Precision medicine for ALS

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Washington University in St. Louis

Genetics and Genomics of Disease
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Disclosures:

Ionis Pharmaceuticals provided the antisense oligos used in these studies and provides research support for my lab.

Regulus Therapeutic has provided the antisense oligos used in the miRNA studies.

Biogen Idec. provides research support for clinical studies.

Cytokinetiics: Consultant

Washington University, Ionis Pharmaceuticals, Regulus Therapeutics have filed patents regarding the use of antisense oligonucleotides in neurodegenerative disease.
Research Focus

Goal: Understand the pathophysiology of and develop novel therapeutic strategies for neurological diseases.

• SOD1 Familial ALS
  - To be described

• miRNAs –
  - Understanding miRNA changes in disease
  - Developing novel tools to understand cell type specific miRNAs

• C9ORF72 –
  - Using neurons directly converted from fibroblasts to understand disease
  - Understanding clinical phenotype and biomarkers

• Tau –
  - Understanding role of tau isoforms
  - Understanding how decreasing tau affects seizures (hyperexcitability)
  - Developing antisense oligo methods of reducing total tau mRNA or changing tau splicing patterns
**Targets**

- **Huntingtin** – Huntington’s Disease
- **Tau** – Alzheimers Disease, FTD, PSP, CBD
- **Prion protein** – prion disease (Creutzfeld-Jacob)
- **SMN** – spinal muscular atrophy
- **Dystrophin** – muscular dystrophy (DMD)
- **TDP-43** - FTD, ALS
- **C9ORF72** – FTD, ALS
- **Myostatin** – muscle diseases
- **TREM2** – AD, Parkinsons, FTD, ALS
- **Many other pathways**
Targeted Therapeutic Approaches

• Consider rationale for the therapeutic
  – Link to human disease?
  – Likely safe?
• Develop a method to engage that target
• Develop a method to measure the target in living humans
• Applies more broadly?
• Understand patient population
• Focused clinical trial
Methods to Increase/Replace Proteins

- Small molecules
- Viral delivery
- Change splicing (Small molecules/Antisense oligonucleotides)
Methods to Clear/Improve Toxic Proteins

- Small molecules
- Use the immune system (vaccination or passive immunization)
- RNA interference
- Antisense oligonucleotides
Antisense Oligonucleotides

Current chemistries
- 10 fold increase in potency
- 10 fold increase in duration of action
- Marked decrease in toxicities
- Increase in therapeutic index
- Clinical experience 1000+ patients outside of CNS
Amyotrophic Lateral Sclerosis

- Progressive degenerative disease
  - resulting in stiffness, weakness, and death in 2-5 years from respiratory failure
- No adequate current therapies
- Loss of neurons in the brain and spinal cord in the motor pathways
- 10% ALS familial / 90% Sporadic
- 15-20% of familial ALS caused by superoxide dismutase 1 (SOD1) mutations

Lou Gehrig
ALS is many diseases

• Very obvious: patients are very different
  – Sites of onset, age at onset, speed of progression
  – So…it’s clear we should be “splitters”

• However, ALS is rare.
  – So…we’ve had to be “lumpers”
    • To study genetic causes: get 5000 patients of all types and look for shared genes
    • To study therapies: get everyone meeting inclusion criteria and see if on average, a drug works
Studies to Understand Subsets of ALS

Better Subgroups:
- Genetic
- Protein expression
- Disease features

20,000

New gene discoveries

Genomic Translation for ALS care

“Precision Medicine”
- information back into ALS clinics
- influence and improve ALS care

PROJECT MinE
Make it yours

CREATE
Treatments for ALS & Related Disorders

ANSWER ALS
ALS is many diseases

• Why is recognizing “splitting” important?
  - Different types may have very different causes
  - Different types may have very different outcomes
  - Different types may require different therapies

• Progress is being made in finding subtypes beyond what human experience can identify
Properties of SOD1

- Soluble homodimers (153aa)
- Very stably folded protein
- Binds one Cu and one Zn; active site is Cu
- Abundant (~1% of brain protein)
- Ubiquitous, Cytosolic
Rationale for Decreasing SOD1 as a Therapy for SOD1-Mediated ALS

• Mutant Superoxide Dismutase 1 (SOD1) causes disease by acquisition of a toxic property that is independent of dismutase activity
• Decreasing SOD1 likely to ameliorate disease
• Likely safe to decrease SOD1
Oxidized/misfolded superoxide dismutase-1: the cause of all amyotrophic lateral sclerosis?

Kabashi E, Valdmanis PN, Dion P, Rouleau GA.
Gene Targeted Therapy for ALS

• Preclinical SOD1 Antisense oligo data
  - decrease SOD1 in vivo
  - distribute widely
  - neuroprotective

• Phase I Clinical Trial

• Other SOD1 studies to enable Phase II
Inhibition of SOD1 mRNA after antisense oligo treatment in vitro

Effective oligos that suppress SOD1 mRNA levels

Untreated Control

Control Oligo

r/hSOD1^{146144}
r/hSOD1^{146145}
rSOD1^{146192}

%Control Expression

5'-UTR
650 nt
3'-UTR

Intron Targeting ASOs
Intraperitoneal Administration of Antisense Oligo

SOD1 mRNA % Saline Control

Liver
Kidney
Brain

r/hSOD1146144
r/hSOD1146145
rSOD1146192
SOD1 scrambled (Control)
Delivery of Oligos into CNS

Continuous infusion into right lateral ventricle

Cervical
Thoracic
Lumbar
Sacral

Continuous infusion into Spinal Cord
Delivery of Oligos to Rats/Mice

Anti-sense Oligonucleotides (ASOs)

Catheter

Alzet Pump

Lateral Ventricle
Delivery by intraventricular administration to Rhesus monkey spinal cord

Anti Oligo

Anti-GFAP

F

G

Oligo Treated

H

I

Saline Treated

Lumbar Ventral Horn
Intraventricular infusion delivers oligos widely

**Hippocampus**
- D. Pyramidal neuron
- D. Dentate granular neuron

**Substantia nigra**
- E. Dendritic neurons

**Brainstem (Pons)**
- F. Pontine nuclei

**Cerebellum**
- G. Granular neurons
  - Purkinje cells

**Rhesus monkey brain**

Anti oligo antibody: monoclonal antibody that specifically recognizes modified oligos 100 micrograms infused per day intraventricularly for 14 days
CSF infusion delivers SOD1 Antisense oligos widely

Kordasiewicz et al. Neuron 2012
Mutant SOD1 Causes ALS-like phenotype in Rodents

• Mice, rats develop weakness and atrophy
• SOD1\textsuperscript{G93A} Rat

(Howland et al., PNAS, 2002)

Richard Smith, Don Cleveland
Antisense SOD1 oligos decrease SOD1 protein in SOD1\textsuperscript{G93A} rat

Saline  r/hSOD1\textsuperscript{333611}

\begin{itemize}
  \item human SOD1\textsuperscript{G93A}
  \item rat SOD1
  \item \(\alpha\)-tubulin
\end{itemize}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{chart}
\caption{Cervical Spinal Cord}
\end{figure}

\begin{itemize}
  \item SOD1\textsuperscript{Scrambled}
  \item r/hSOD1\textsuperscript{333611}
\end{itemize}

\(N=6, +/- SD\)
Treatment with SOD1 Oligo Extends Survival in SOD1$^{G93A}$ Rat

- **Onset**
  - Saline: 102+/11
  - SOD1 Oligo: 107+/4

- **Early Disease**
  - Saline: 122+/-11
  - SOD1 Oligo: 139+/-5

- **Survival**
  - Saline: 126+/-8
  - SOD1 Oligo: 156+/-12

Doubling of survival *after* onset

N=12
An antisense oligonucleotide against SOD1 delivered intrathecally for patients with SOD1 familial amyotrophic lateral sclerosis: a phase 1, randomised, first-in-man study

Timothy M Miller, Alan Pestrone, William David, Jeffrey Rothstein, Ericka Simpson, Stanley H Appel, Patricia L Andres, Katy Mahoney, Peggy Allred, Katie Alexander, Lyle W Ostrow, David Schoenfeld, Eric A Macklin, Daniel A Norris, Georgios Manousakis, Matthew Crisp, Richard Smith, C Frank Bennett, Kathie M Bishop, Merit E Cudkowicz

www.thelancet.com/neurology Published online March 29, 2013
Antisense Oligonucleotide in CNS in Humans

- 32 subjects, 21 individuals
Antisense Oligonucleotide in CNS in Humans

- 32 subjects, 21 individuals
- Received single, dose of Antisense oligonucleotide designed to lower SOD1 levels
- Intrathecal infusion for 12 hours
- Randomized, double-blind, placebo
- Doses (0.15 mg, 0.50 mg, 1.50 mg, 3.00 mg)
Intrathecal Infusion
# Treatment-emergent Adverse Events

Adverse events listed are those that occurred with a frequency >5% (i.e. occurring in >1 ISIS-SOD1\textsubscript{Rx} patient) or were CTCAE grade 3 or greater in severity.

<table>
<thead>
<tr>
<th>Adverse Event Term</th>
<th>ISIS-SOD1\textsubscript{Rx} % (# events)</th>
<th>Placebo % (# events)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-LP Syndrome</td>
<td>33% (8)</td>
<td>38% (5)</td>
</tr>
<tr>
<td>Back Pain</td>
<td>17% (4)</td>
<td>50% (4)</td>
</tr>
<tr>
<td>Nausea</td>
<td>13% (3)</td>
<td>0% (0)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>8% (2)</td>
<td>0% (0)</td>
</tr>
<tr>
<td>Headache</td>
<td>8% (2)</td>
<td>13% (1)</td>
</tr>
<tr>
<td>Fall</td>
<td>8% (2)</td>
<td>0% (0)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>8% (2)</td>
<td>0% (0)</td>
</tr>
<tr>
<td>Cerebral Infarct</td>
<td>0% (0)</td>
<td>13% (1)</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>0% (0)</td>
<td>13% (1)</td>
</tr>
<tr>
<td>Cough</td>
<td>0% (0)</td>
<td>13% (1)</td>
</tr>
</tbody>
</table>

Post-LP syndrome, back pain, and nausea/vomiting incidences are not unexpected given the 17G Tuohy needle used for the infusion.
Pharmacokinetics
Plasma Concentrations Peak at End of 12-hr Infusion

ISIS 333611 Plasma Concentrations from Patients in Cohorts 3 and 4, (1.5 and 3.0 mg/12 hrs) (333611-CS1)

Infusion period

Predicted Cohort 4

Predicted Cohort 3

Mean ± SD

Cohort 1,2 were <LLOD

ISIS 333611 plasma, observed Cohort 3

ISIS 333611 plasma, observed Cohort 4
Conclusions

• SOD1 ASO was very well tolerated at doses up to 3 mg;
  – No safety or tolerability concerns related to ASO were identified

• Dose dependent CSF and plasma concentrations were observed;
  – observed drug concentrations were reasonably consistent with expected values (generally within 2-fold)

• Results from this study suggest that antisense oligonucleotide delivery to the CNS may be a viable therapeutic strategy for neurological disorders
Antisense Oligos: C9ORF72

Targeting RNA Foci in iPSC-Derived Motor Neurons from ALS Patients with a C9ORF72 Repeat Expansion

Dhruv Sareen,1,2 Jacqueline G. O’Rourke,1 Pratap Meera,3 A. K. M. G. Muhammad,1 Shaday Grant,1 Megan Simpkinson,1 Shaughn Bell,1 Sharon Carmona,1 Loren Ornelas,1 Anais Sahabian,1 Tania Gendron,4 Leonard Petrucelli,4 Michael Baughn,5 John Ravits,5 Matthew B. Harms,6 Frank Rigo,7 C. Frank Bennett,7 Thomas S. Otis,3 Clive N. Svendsen,1,2 Robert H. Baloh1,8*

Targeted degradation of sense and antisense C9orf72 RNA foci as therapy for amyotrophic lateral sclerosis and frontotemporal dementia

Clotilde Lagier-Tourenne1,2,4, Michael Baughn1,4, Frank Rigo6, Shuying Sun2,3, Patrick Liu3, Hai-Ri Li3, Jie Jiang2,3, Andy Watt5, Seung Chun3, Melanie Katz1, Jinsong Qiu1, Ying Sun1,3, Shuo-Chien Ling1,3, Qiang Zhu2,3, Magdalini Polymenidou2,3,4, Kevin Drenner1,2, Jonathan W. Artates2,3, Melissa M. McAlonis2,3, Sebastian Markmiller3, Kasey R. Hutt2, Donald P. Pizzo6, Janet Cady7, Matthew B. Harms7, Robert H. Baloh6, Scott R. VandenBerg6, Gene W. Yeo5, Xiang-Dong Fu4, C. Frank Bennett3, Don W. Cleveland1,2,3,4, and John Ravits1,4

RNA Toxicity from the ALS/FTD C9ORF72 Expansion Is Mitigated by Antisense Intervention

Christopher J. Donnelly,1,5 Ping-Wu Zhang,1,5 Jacqueline T. Pham,3 Aaron R. Heusler,4 Nipun A. Mistry,1,5 Svetlana Vidensky,1,5 Elizabeth L. Daley,1,5 Erin M. Poth,7 Benjamin Hoover,1,5 Daniel M. Fines,1,5 Nicholas Maragakis,1 Pentti J. Tienari,6 Leonard Petrucelli,7 Bryan J. Traynor,1,8 Jiou Wang,2,4 Frank Rigo,9 C. Frank Bennett,9 Seth Blackshaw,2 Rita Sattler,1,5,10,* and Jeffrey D. Rothstein1,2,3,5,10,*
Planning for SOD1 Phase II

- Natural history of SOD1
- SOD1 as a pharmacodynamics marker?
SOD1 as a Biomarker in CSF

• Does SOD1 in CSF reflect brain SOD1?

• Is SOD1 stable over time?
Antisense Oligo Decreases SOD1 in CSF

Winer et al., JAMA Neurology 2013
Antisense Oligo Decreases SOD1 in CSF

C

Human SOD1 Protein in Rat CSF

D

Human SOD1 in Rat CSF vs Brain

Human SOD1 in Brain, y

Human SOD1 in CSF, %

R² = 0.3011

Winer et al., JAMA Neurology 2013
SOD1 in CSF Varies Little Over Time

Average 7%
CSF SOD1 as a Pharmacodynamic Marker

• SOD1 Knockdown in brain leads to knockdown in CSF
• SOD1 CSF varies little with repeat measurements

SOD1 half life?
Measuring SOD1 Protein Half-Life in People

David Holtzman, Randy Bateman
SOD1 Levels by Mass Spec

- Immunoprecipitate SOD1
- Identify reliable proteolytic fragment (peptide) containing Leucine
- Measure $^{13}$C-Leucine SOD1 compared to $^{12}$C-Leucine SOD1 (mass spec shift of 6)

Collaboration with Randy Bateman, Kwasi Mawuenyega, Bruce Patterson, Kevin Yarasheski
SOD1 is not adequately labeled in 6 hours.
SOD1 Synthesis Rate in Brain in Rats

SOD1\textsuperscript{WT} Rats labeled with $^{13}$C Leucine for 3 days or 7 days (N=4)

Synthesis rate = 1.71\% per day
$t_{1/2}$ = 29.3 days
Does the half-life matter?

Simulated Effect of SOD1 Protein Half-life on Protein Levels

- Time to Maximum Inhibition:
  - 143 days - 90 day $t_{1/2}$
  - 129 days - 60 day $t_{1/2}$
  - 111 days - 30 day $t_{1/2}$

Dan Norris, Ionis
Stable Isotope Labeling Kinetics (SILK)

A

Label with $^{13}$C$_6$-leucine

Perfuse and collect tissues

Immunoprecipitate SOD1

Elute and digest with GluC

LC-MS

B

Input

IP

SOD1

C

Signal Intensity

+6

m/z

Labeled SOD1

Unlabeled SOD1

Matt Crisp, Kwasi Mawuenyega
<table>
<thead>
<tr>
<th>Leucine-free diet acclimation (1-2 weeks)</th>
<th>Pulse (7 days)</th>
<th>Chase</th>
</tr>
</thead>
</table>

**SILK Study Design**
SILK Study Design

Leucine-free diet acclimation (1-2 weeks) → Pulse (7 days) → Chase

- 12C₆-leucine
- 13C₆-leucine

Labeled SOD1
Unlabeled SOD1

Rats
- SOD1WT
- SOD1G93A

Tissues
- Spinal Cord, Cortex, Liver, Kidney, Plasma
SOD1 animals received sufficient label

SOD1<sup>WT</sup>

Plasma Leucine

SOD1<sup>G93A</sup>

Plasma Leucine

Bob Chott, PhD (Yarasheski Lab)
SOD1 turnover is relatively rapid in unaffected tissues.

**SOD1\(^{WT}\)**
- Plasma Leucine
- Liver

**SOD1\(^{G93A}\)**
- Plasma Leucine
- Liver
SOD1 turnover is relatively rapid in unaffected tissues

**SOD1\(^{WT}\)**

- Plasma Leucine
- Liver
- Kidney

**SOD1\(^{G93A}\)**

- Plasma Leucine
- Liver
- Kidney
SOD1 turnover is slowest in affected tissues

SOD1 \(^{WT}\)

- Plasma Leucine
- Liver
- Kidney
- Cortex

SOD1 \(^{G93A}\)

- Plasma Leucine
- Liver
- Kidney
- Cortex
SOD1 turnover is slowest in affected tissues
Turnover of misfolded SOD1 G93A is accelerated in the spinal cord

SOD1\textsuperscript{G93A} Spinal Cord

- Plasma Leucine
- Lumbar SC
- Misfolded SC

B8H10, mouse monoclonal, MM-0070 MédiMabs
Misfolded SOD1 pools are turned over faster in non-affected tissues

SOD1$^{G93A}$ Spinal Cord

SOD1$^{G93A}$ Liver

- Plasma Leucine
- Lumbar SC
- Misfolded SC
- Liver
- Misfolded Liver
<table>
<thead>
<tr>
<th></th>
<th><strong>SOD1 WT</strong></th>
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<th><strong>SOD1 G93A</strong></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>FTR (pools/d)</td>
<td>95% CI</td>
<td>Half-life (d)</td>
<td>FTR (pools/d)</td>
<td>95% CI</td>
<td>Half-life (d)</td>
</tr>
<tr>
<td>Liver total protein</td>
<td>1.265</td>
<td>1.121 - 1.409</td>
<td>0.55</td>
<td>0.711</td>
<td>0.655 - 0.767</td>
<td>0.97</td>
</tr>
<tr>
<td>Cortex total protein</td>
<td>0.177</td>
<td>0.167 - 0.186</td>
<td>3.92</td>
<td>0.127</td>
<td>0.120 - 0.135</td>
<td>5.44</td>
</tr>
<tr>
<td>Spinal cord total protein</td>
<td>0.087</td>
<td>0.081 - 0.094</td>
<td>7.95</td>
<td>0.096</td>
<td>0.088 - 0.104</td>
<td>7.21</td>
</tr>
<tr>
<td></td>
<td>SOD1 WT</td>
<td></td>
<td></td>
<td>SOD1 G93A</td>
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<td>95% CI</td>
<td>Half-life (d)</td>
</tr>
<tr>
<td>Liver SOD1</td>
<td>0.397</td>
<td>0.379 - 0.415</td>
<td>1.75</td>
<td>0.485</td>
<td>0.457 - 0.514</td>
<td>1.43</td>
</tr>
<tr>
<td>Kidney SOD1</td>
<td>0.205</td>
<td>0.197 - 0.213</td>
<td>3.38</td>
<td>0.188</td>
<td>0.180 - 0.196</td>
<td>3.68</td>
</tr>
<tr>
<td>Cortex SOD1</td>
<td>0.074</td>
<td>0.071 - 0.078</td>
<td>9.33</td>
<td>0.104</td>
<td>0.098 - 0.109</td>
<td>6.69</td>
</tr>
<tr>
<td>CSF SOD1</td>
<td>0.047</td>
<td>0.044 - 0.050</td>
<td>14.86</td>
<td>0.074</td>
<td>0.070 - 0.077</td>
<td>9.38</td>
</tr>
<tr>
<td>Spinal cord SOD1</td>
<td>0.044</td>
<td>0.041 - 0.046</td>
<td>15.86</td>
<td>0.077</td>
<td>0.073 - 0.081</td>
<td>8.98</td>
</tr>
<tr>
<td></td>
<td>SOD1 WT</td>
<td></td>
<td></td>
<td>SOD1 G93A</td>
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<td>95% CI</td>
<td>Half-life (d)</td>
<td>FTR (pools/d)</td>
<td>95% CI</td>
<td>Half-life (d)</td>
</tr>
<tr>
<td>Liver misfolded SOD1</td>
<td>0.868</td>
<td>0.795 – 0.941</td>
<td>0.80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spinal cord misfolded SOD1</td>
<td>0.325</td>
<td>0.309 – 0.342</td>
<td>2.13</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
SOD1 G93A turnover is slowest in affected tissues

- Cortex: 7 days
- Spinal Cord: 9 days
- Liver: 1.4 days
- Kidney: 3.7 days
Measuring SOD1 turnover in human CSF

Animal data indicated a long half-life

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td>CSF SOD1</td>
<td>0.047</td>
</tr>
<tr>
<td>Spinal cord SOD1</td>
<td>0.044</td>
</tr>
</tbody>
</table>

Human labeling adapted from animal study

10 days – low leucine diet (2000 mg) with $^{13}$C$_6$-leucine administered 3X daily (1000 mg total)

Plasma and CSF collected at end of labeling and up to 84 days later
<table>
<thead>
<tr>
<th>Day</th>
<th>CSF Samples</th>
<th>Plasma Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>10</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>14</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>28</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>42</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>84</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

**CSF SOD1 Half-life in Humans**

Oral $^{13}C_6$-leucine Labeling

Predicted $^{13}C_6$-leucine labeled SOD1(2%)
Measuring SOD1 turnover in human CSF
SOD1 Labeling in Humans

![Graph showing human subject labeling over time.
Legend:
- NC02 Plasma Leucine
- NC02 CSF Total Protein
- NC02 Plasma Total Protein]
SOD1 Labeling in Humans

Human Subject Labeling

- C-13 Label (%)
- Time (days)
- End of Pulse

Lines and markers represent:
- SOD1 NC02
- NC02 Plasma Leucine
- NC02 CSF Total Protein
- NC02 Plasma Total Protein
SOD1 Labeling in Humans

**Human Subject Labeling**

- **SOD1 NC05**
- **SOD1 NC02**
- **SOD1 NC03**

- **NC02 Plasma Leucine**
- **NC02 CSF Total Protein**
- **NC02 Plasma Total Protein**

**SOD1 CSF Half life**

23+/-8 days
Conclusions/Next steps

- SOD1 CSF levels Stable
- Development of SILK paradigm in animals and human
- SOD1 turnover is slowest in affected tissues
- SOD1 turnover in CSF from human subjects is 4 fold slower than CSF total protein
- Half-life of SOD1 in humans:
  - In SOD1 ALS underway
Funding
Hope Center
Project5 for ALS
ALS Association
Muscular Dystrophy Association
Packard Center for ALS
Target ALS
Weston Foundation
Tau Consortium
NIH/NINDS
NIH/NIA

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Mariah Lawler
Kathleen Schoch
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Carey Shaner
Tao Shen
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Leah Winer, C. Kebodeaux

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Randy Bateman, Dave Brody, John Cirrito,
Marc Diamond, Joe Dougherty, Matt Harms,
Dave Holtzman, Bruce Patterson, Chris Weihl,
Mike Wong, Greg Wu, Kevin Yarasheski

MGH Merit Cudkowicz – Co-PI Phase I study
Pat Andres, Katy Mahoney
Eric Macklin, David Schoenfeld

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Univ. Pittsburg
Bob Bowser, David Lacomis

UCSD Don Cleveland, Richard Smith

Isis Pharmaceuticals
Frank Bennett, Kathie Bishop, Frank Rigo
Holly Kordasiewicz, Eric Swayze

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microRNAs

• Discovered in 1993
  - 2\textsuperscript{nd} discovered in 2000

• Translational repressors;
  18-22nt long

• Partial complementarity
  - Seed region
  - Typically 200-300 mRNAs
miRNA Antisense Oligonucleotide Safety:

- Phase 2a by Santaris Pharma, 36 patients with chronic HCV genotype 1 infection.

miRNAs as Targets for ALS Therapeutics

• Identify dysregulated microRNAs in ALS
  - In rodent model and in patients

• Develop method for inhibiting these miRNAs throughout CNS

• Determine if these miRNAs negatively or positively influence disease progression
Human Tissues Identifies 6 Best Targets

Human SC

Koval et al. Hum Mol Genet 2013
MiR-155 is increased in human ALS
DeVos and Miller, 2013
anti-miR-155 is functional throughout CNS
Anti-miR-155 is present in all cell types
Anti-Mir-155 Does not Change Onset

**Weight Peak**

- Cumulative onset vs Age (d)
- Green line: saline
- Blue line: anti-miR-155
- n.s. indicates no significant difference

**Neuroscore 1**

- Cumulative onset vs Age (d)
- Green line: saline
- Blue line: anti-miR-155
- n.s. indicates no significant difference

**SOD1<sup>G93A</sup>** mice, treated at 60 days of age both intraventricularly and intraperitoneally
Anti-miR-155 Extends Disease Duration

10 day extension  
\( p = 0.007 \)

38% increase  
\( p < 0.001 \)
Conclusions

• miRNAs are dysregulated in ALS in both the rodent model and in patients

• miRNAs can be inhibited broadly in the CNS with antisense oligonucleotides

• miR-155 remains an exciting therapeutic target
  - miR-155 negatively contributes to disease
  - Implications for both sALS and fALS
  - Significant increase in survival
  - Can read miR-155 in peripheral blood cells
Remaining questions

• Mechanism of how miR-155 influences disease
• Which CNS cells express miR-155?
• Other miRNAs?
• miR-155 clinical trial?
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