|   | Introducing ALS: Is ALS one disease? | Biology of ALS: Complex Multisystem Disorder | Treatment of ALS |
Amyotrophic Lateral Sclerosis: no longer considered a neuromuscular disorder: Neurogenerative

- Upper and lower motor neurons
  <1 year to > 10 years

- 20-80 yrs onset, median 54

- Association with FTD/IBM/Pagets

- 1:300 lifetime risk males; 1:800 females
In 30 years there has been an exponential increase in yearly ALS publications.

![Graph showing the increase in ALS publications from 1980 to 2010. The x-axis represents years, and the y-axis represents the number of references. Key terms highlighted include: SOD1, ALS Mouse, Riluzole, >25 genes, Drugs, stem cells, Gene inactivation, and Trial groups.]
Multiple ALS therapies have been tested

One approved (riluzole) and five with some positive Phase II human data*

<table>
<thead>
<tr>
<th>Anti-glutamate&lt;sup&gt;1,2,3,4&lt;/sup&gt;</th>
<th>Growth factors&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Anti-inflammatory&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riluzole</td>
<td>BDNF-IT</td>
<td>Celebrex</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>SC</td>
<td>Minocycline</td>
</tr>
<tr>
<td>Topiramate</td>
<td>CNTF</td>
<td>Nimesulide</td>
</tr>
<tr>
<td>Dextro-methorphan</td>
<td>IGF-1</td>
<td>*NP001</td>
</tr>
<tr>
<td>ONO-2506</td>
<td>Xaliproden</td>
<td></td>
</tr>
<tr>
<td>Memantine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Talampanel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Ceftriaxone</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anti-oxidants/ bioenergetics&lt;sup&gt;1,2,5&lt;/sup&gt;</th>
<th>Anti-apoptotic&lt;sup&gt;1,6&lt;/sup&gt;</th>
<th>Protein aggregation&lt;sup&gt;2,3,7,8&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatine (5 and 10 g)</td>
<td>TCH346</td>
<td>Arimoclool</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Pentoxyfilline</td>
<td>*Lithium</td>
</tr>
<tr>
<td>Acetylcysteine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coenzyme Q</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olexosome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Dexpramipexole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Tamoxifen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*MCI-186</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Dexpramipexole: Phase 2 Results inform Phase 3 Endpoint (CAFS)

ALSFRS-R (Measure of functional decline adjusted for mortality):
Trend towards a dose-dependent improvement in function at 12 weeks (Part 1)

Survival:
Trend towards dose-dependent improvement in survival at 6 months (150 mg BID vs 25 mg BID; Part 2)

*P = 0.046
*P = 0.07

Nature Medicine 2011; 17: 1652
Phase III: No Effect on CAFS through 12 months was Demonstrated with Dexpramipexole
d
Least Square Mean Rank Score

\[ p = 0.8568 \]

- **Placebo** (n = 468)
- **DEX 150 mg twice daily** (n = 473)

\[ 438.84 \pm \text{SE} \]
\[ 441.76 \pm \text{SE} \]

*Dexpramipexole dihydrochloride 150 mg CAFS-Combined Assessment of Function and Survival, Dex, dexpramipexile Analysis used efficacy population: all subjects who received ≥ dose of drug (or placebo) with ≥1 post-dosing evaluation.
# Phase II and EMPOWER: Covariate distribution across studies

<table>
<thead>
<tr>
<th>Subgroup (Baseline characteristic)</th>
<th>Phase 2</th>
<th>EMPOWER</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ALSFRS-R</td>
<td>38.0</td>
<td>38.2</td>
<td>0.78</td>
</tr>
<tr>
<td>Mean age</td>
<td>57.0</td>
<td>57.1</td>
<td>0.93</td>
</tr>
<tr>
<td>Site of onset (limb)</td>
<td>82%</td>
<td>77%</td>
<td>0.20</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>64%</td>
<td>64%</td>
<td>0.92</td>
</tr>
<tr>
<td>Mean symptom duration</td>
<td>14.1 months</td>
<td>15.2 months</td>
<td>0.038</td>
</tr>
<tr>
<td>Riluzole (yes)</td>
<td>61%</td>
<td>75%</td>
<td>0.002</td>
</tr>
<tr>
<td>El Escorial (definite)</td>
<td>46%</td>
<td>32%</td>
<td>0.005</td>
</tr>
</tbody>
</table>
Post-Hoc Exploratory analyses: EMPOWER subgroups by El Escorial definite, symptom duration and riluzole use
EMPOWER: CAFS improved in El Escorial definite subjects

Summary of joint rank (CAFS) through 12 months (Phase 3 study)*

<table>
<thead>
<tr>
<th>Category</th>
<th>% Improvement of DEX compared to PBO</th>
<th>p-value</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficacy</td>
<td>0.6%</td>
<td>0.8569</td>
<td>948</td>
</tr>
<tr>
<td>Definite</td>
<td>6.6%</td>
<td>0.3376</td>
<td>303</td>
</tr>
<tr>
<td>Definite &lt;18 months</td>
<td>7.6%</td>
<td>0.3698</td>
<td>198</td>
</tr>
<tr>
<td>Definite + riluzole</td>
<td>13.4%</td>
<td>0.0919</td>
<td>231</td>
</tr>
<tr>
<td>Definite + riluzole &lt;18 months</td>
<td>19.1%</td>
<td>0.0566</td>
<td>147</td>
</tr>
</tbody>
</table>

*Covariates specified in EMPOWER SAP used to calculate p-values by ANCOVA
EMPOWER clinical effect: rate of ALSFRS-R decline reduced in El Escorial definite subjects

Reduction in slope of decline through 12 months*

<table>
<thead>
<tr>
<th>Group</th>
<th>% improvement of DEX compared to PBO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficacy population</td>
<td>1.72%</td>
</tr>
<tr>
<td>Definite</td>
<td>8.69%</td>
</tr>
<tr>
<td>Definite &lt;18m duration</td>
<td>13.90%</td>
</tr>
<tr>
<td>Definite + riluzole</td>
<td>17.23%</td>
</tr>
<tr>
<td>Definite + riluzole &lt;18m duration</td>
<td>25.65%</td>
</tr>
</tbody>
</table>

*n = 941, p = 0.731
*n = 303, p = 0.2313
*n = 198, p = 0.1111
*n = 231, p = 0.0289
*n = 147, p = 0.0055

*Covariates specified in EMPOWER SAP used to calculate p-values by ANCOVA
EMPOWER clinical effect: hazard for mortality reduced in El Escorial definite subjects

<table>
<thead>
<tr>
<th></th>
<th>Reduction in Hazard Ratio (%)</th>
<th>n</th>
<th>HR</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITT population</td>
<td>1.6%</td>
<td>942</td>
<td>0.984</td>
<td>0.9091</td>
</tr>
<tr>
<td>Definite</td>
<td>23.7%</td>
<td>303</td>
<td>0.763</td>
<td>0.2627</td>
</tr>
<tr>
<td>Definite &lt;18m duration</td>
<td>22.2%</td>
<td>198</td>
<td>0.778</td>
<td>0.3732</td>
</tr>
<tr>
<td>Definite + riluzole</td>
<td>37.3%</td>
<td>231</td>
<td>0.627</td>
<td>0.103</td>
</tr>
<tr>
<td>Definite + riluzole &lt;18m duration</td>
<td>44.9%</td>
<td>147</td>
<td>0.551</td>
<td>0.0797</td>
</tr>
</tbody>
</table>

*Covariates specified in EMPOWER SAP used to calculate p-values by ANCOVA
Ceftriaxone, Upregulates mRNA for glutamate transporter on astrocytes

**Positive Phase II**
Phase II, 38% slowing, p 0.04

**Negative phase III**
Phase III, 8.2%, p 0.20

NINDS Supported
Study stopped early for Futility Interim Survival Analysis

Log-Rank
P = 0.3680

Post-Hoc Covariate Analysis Suggests (p=0.08) 20% Reduction in Hazard Rate for Ceftriaxone Group
NP001 (targets activated macrophages)
Phase II Post Hoc "Responder" Analysis

- 2.5 times higher in high dose group relative to placebo
- Patients with "stable" disease (no change or improved on ALSFRS-R score through Month 6)
What are some of the challenges for therapy development in ALS?

• Disease heterogeneity
• Late diagnosis
• Choosing Therapy: **Uncertainty of cause and targets**
• CNS Therapy Delivery – dosing
• Lack of Biomarkers
ALS Trial Challenges: Disease Heterogeneity

- Heterogeneity is high
- Favorable Prognostic factors
  - Higher Uric acid, creatine, WBC and BMI
  - Eph4A mutations
- Small phase II studies or open label studies don’t predict success in phase III

Sample Size for ALSFRS-R
(90% power, 5% p 1 year, 2 arms)

30% (1.0 →0.7)  356 patients
40% (1.0 →0.6)  200 patients

http://hedwig.mgh.harvard.edu/biostatistics/software
There are many clinical phenotypes

- Neurologist classification
  - Clinical experience
The rate of gene discovery in ALS is increasing: >25 ALS genes

Each gene defines pathways and treatment targets.
Genes associated with mutations linked to MND syndromes show range of clinical phenotypes and neuropathological protein aggregates.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Clinical Involvement</th>
<th>Course</th>
<th>Neuropathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD</td>
<td>LMN, UMN</td>
<td>A4V &lt; 9 months</td>
<td>SOD1 inclusions; no TDP43</td>
</tr>
<tr>
<td>FUS</td>
<td>Primarily LMN, FTD</td>
<td>Juvenile onset</td>
<td>FUS aggregates, no TDP43</td>
</tr>
<tr>
<td>C9orf72</td>
<td>LMN, UMN, FTD</td>
<td>Young onset; shortened survival</td>
<td>TDP43 aggregates; widespread – cerebellar and hippocampal</td>
</tr>
<tr>
<td>UBQLN2</td>
<td>LMN, UMN, FTD</td>
<td>X-linked, juvenile and adult onset</td>
<td>Ubiquilin-2, tdp43 and FUS</td>
</tr>
</tbody>
</table>
• >60% of families known genetic mutations

• 7-10% “sporadic” ALS have C9orf72 mutations

Courtesy of Bryan Traynor, MD
Diagnostic delay in ALS is very long (12 months)

Longer in Older patients, Males, and Limb-Onset Patients see an average of 3 specialists before ALS diagnosis

Nazem Atassi, MD, ABF fellow
Multiple Complex Pathways. Does “death” differ in different compartments?

- Astrocytosis
- Microglial Activation
- Axon Transport
- Protein Aggregation
- Oxidative Stress
- ER Stress
- Mitochondrial Dysfunction
- RNA Processing
- De Myelination
- Muscle Wasting
- Synaptic Die Back
- Micro hemorrhaging
- Leukocyte infiltration

Courtesy of Steve Perrin, PhD and ALSTDI
Lack of ALS drugs due to Complex Biology

• NOT because of FDA, clinical trial design, clinical investigators or expanded access
• The biology of ALS is extremely complex
Many new models and tools to study ALS biology

Rodent Models: SOD1, TDP43
Fly and Worm Models
Induced pluripotent stem cells

Dimos et al Science 2008
Studies using 7T-MR Spectroscopy, 7T-Spine, & PET Imaging reveal abnormalities in glia and corticospinal tracks

A. Myoinositol/Cr (glial marker) increased in the motor cortex in people with ALS.

B. MI/Cr levels correlate with increased reflexes.

High PBR28 uptake in the motor cortex

Poster P06.132

T2* high intensity in the cervical CST

In press

Lawson R, Atassi N et al.
Treatments
Treatment of ALS is Improving

Northeast ALS Consortium (NEALS)

- Symptomatic
- Neuroprotective / Restorative
  - Drugs
  - Stem cells
  - Inactivation of toxic gene products
...the ALS pipeline has many treatments in development for ALS

- Phase III
  - MCI-186
- Phase II
  - NP001
  - GSK1223249
  - Gilenya
  - Tamoxifen/Creatine
  - Mexiletine
  - Nuedexta
  - Tirasemtiv (formerly known as CK-2017357)
  - Immunosuppression (4)
  - Ursodiol
- NurOwn™
- Neuralstem
- High fat nutrition
- Exercise
- Diaphragm pacing

- FALS SOD1
  - ISIS333611
  - Arimoclomol

- FALS/SALS – C90rf72

www.alsconsortium.org
www.clinicaltrials.gov
Two Regulatory Approved Phase I/II stem cells studies in ALS: Neuralstem and BrainStorm

- Neural stem cells engrafted into the spinal cord
- NurOwn™ Adult bone marrow-derived Mesenchymal Stromal Cells (MSCs)
Summary of Phase 2a Studies of Tirasemtiv

1. Tirasemtiv may improve breathing measures, function in general as measured by ASLFRS-R, strength, and endurance

2. Tirasemtiv increases riluzole concentrations, but reducing riluzole dose by half results in levels similar to full dose riluzole alone

3. The initial small studies supported going forward and studying the effect of tirasemtiv in more patients for a longer time period
BENEFIT-ALS: Blinded Evaluation of Neuromuscular Effects and Functional Improvement with *Tirasemtiv* in ALS

- ~400 Patients with ALS
  - Multi-national, double-blind, placebo-controlled trial

- 1 week open-label treatment with *tirasemtiv* at 125 mg twice daily
- 12 weeks double-blind, twice daily oral ascending doses of *tirasemtiv* vs. placebo
Important Inclusion Criteria
1. Definite, possible, or probable ALS
2. A slow vital capacity of 60% or greater
3. Because we are interested in how tirasemtiv may change function in ALS, patients must have some functional abilities that are impaired but still sensitive to improvement
4. Since handgrip endurance is an important measure, patients must have good but not normal maximum handgrip strength

Where Are We Now?
1. 42 centers activated in North America, 3 centers activated in Europe
2. So far, 180 patients have been screened, and 112 patients have been enrolled (as of March 12, 2013)
3. Enrollment expected to be completed by the end of June, 2013
Gilenya Phase IIa Study: Goals and Objectives

- **Primary Objectives:** To evaluate the acute safety and tolerability of fingolimod therapy in patients with ALS

- **Secondary Objectives:** To evaluate the effect of fingolimod on circulating WBC (including lymphocyte sub-populations) over one month in patients with ALS

- **Trial Goal** is to facilitate a Phase IIb Trial of Gilenya for the treatment of patients with ALS
Gilenya Study Sites

- Massachusetts General Hospital
- University of California Irvine
- Georgia Health Sciences University
- Methodist Neurological Institute

Many more wonderful sites were interested. We hope to include most of them in a subsequent, larger trial.
Mexiletine (Dr. Weiss)

**Purpose:** To determine whether mexiletine is safe and tolerable for patients with Sporadic Amyotrophic Lateral Sclerosis (SALS).

**Mexiletine:**

- Is a use-dependent sodium channel blocker that is FDA-approved for the treatment of cardiac arrhythmias
- Showed prolonged survival in animal models of ALS
- ~ 60 eligible subjects w/ SALS will be recruited at multiple US centers
  - 12 weeks: Mexiletine (300 mg/day or 900 mg/day) or placebo
**Purpose:** To determine whether Nuedexta® improves speech, swallowing, and saliva control in subjects with ALS. (Phase II)

- 60 participants
  - 28 days (±3 days) with either Nuedexta® or placebo.
  - Followed-by 10-15 day washout period will then occur.
  - After the washout period, participants will be crossed-over to the other treatment arm for an additional 28 days (±3 days) of treatment.
  - A follow-up telephone call will occur approximately 28 days after completion of the study.

- California Pacific Medical Center
  San Francisco, CA
- Hennepin County Medical Center
  Minneapolis, MN
- Neurology Associates
  Lincoln, NE
- Cleveland Clinic
  Cleveland, OH
- Providence ALS Center
  Portland, OR
- Georgetown University
  Washington D.C.
Ozanezumab (GSK1223249) monoclonal antibody against Nogo-A

48-week, randomized, multi-center, double-blind, placebo-controlled, parallel group trial investigating efficacy and safety of IV GSK1223249 compared to placebo in participants with ALS

Eligible subjects will be randomized (1:1) to receive either IV placebo or IV ozanezumab every 2 weeks over 48 weeks.

Approx 294 eligible subjects will be randomized from approximately 37 centers worldwide.

Primary objective: assess the effect of ozanezumab on the physical function and survival of ALS subjects over a treatment period of 48 weeks, measured by the ALSFRS-R.

Currently 18 international sites listed on clinicaltrials.gov / NEALS
What was learned/gained from past human studies?

- Two therapies (riluzole and nuedexta) and multidisciplinary care
- Large experience in trials in ALS/Regulatory pathway
- Genetic studies define multiple critical pathways in ALS
  - New opportunities for the development of animal and cell-based models.
- Not one disease
  - Treatments -differentiated -- different cohorts
  - Treatments -target different pathways – cocktail
  - Timing of treatments may need to be tailored
Therapy for ALS in 1, 2, 5 and 10 years

- Cocktail therapy
- Cohort enrichment for relevant pathology
- Target engagement and pharmacodynamic biomarkers
- Symptomatic treatments

Courtesy Doug Kerr
Thank you!
The Northeast ALS Consortium
www.alsconsortium.org

Facebook: www.facebook.com/NortheastALSConsortium
Twitter: @NEALSConsortium

Questions: alstrials@partners.org