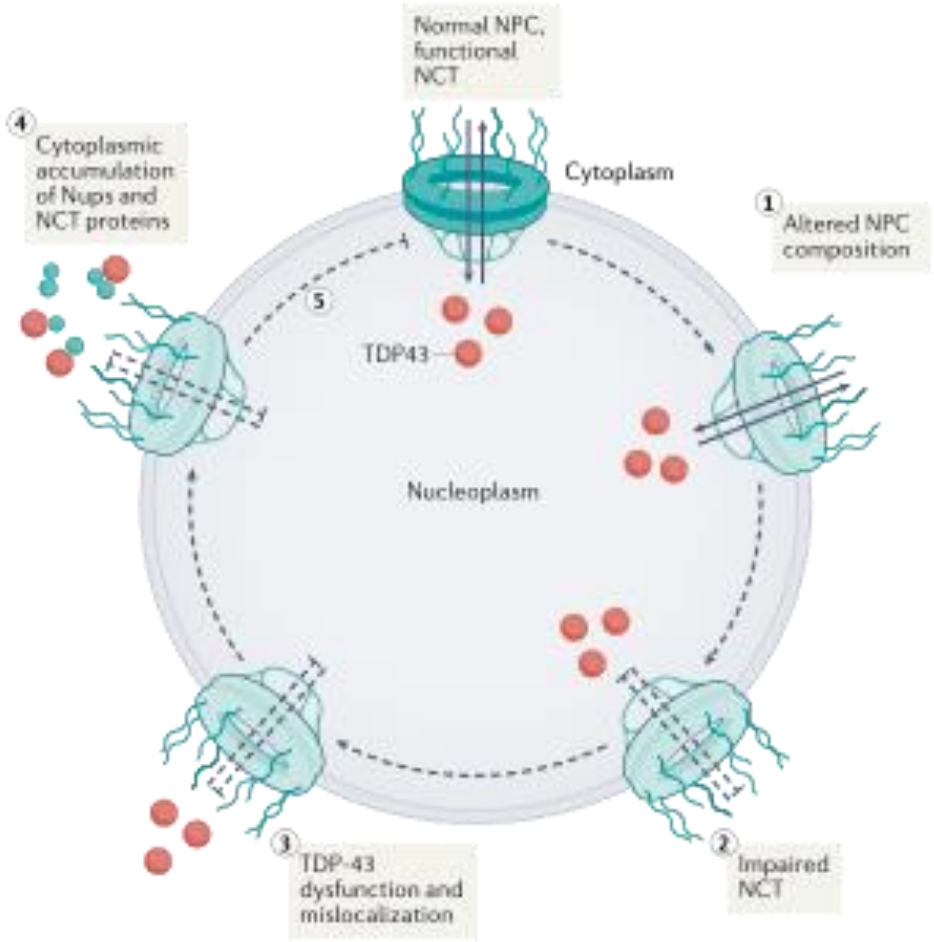
Nuclear Pore



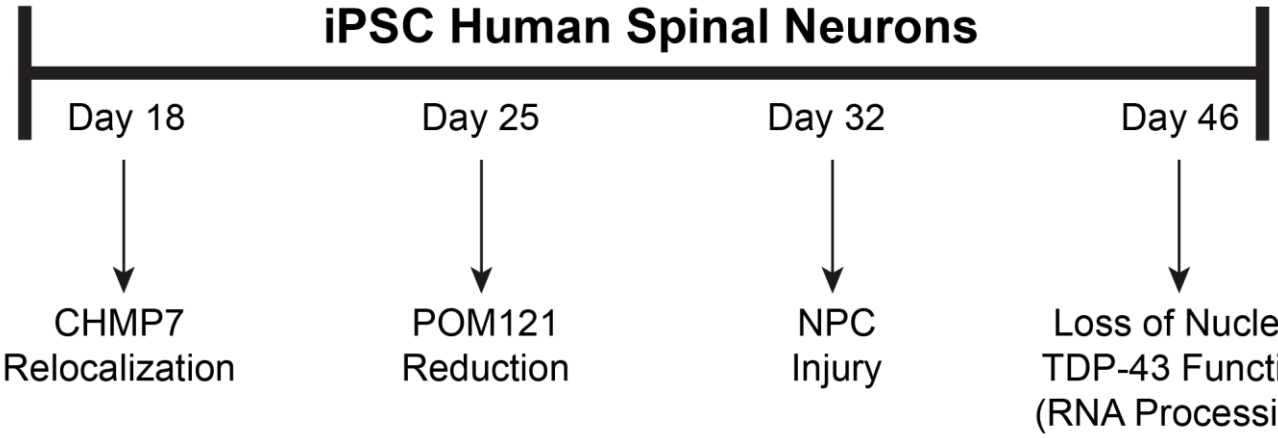
- The largest eukaryotic protein complex: mass greater than 120 MDa
- Made up of ~30 distinct proteins (total of more than 1000 protein molecules)
  - Highly organized with eightfold rotational symmetry
  - Exceptionally long lived with half lives ranging from months to years
- Comprised of multiple domains organized into subcomplexes:
  - Cytoplasmic Ring and Filaments
  - Nup62 Complex (Central Channel)
  - Y complex (Nup107-Nup160 complex, Outer Ring)
  - Nup93 complex (Inner Ring)
  - Transmembrane Ring
  - Nuclear Basket
- Functions to organize, coordinate, and control multiple cellular functions including nucleocytoplasmic transport, genome organization, and gene expression

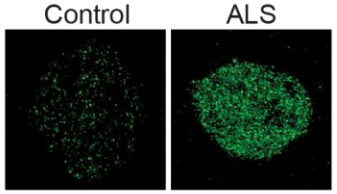
Loss of nuclear TDP43 is a result of nuclear pore damage

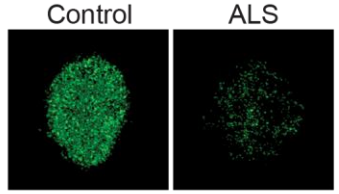
Relationship of nuclear pore complex injury and subsequent TDP43 dysfunction



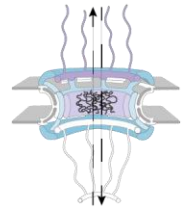
CHMP7 nuclear localization initiates disease cascade

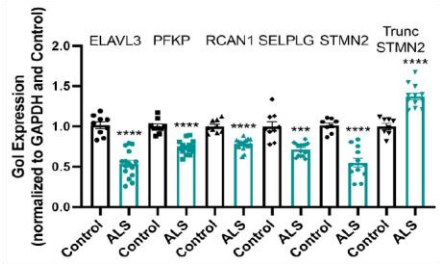






Initiation of NPC injury: POM121 reduction

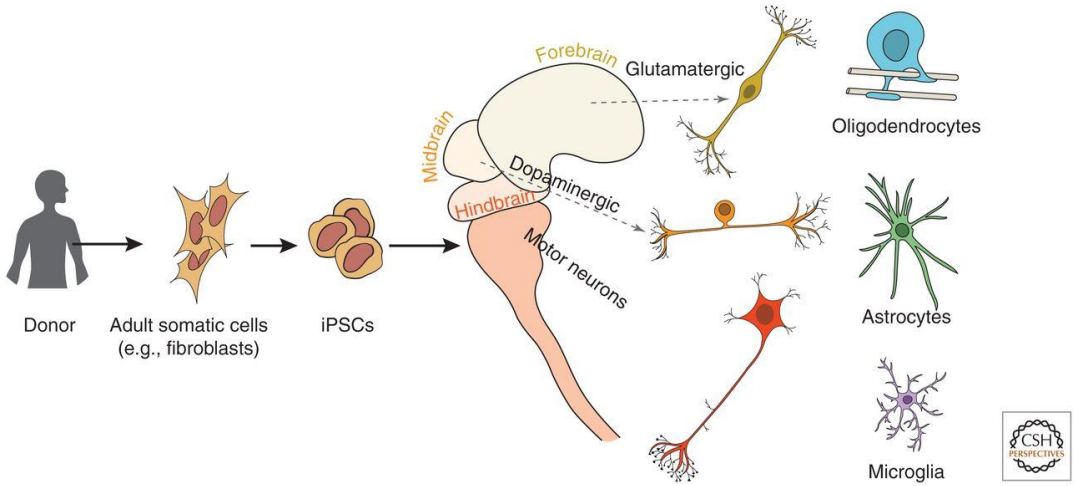




CHMP7 may be the most upstream pathophysiological event in sALS

(Coyne te al, Sci Transl Med, 2021)

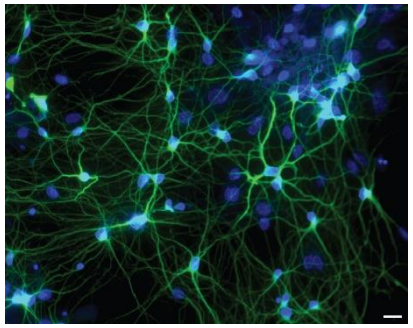
Human ALS iPS Library and Tissues:
Answer ALS:Tools for genetic and sporadic forms of ALS



ALS iPS Cell Bank (>1000 lines)

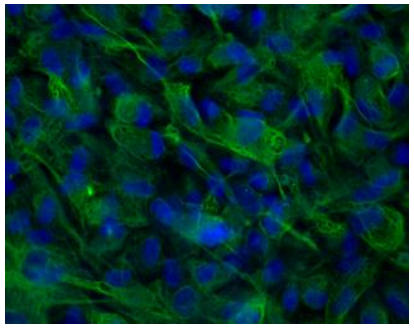
- Library of >40 fALS and >800 sALS iPS lines (**Answer ALS**)
- Mutations: SOD1, FUS, TDP43, C9orf72
- >30 C9orf72 (ALS and FTD) iPS lines (neurons/glia)
- *ALS Autopsy bank (>90 full autopsies; >15 C9orf72)*

- *Disease modeling*
- *Does C9orf72 recapitulate human brain pathology?*
- *Drug Screening*



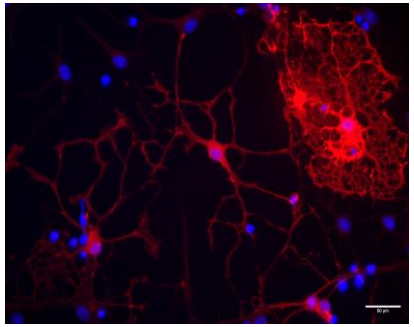
MAP2/DAPI

iPS neurons
(Motor + Cortical neurons)



GFAP/DAPI

iPS astrocytes



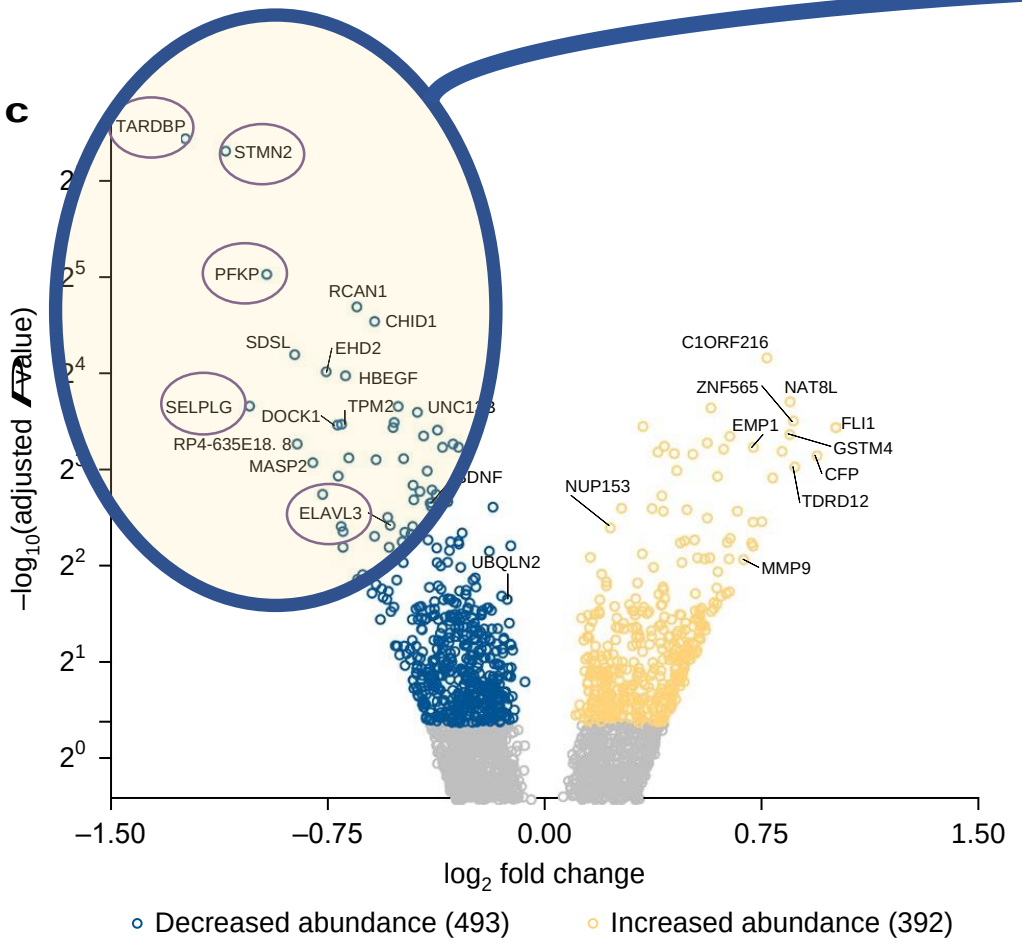
MBP/DAPI

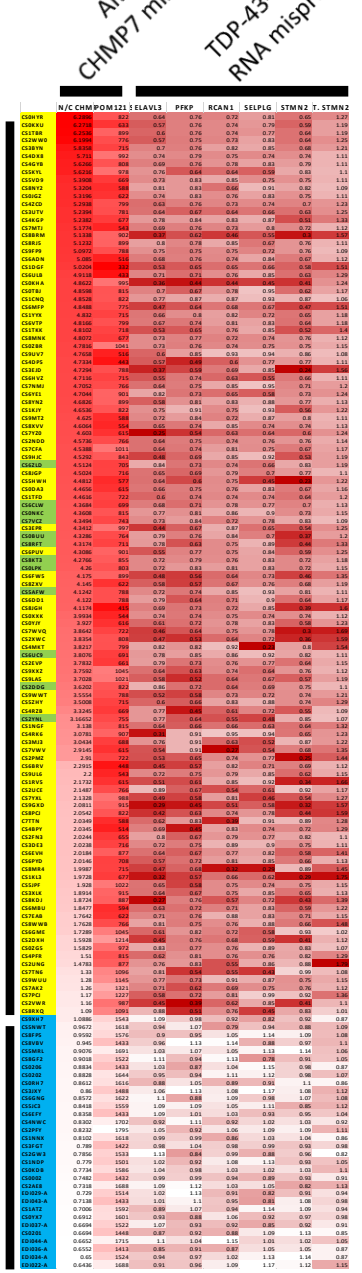
iPS oligodendrocytes

Altered TDP-43 dependant RNA species in ≥*150 ALS patient iPSC derived spinal neurons (like a biopsy)*→ Highly variable changes

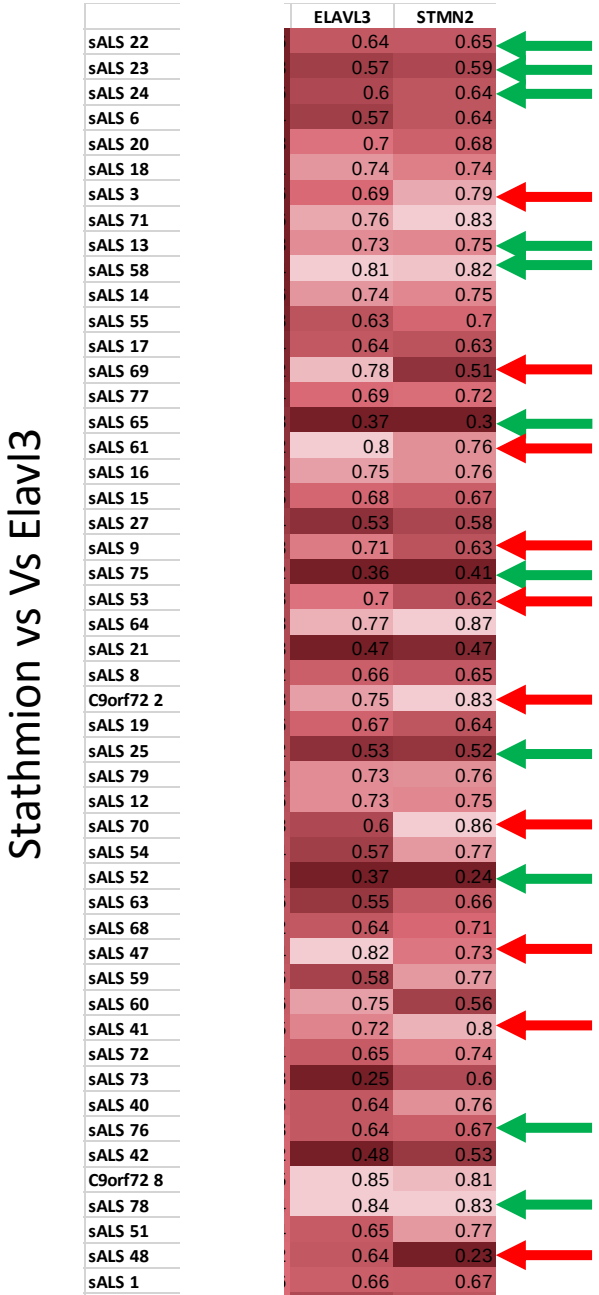
- Multiple different TDP-43 dependent RNA species:
  - ELAVL3, PFKP, RCAN1, SELPLG, STMN2
  - (8 others not shown)
- ?Are these correlated with clinical disease parameters?

- *ALL REPAIRED WITH THERAPY*





However → Significant lack of concordance between different TDP-43 misprocessed RNA species



Implications

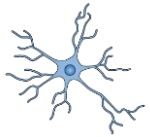
- Not all sALS patient have “equal” alteration of TDP-43 misprocessing
- Thus- a need to “biomarkers” of TDP-43 function
- Possibly choose patients based on detailed knowledge of their specific profile:
  - e.g. high stathmin vs loss stathmin change in upcoming ASO trial.
- BUT- simply looking at one RNA species may be misleading
- Also-- repairing Stathmin alone will not affect the other misprocessed species.

Similar changes amoung misprocessed RNA species

No relationship between misprocessed RNA

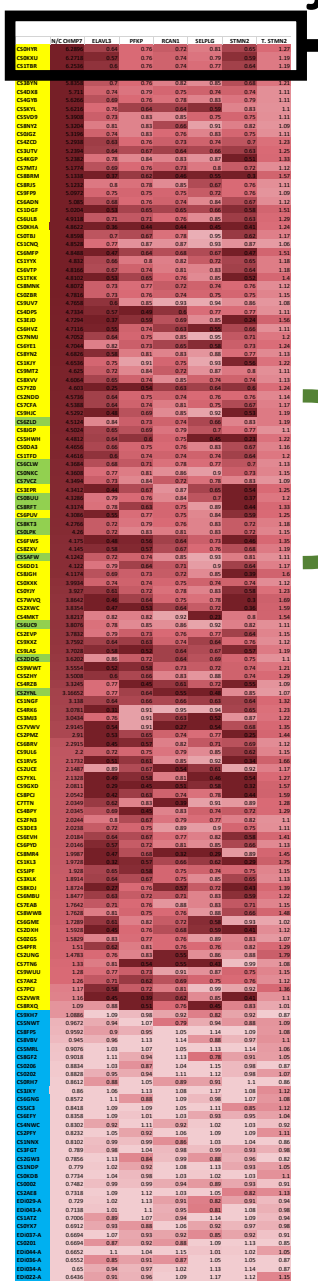
N=122 sporadic and C9orf72 ALS “iPS biopsies”

Molecular hallmarks of TDP-43 dysfunction correlate with CHMP7 pathology and NPC injury in iPSNs→ All Robustly repaired with CHMP7 ASO



sALS/C9orf72

Control



N/C CHMP7	NPC Injury	ELAVL3	PFKP	RCAN1	SELPLG	STMN2	T. STMN2

Treated 32 “patients” (iPS lines) with CHMP7 ASO→
Complete repair of TDP-43 misprocessing!

- 122 ALS lines (12 C9orf72; 110 sALS)
- 35 controls

(Coyne and Rothstein, unpublished)

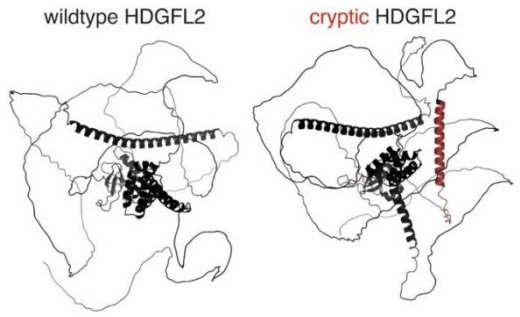
~90% KD of CHMP7

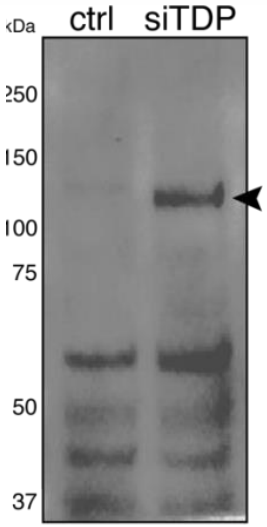
sALS/C9orf72

Control

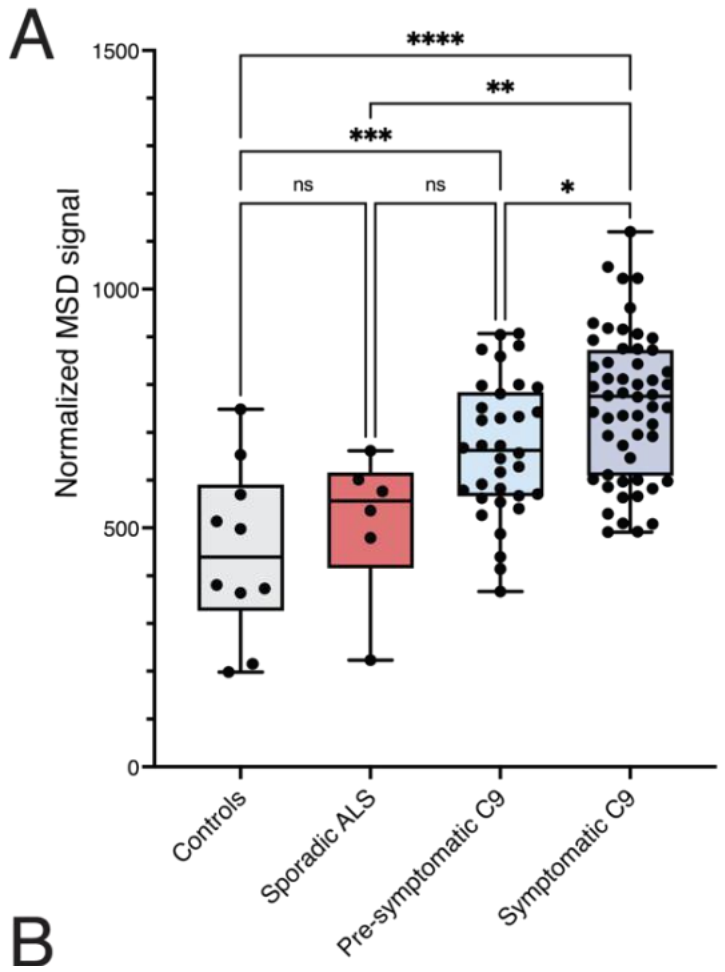
CS4PFR	85.4	0.89	0.98	1.04	0.91	0.94	0.86
CS6MBU	82.7	0.93	1.05	1.01	1.08	0.87	1.03
CS5JPF	91.6	0.99	1.04	0.92	1.05	0.82	1.05
CS1KL3	93.8	1.07	0.96	0.93	0.96	0.99	0.91
CS8MR4	94.5	1.04	0.92	0.98	0.92	1.06	1.09
CS6PYD	96.2	0.82	0.93	0.87	0.87	1.01	1.01
CS6EVH	98.1	0.87	1.07	0.96	0.89	0.93	0.86
CS2FN3	92.2	0.91	1.06	0.95	0.83	0.98	0.92
CS9GXD	88.7	0.83	1.05	0.89	1.05	0.82	1.15
CS2YNL	86.1	0.99	0.98	0.94	1.08	0.94	1.03
CS2EVP	87.4	1.08	0.92	0.87	0.97	0.95	0.91
CS6UC9	83.3	1.09	0.97	0.95	0.92	0.97	0.84
CS2XWC	95.1	1.1	0.96	1.11	0.87	0.86	1.07
CS0YJY	96.8	0.87	1.05	1.09	0.96	0.99	0.88
CS0XXK	92.2	0.93	1.04	0.94	0.95	1.04	0.94
CS0BUU	90.6	0.92	1.03	1.15	0.93	1.03	1.01
CS3EPR	91.5	0.89	0.99	1.07	1.06	1.06	0.94
CS7VCZ	95.8	0.87	0.95	1.03	1.01	0.93	0.96
CS0NKC	87.6	1.05	0.96	0.94	0.88	0.97	1.01
CS6CLW	88.2	1.03	0.98	0.92	0.94	0.94	1.08
CS8JGP	90.4	0.88	1.03	0.96	0.97	1.02	0.94
CS6ZLD	93.4	0.92	1.02	0.95	1.08	1.05	1.03
CS1KJY	97.6	0.99	0.97	0.88	1.03	1.08	0.82
CS4KGP	95.1	1.05	1.13	0.91	0.94	1.09	0.97
CS3UTV	94.3	1.06	0.85	0.95	0.97	1.06	0.96
CS4ZCD	92.8	0.88	0.96	0.88	0.89	0.93	0.81
CS0JGZ	98.7	0.83	0.81	0.93	0.86	0.92	0.93
CS3BYN	93.4	0.91	0.78	0.95	0.95	0.87	1.04
CS2WW0	84.6	0.96	0.94	1.09	0.91	0.99	1.08
CS1TBR	87.7	0.82	0.99	1.02	0.99	0.89	0.93
CS0KXU	85.2	0.99	1.02	1.01	1.05	0.94	0.99
CS0HYR	93.3	1.08	1.04	0.94	1.04	0.92	0.97
EDi022-A	90.2	0.95	0.98	1.06	1.05	0.88	1.02
EDi034-A	92.8	0.97	1.05	0.95	1.01	0.95	0.91
EDi036-A	87.6	0.92	0.87	0.88	0.98	0.92	0.83
CS0201	95.4	0.88	0.99	0.92	0.86	1.03	0.97
CS1ATZ	98.3	1.05	1.1	1.13	0.91	1.07	0.92
EDi043-A	93.1	0.94	0.94	0.98	0.94	0.91	1.1
CS2AE8	85.4	1.01	0.97	0.96	1.05	0.94	1.05
CS0002	94.3	0.94	1.15	0.83	1.08	0.97	1.04
CS0202	92.8	0.98	0.87	0.94	0.99	0.96	1.03
CS0206	90.1	1.05	0.91	0.91	0.93	1.08	0.93
CS9XH7	97.6	1.1	0.93	0.87	0.87	1.04	0.99
CS8PAA	95.4	0.92	0.96	0.99	0.96	0.83	0.87

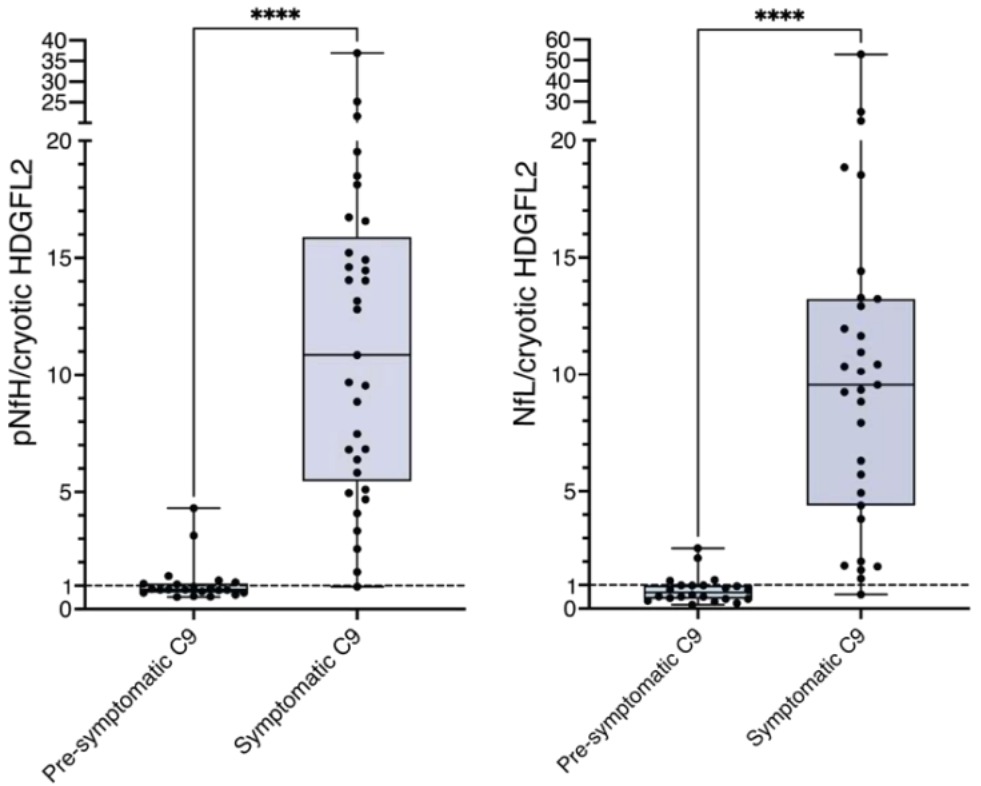
Development ot TDP-43 Biomarkers:
TDP-43 loss of function generated cryptic peptide in sALS and C9 ALS CSF





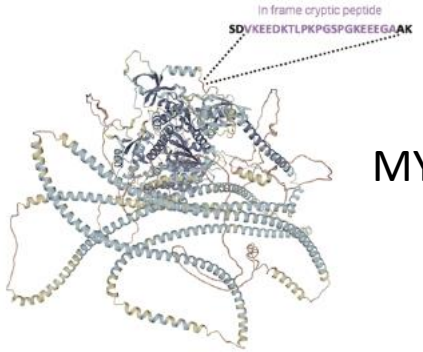
cryptic
HDGFL2 Ab





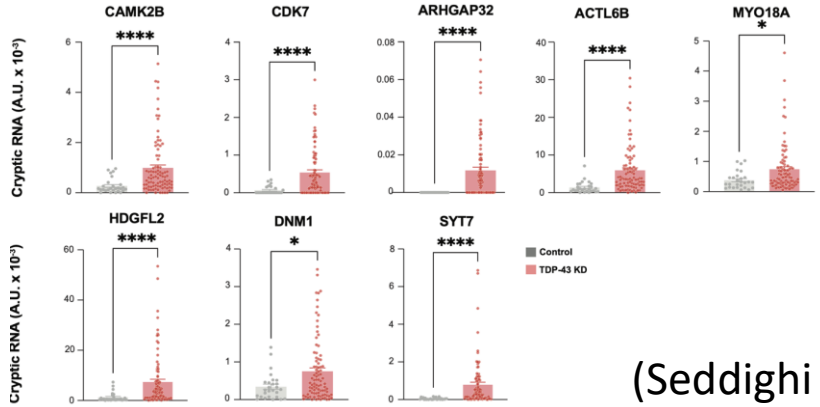
Irwin et al, BioRxiv, 2023

Identification of multiple TDP-43 dependent cryptic peptides in ALS CSF



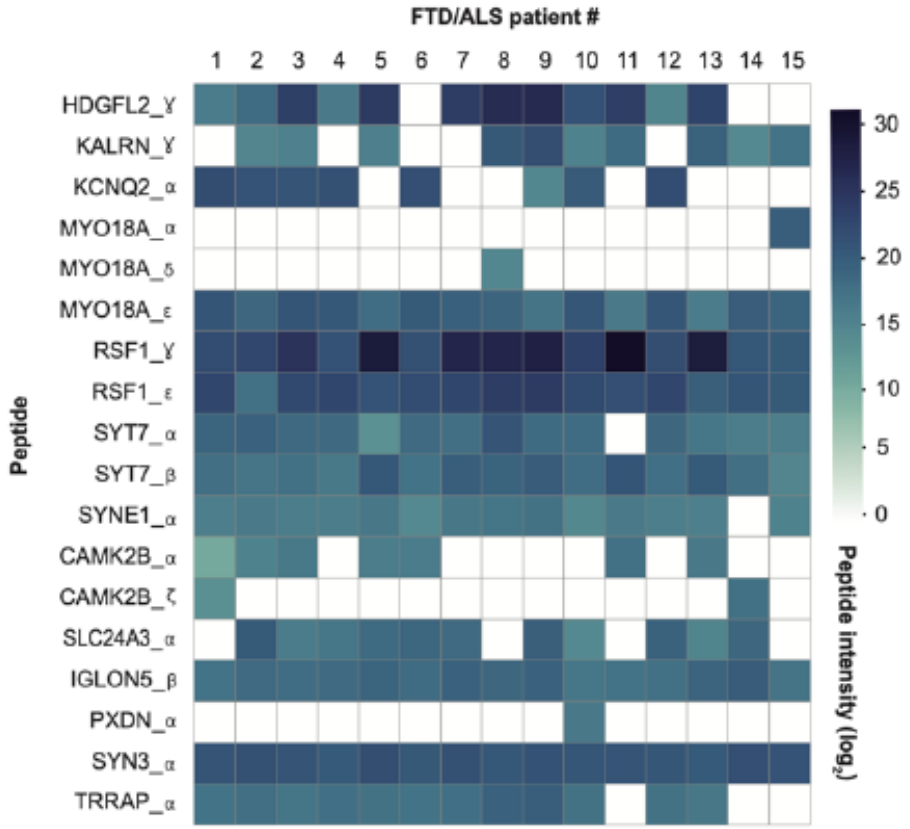
MYO18 cryptic peptide

Detection of cryptic peptides RNA in ALS patients



(Seddighi et al, BioRxiv, 2023)

Detection of cryptic peptides in ALS CSF (Mass spect)



Functional Biomarkers for sALS: TDP-43

- Multiple TDP-43 readouts coming:
  - cryptic peptides (e.g. ELISA), RNA analytics
- Needed studies
  - The first two identified- more are likely to come
  - Need data on reliability, reproducibility, sensitivity
    - Banked CSF may be used
  - Correlation with disease parameters
    - rate of progression, clinical subtypes, age, sex, etc
  - Response to drugs ??
    - (invitro pending (e.g. CHMP7 ASO)
  - Correlation with existing biomarkers: NFL?, inflammation, etc
- Will require CSF testing

Regimen F Drug Science Q&A Webinar

Register: https://partners.zoom.us/webinar/register/WN_I8oqKOrRRpOT2LU3autvLw

When: Monday, March 27th at 5-6 PM Eastern Time

Topic: Regimen F Drug Science and MOA Public Webinar