Translational medicine and ALS clinical trials

Timothy Miller, MD, PhD
Washington University in St. Louis

Genetics and Genomics of Disease
October 6, 2014

millert@neuro.wustl.edu
Disclosures:

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

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

Biogen Idec. provides research support for clinical studies.

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

• Define a clear target
• 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
DeVos and Miller, 2013
DeVos and Miller, 2013
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
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
SOD1 in Sporadic ALS

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

Intron Targeting ASOs

Control Oligo

%Control Expression

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

5’-UTR

3’-UTR

650 nt
Intraperitoneal Administration of Antisense Oligo

Liver
Kidney
Brain

SOD1 mRNA % Saline Control

r/hSOD1\textsuperscript{146144}  r/hSOD1\textsuperscript{146145}  rSOD1\textsuperscript{146192}  SOD1\textsuperscript{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

<table>
<thead>
<tr>
<th>Anti Oligo</th>
<th>Anti-GFAP</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="F" alt="Image" /></td>
<td><img src="G" alt="Image" /></td>
</tr>
<tr>
<td><img src="H" alt="Image" /></td>
<td><img src="I" alt="Image" /></td>
</tr>
</tbody>
</table>

Oligo Treated

Saline Treated

Lumbar Ventral Horn
Intraventricular infusion delivers oligos widely

<table>
<thead>
<tr>
<th>Hippocampus</th>
<th>Substantia nigra</th>
<th>Brainstem (Pons)</th>
<th>Cerebellum</th>
</tr>
</thead>
<tbody>
<tr>
<td>D Pyramidal neuron</td>
<td>E Dendritic neurons</td>
<td>F Pontine nuclei</td>
<td>G Granular neurons</td>
</tr>
<tr>
<td>Dentate granular neuron</td>
<td></td>
<td></td>
<td>Purkinje cells</td>
</tr>
</tbody>
</table>

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
- $\text{SOD1}^{G93A}$ Rat

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

- Human SOD1\textsuperscript{G93A}
- Rat SOD1
- \(\alpha\)-tubulin

Cervical Spinal Cord

SOD1\textsuperscript{G93A} Protein (% Control)

Saline  r/hSOD1\textsuperscript{333611}

\(N=6, +/- SD\)
Treatment with SOD1 Oligo Extends Survival in SOD1\textsuperscript{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 Pestronk, 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
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

<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>

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. Post-LP syndrome, back pain, and nausea/vomiting incidences are not unexpected given the 17G Tuohy needle used for the infusion.
**ISIS-SOD1\textsubscript{Rx} Adverse Events are not Dose-Related**

<table>
<thead>
<tr>
<th>Adverse Event Term</th>
<th>ISIS-SOD1\textsubscript{Rx} % (# events)</th>
<th>Cohort Frequency # events in Cohorts (1, 2, 3, 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-LP Syndrome</td>
<td>33% (8)</td>
<td>(4, 2, 1, 1)</td>
</tr>
<tr>
<td>Back Pain</td>
<td>17% (4)</td>
<td>(2, 1, 1, 0)</td>
</tr>
<tr>
<td>Nausea</td>
<td>13% (3)</td>
<td>(2, 0, 1, 0)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>8% (2)</td>
<td>(2, 0, 0, 0)</td>
</tr>
<tr>
<td>Headache</td>
<td>8% (2)</td>
<td>(0, 2, 0, 0)</td>
</tr>
<tr>
<td>Fall</td>
<td>8% (2)</td>
<td>(1, 1, 0, 0)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>8% (2)</td>
<td>(1, 0, 0, 1)</td>
</tr>
</tbody>
</table>
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 3
- Predicted Cohort 4
- Mean ± SD

Cohort 1,2 were <LLOD
Pharmacokinetics - CSF

Graph showing the relationship between the amount delivered intrathecally (mg) and the concentration at the end of infusion (μg/mL) for different cohorts.
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 Sarneen,1,2 Jacqueline G. O’Rourke,1 Pratap Meera,3 A. K. M. G. Muhammad,1 Shadray 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. Svedesen,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,*, Michael Baughn1,*, Frank Rigo6, Shuying Sun2,3, Patrick Liu5, Hai-Ri Li3, Jie Jiang2,1, Andy Watt5, Seung Chun5, Melanie Katz5, Jinsong Qiu5, Ying Sun1,3, Shuo-Chien Ling1,3, Qiang Zhu2,3, Magdalini Polymenidou2,3,*, Kevin Drenna1,2, Jonathan W. Artates2,3, Melissa M. McAlonis2,3, Sebastian Markmiller3, Kasey R. Hutt3, Donald P. Pizzo6, Janet Cady7, Matthew B. Harms7, Robert H. Baloh6, Scott R. VandenBerg6, Gene W. Yeo5, Xiang-Dong Fu5, C. Frank Bennett7, Don W. Cleveland1,2,3,*, and John Ravits1,8

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,2 Benjamin Hoover,1,5 Daniel M. Fines,1,5 Nicholas Maragakis,1 Pentti J. Tenari,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

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

Average 7%

Bob Bowser
David Lacomis
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?
microRNAs

- Discovered in 1993
  - 2nd 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 microRNAs throughout CNS

• Determine if these microRNAs negatively or positively influence disease progression
10 array hits confirmed

Mouse SC

Rat SC

Symptomatic SOD1\textsuperscript{G93A} mice and rats
Human Tissues Identifies 6 Best Targets

**miR-24-2**
**miR-142-3p**
**miR-146a**
**miR-146b**
**miR-155**

Koval et al. Hum Mol Genet 2013
MiR-155 is increased in human ALS
anti-miR-155 is functional throughout CNS

Cortex

-RQ- SHIP  PU.1  Card11  CyR61
Saline Scrambled anti-miR-155

SHIP1

-RQ- Parietal Cortex  Occipital Cortex  Cerebellum  Cervical SC  Lumbar SC
Saline Scrambled anti-miR-155
Cy3-anti-miR-155 distributes throughout CNS

- Subventricular zone
- Cortex
- Hippocampus
- Lumbar spinal cord
Anti-miR-155 is present in all cell types tested.
Anti-Mir-155 Does not Change Onset

Weight Peak

Neuroscore 1

SOD1^{G93A} 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?
Acknowledgements

Funding
Project5 for ALS
ALS Association
Muscular Dystrophy Association
Packard Center for ALS
Target ALS
Weston Foundation
Tau Consortium
NIH/NINDS
NIH/NIA

millert@neuro.wustl.edu
Acknowledgements

Miller Lab
Taha Bali
Matt Crisp
Sarah DeVos
Caroline Drain
Jennifer Jockel-Balsarotti
Erica Koval
Mariah Lawler
Kathleen Schoch
Wade Self
Carey Shaner
Tao Shen
Amy Wegener
Former Miller Lab
Peggy Allred, Dushyanth Srinivasan
Leah Winer, C. Kebodeaux

Washington University Colleagues
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

Northeast ALS Consortium (NEALS)

Univ. Pittsburg
Bob Bowser, David Lacomis

UCSD Don Cleveland, Richard Smith
Isis Pharmaceuticals
Frank Bennett, Kathie Bishop, Frank Rigo

millert@neuro.wustl.edu