Anti CD40L Monoclonal Antibody Therapy Improves Disease Progression and Survival in a Murine Model of ALS

RAC Meeting
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Neurodegeneration......
A complex process......

De Myelination
Synaptic Die Back
Axon Transport
RNA Processing
Muscle Wasting
Micro hemorrhaging

Oxidative Stress
Astrocytosis
ER Stress
Mitochondrial Dysfunction
Protein Aggregation
Microglial Activation
Leukocyte infiltration
ALS-TDI Facilitates Clinical Translation

De-Risk the Opportunity

Disease ⇒ Find a Drug Target ⇒ Build Drug ⇒ Pre-Clinical Development ⇒ Clinical Development

Basic Research Pre-Clinical Development Clinical Translational Research

Drug Development Gap

Founded 1999 by Heywood Family
• Annual budget of ~$10 million
• 42 employees
• 25 full-time scientists

Systems Biology Approach to Therapeutic Discovery
Target Discovery in ALS

• Create a comprehensive data warehouse
• Gene Expression Studies
  • hSOD1<sup>G93A</sup> model
    • Brain, spinal cord, skeletal muscle, blood, adipose, sciatic nerve
    • LCM motor neurons, neuromuscular junctions, glia
    • 8 time points, 6 replicates per group
    • non transgenics, wtSOD1 transgenic
  • Murine model Traumatic brain injury
    • Murine model Parkinson
    • Murine model Muscular Dystrophy
    • Murine model Spinal Ataxia
    • Murine model Multiple Sclerosis
    • Murine <sup>Loa</sup> dynein mutation
      • Brain, spinal cord, skeletal muscle, blood, adipose, sciatic nerve
      • LCM motor neurons, neuromuscular junctions, glia
      • 6 time points, 6 replicates per group
  • Human ALS blood (280 samples, and 80 controls)
  • Human ALS muscle biopsies (24 samples, 40 controls)
  • Human Muscular Dystrophy (240 samples)
  • Human Parkinson’s (240 samples)
  • Human Alzheimers (52 samples)
• Proteomics Studies (LUMINEX)
  • hSOD1<sup>G93A</sup> model
    • spinal cord, Blood
    • Human ALS plasma samples

Study Design: Whole Genome Expression Profiling SOD1 Mice

- Animals re genotyped for copy number and assigned to a study
- Disease onset Tail Paralysis
- End stage disease
- d30 d45 d60 d70-80 d80-120 d120-150
- Animals Genotyped
- Treatment Initiation
- Disease progression Paralysis hind limbs to forelimbs

- 5 non transgenics, 5 SOD1<sup>G93A</sup> and 5 wtSOD1 transgenic animals per group
- Groups harvested at 10 day intervals starting at day 50
- Tissues extracted and flash frozen on dry ice
- Brain, spinal cord, skeletal muscle, brown fat, white fat, sciatic nerve, blood
- Laser captured motor neurons and surrounding tissue
- Profiled on Affymetrix MOE430vII gene chips and Affymetrix Ex1.0 exon arrays
**SOD1^G93A Tissue Interactome**

- **3 Tissues:**
  - muscle
  - spinal cord
  - sciatic nerve
- **8 time points:**
  - days 30, 50, 60
  - 80, 90, 100, 110, 120
- **45,000 genes**
- **Affy:** MOE430v2
- **SOM Clustering of TxP**
  - 100 clusters per tissue
- **498 biological pathways**
  - Kegg, BioCarta
- **Drug bank interactions**
  - 10,500 drugs
- **22,830 nodes**
- **52,857 interactions**

**Statistics**
- **Limma package (R)**
  - Estimate changes in mRNA
- **GlobalTest (R)**
  - Estimate changes in biological pathways based on geometric mean

**Computational Biology Workbench**

**Scitegic Pipeline Pilot**

- **Data analysis Pipes**
- **Gene Expression changes**
**SOD1G93A Tissue Interactome**

- 3 Tissues:
  - 3 time points:
  - 45,000 genes
  - 100 clusters per tissue
  - 498 biological pathways
  - 10,500 drugs
  - 22,830 nodes
  - 52,857 interactions

- Computational Biology Workbench
- Scitegic Pipeline Pilot
  - Data analysis Pipes
  - Gene Expression changes

**GSEA: Statistical Modeling**

**Statistical Analysis**
- Goeman et al (Bioinformatics 2004)

- Q score: variance in geometric means

**Example: Single pathway**
- 49 genes
- 1 time point day 90
- 1 tissue spinal cord

\[ Q = \frac{1}{\mu_2} \sum_{i=1}^{n} \sum_{j=1}^{s} R_{ij}(Y_i - \mu)(Y_j - \mu) \]

Where:
- i and j are replicate samples
- R is the covariance matrix for x number genes
- Y are the biological classes

**Data Visualization**
- Spotfire
- Cytoscape

**Bioconductor (R) Limma, Affy, SimpleAffy**
GSEA: Statistical Modeling

Statistical Analysis
- Goeman et al (Bioinformatics 2004)
- 498 biological pathways
- 3 tissues, 4 time points
- Plotted histograms of Q scores over time

• Randomized genotypes and recalculated
**SOD1<sup>G93A</sup> Tissue Interactome**

- Query
- Pathways at days 80 and 90
  - Q score >40
  - All 3 tissues
- Cut off of 40 is significant
- Determined by sample randomization
- 5 pathways pass filter
- 95 genes total
- Related biological function
- Activation of the costimulatory Pathway and humoral response

**Activation of Costimulatory Pathway**

- Drugable pathway present in 3 diseased tissues in the hSOD1 preclinical model
Localization of mRNAs in hSOD1<sup>G93A</sup> Spinal Cord

- **A**: SOD1: DNAhc5
- **B**: SOD1: CD44
- **C**: SOD1: GFAP
- **D**: SOD1: CD68
- **E**: SOD1: CXCL10
- **F**: SOD1: CD11c
- **G**: SOD1: CCL3
- **H**: wt: CCL3
- **I**: SOD1: ITGAM
- **J**: wt: ITGAM

Macrophage Progressively Accumulate in Nerves

- **Day 60**: Red: S100b, Green: CD68, Blue: DAPI
- **Day 80**: Red: S100b, Green: CD68, Blue: DAPI
- **Day 100**: Red: S100b, Green: CD68, Blue: DAPI
ALS, a non Cell Autonomous Disease...

(A) Chimeric wild type/SOD1 chimeras wt glial surrounding SOD1 expressing MNs improved survival Clement et al., 2006).

(B) Transplantation of wt but not mSOD1 bone marrow into mSOD1(tg93a/Pu1-/- mice slows disease progression and improves survival (Beers, 2006).

(C) Cre-Lox mediated excision and loss of expression in microglial cells slows disease progression and improves survival (Boillee et al., 2006).

(D) Cre-Lox mediated excision and loss of expression in astrocytes cells slows disease progression and improves survival (Yamanaka et al., 2008).

(A) Crossing the mSOD1 transgenic into Rag2-/- mice exacerbates disease progression (Beers et al., 2006).

(B) Crossing the mSOD1 transgenic into CD4-/- mice exacerbates disease progression (Beers et al., 2006).

(C) Crossing the mSOD1 transgenic into the TCRb-/- exacerbates disease progression (Chiu et al., 2008).

(D) Autologous transplant of wild type Tregs or Teffs into mSOD1 animals slows disease progression and improves survival in mSOD1 mice (Banerjee et al., 2008).
Costimulatory Pathway is Increased in a Subset of ALS

Biomarker Development Efforts

• Molecular Profiling
  • 300 ALS blood samples
  • 50 skeletal muscle biopsies
  • Skin and adipose collections 2010
  • Costimulatory signature is present in 40% of samples

• Goal
  • Utilize historical database to facilitate phase II design
  • Patient enrollment and drug response

Variables Contributing to Noise in the Model

- Tg ALS Death (278)
- Control non-ALS Death (278)
- Variable #1: Exclusion Criteria
- Variable #2: Low Copy Animals
- Variable #3: Gender
- Variable #4: Litter

Age at Death (Days)

ALS deaths 87%
non-ALS deaths 10%

2.6% low-copy Tg's
Control Noise Variables in the Preclinical Model

Optimized Study Design
1. 48 total mice.
2. Tx group 12m+12f
3. Control group 12m+12f.
4. Gender & litter matching
5. Blinded scoring
6. Confirm transgene copy number
7. Censor non ALS deaths
8. Log rank and Cox proportional hazard model for statistical testing

Scott et al., 2008 ALS J.
**In Vivo Experiments: Blocking mAb to CD40L**

- Pharmacokinetic (Pk) Analysis in mSOD1 mice
  - Determine ½ life of the drug in mice
  - Determine biodistribution, tolerability
- Dose Ranging Efficacy Studies
  - A1) Female 1 mg/kg
  - A2) Male 1.34 mg/kg
  - B1) Female 2 mg/kg
  - B2) Male 2.67 mg/kg
  - C1) Female 4 mg/kg
  - C2) Male 5.35 mg/kg
- Biomarker Drug Response
  - Dose dependent marker
  - Amenable to clinical development
  - Shorten and facilitate phase II trial

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**Anti CD40L Treatment is Efficacious in SOD1^{G93A} Mice**

**Females:**
- 5.22 mg/kg loading dose
- 1 mg/kg weekly IP

**Males:**
- 6.75 mg/kg loading dose
- 1.34 mg/kg weekly IP

Day 50 start

A. Time required to attain peak body weight.
   - Time to peak was not significantly changed
B. Time from peak body weight until death.
   - BW maintenance was significantly improved
C. Time to disease onset (Ns = 2).
   - Disease onset was significantly delayed by
D. Survival was significantly prolonged

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$pVal=0.286$  $pVal=0.046$  $pVal=0.001$  $pVal=0.003$
**Meta Analysis of Anti CD40L Treatment**

- 30 female MR1 treated mice
- 30 litter matched controls
- 500 historical female controls
- **Median Survival**
  - Female control: 127 days
  - MR1 treated: 139 days
  - pValue: 0.0002

- **SIM LIMS historical female data**
- Monte Carlo analysis
- 18 non treated females
- Random assign treatment/control
- Frequency of detecting a false positive

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**ALS TDI Preclinical Drug Screening Pipeline**

- Screened ~100 compounds
- Repeated preclinical studies

MR1 is the only compound TDI has tested that:
- Delayed disease onset
- Delay body weight loss
- Improve survival
Anti CD40L Treatment Improves Motor Neuron Survival

- Lumbar Spinal Cord
- 103 days,
- 53 days treatment 1mg/kg

Anti CD40L Treatment Decreases Astrocytosis in the Lumbar Spinal Cord

- GFAP, Dapi
- Lumbar Spinal Cord
- 103 days,
- 53 days treatment 1mg/kg
Anti CD40L Treatment Modulates Costimulatory Gene Expression in the Spinal Cord

- Anti CD40L Treatment started on day 50
- 1 mg/kg weekly
- 10 mg/kg weekly
- 5 animals per group
- MOE430vII Affymetrix Genechips

- Anti CD40L treatment
- Decreases expression of genes in the costimulatory pathway
- An average of 40% reduction in gene expression across the pathway

Anti CD40L Treatment Reduces Axonal Recruitment of Macrophages

No treatment: SOD1_Tg
Anti CD40L treatment: SOD1_Tg

CD68+ Cell Counts By Treatment

All age matched females, 1 mg/kg /week, i.p.
50 day start, sacrificed at 103 days
Biological replicates; double blind analysis
Decreased expression of Immunomodulatory Signaling Molecules in Blood After Anti CD40L Treatment

**Functional Enrichment Classification**
- Cytokine-Cytokine signaling: 7.6E-8
- Immune response: 1.4E-4
- Chemotaxis: 8.7E-2

**Analyte pValues (BH corrected)**
- M-CSF: 3.72E-08
- Lymphotactin: 6.24E-05
- IgA: 0.000124
- CD40 Ligand: 0.000186
- VCAM-1: 0.000332
- VEGF: 0.000863
- C4D0: 0.001745
- SDF: 0.00282
- Endothelin-1: 0.003961
- Eotaxin: 0.004272
- IP-10: 0.007369
- MDC: 0.008528
- ILF: 0.00831

**Summary: Preclinical Development of a Blocking Ab to CD40L**

- Comprehensive and unbiased molecular profiling can identify molecular pathways amenable to therapeutic intervention
- Blocking CD40L signaling is efficacious in the hSOD1G93A preclinical model
  - The Costimulatory pathway is activated in spinal cord, muscle and sciatic nerve in hSOD1G93A Mice
  - The activation is focal in nature in the CNS and periphery
  - The expression of genes in the Costimulatory Pathway are increased in the blood of 40% of ALS patients
  - Treatment with a blocking Ab to CD40L delays disease onset, slows progression, and improves survival
  - Treatment with a blocking Ab to CD40L decreases the accumulation of CD68 positive cells in peripheral nerve during disease progression
  - Treatment with a blocking Ab to CD40L decreases astrocytosis in the lumbar spinal cord
  - Treatment with a blocking Ab to CD40L decreases the abundance of chemotactic proteins in the serum
- What effect does blocking the signaling of other components in this pathway do?
  - Work in progress: CTLA4-FC (Abetacept)
  - Rapamycin, Fk506
  - T Cell modulating Abs
Standardized PD Design

- Discovery Tools
- Therapeutic Development
- Pk Studies
- Tolerability Studies
- Efficacy Studies
- PD Studies

Affymetrix 45,000 genes
Tissues: SC, SN, GS

QPCR 2 LDA Panels
Tissues: SC, SN, GS

ISH 48 genes
SC

ELISA 94 analytes
Tissues: BD, SC

IHC 3-6 proteins
Tissues: SC, SN

Knowledge Sphere

ALS TDI 00903 Day 50 Start

- Single dose day 50

- Single dose day 70
In Situ Hybridization

Biomarkers of drug response

- ALS TDI has screened > 2000 genes by ISH
  - SOD1 versus wt spinal cord
  - Days 50 and 90
- Picked a panel of 48 genes with increased expression in SOD1 versus wt animals
- All drug studies now are surveyed for this panel of genes
- Markers of microglial activation, astrocytosis, motor neuron survival, stress

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