HEALEY ALS Platform Trial

Weekly Q&A – March 23, 2023

Healey Center
Sean M. Healey & AMG Center for ALS at Mass General
Guest Speaker

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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

- 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

- >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, ubiquulin, 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
# BioMarkers for ALS: Current status

Stratification vs prognostic vs target engagement vs outcome measures

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

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

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

Nuclear TDP-43
Nuclear Clearing
Cytoplasmic Aggregation

Postmortem Pathology
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

- 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

iPSC Human Spinal Neurons

Day 18
CHMP7 Relocalization

Day 25
POM121 Reduction

Day 32
NPC Injury

Day 46
Loss of Nuclear TDP-43 Function (RNA Processing)

Initiation of NPC injury: POM121 reduction

CHMP7 may be the most upstream pathophysiological event in sALS

(Coyne et al, Sci Transl Med, 2021)
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 among 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

~90% KD of CHMP7

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

Irwin et al, BioRxiv, 2023
Identification of multiple TDP-43 dependent cryptic peptides in ALS CSF

Detection of cryptic peptides in ALS CSF (Mass spect)

Myo18A cryptic peptide

Detection of cryptic peptides RNA in ALS patients

(Seddighi et al, BioRxiv, 2023)
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