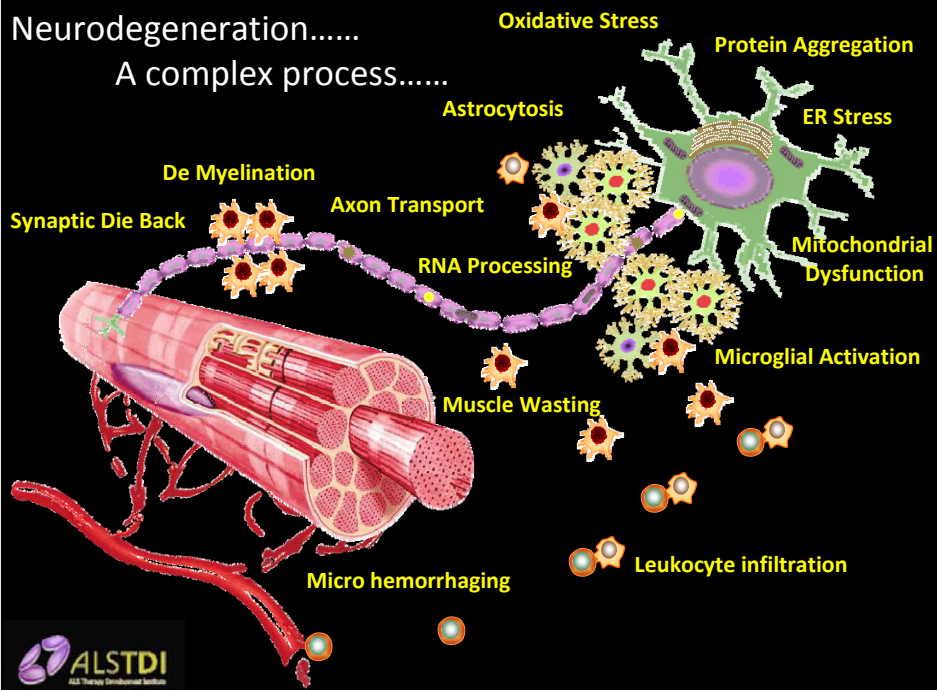


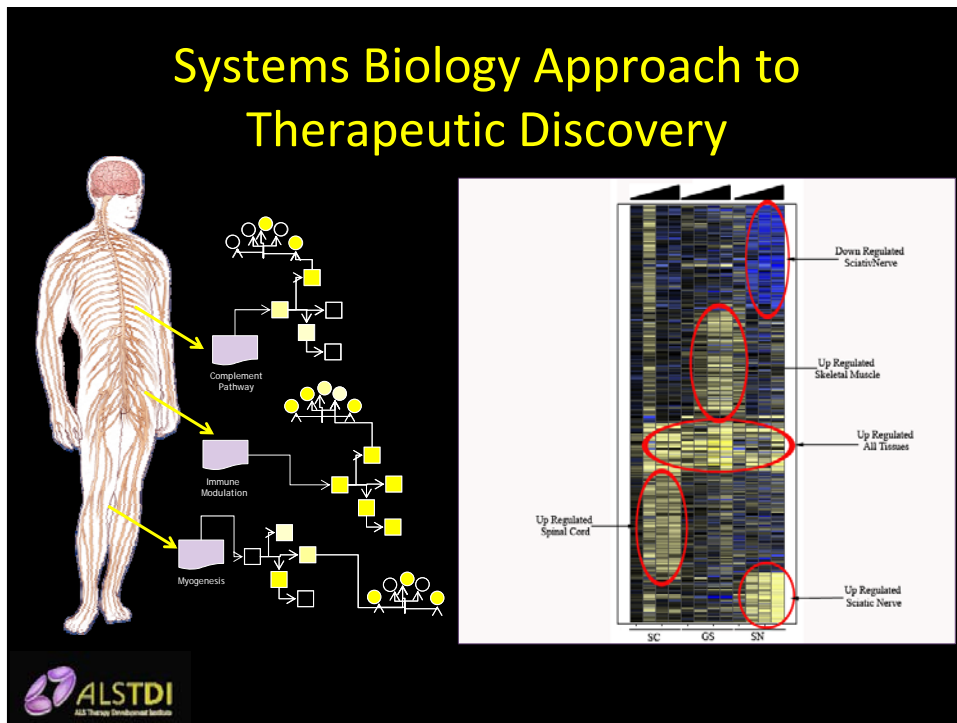
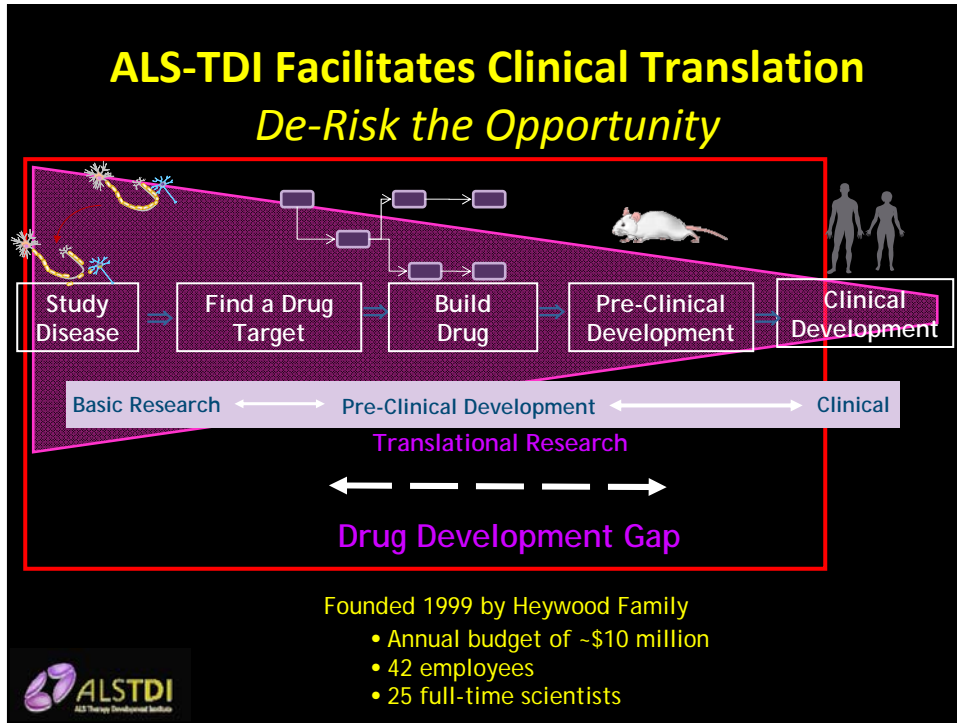
Anti CD40L Monoclonal Antibody Therapy Improves Disease Progression and Survival in a Murine Model of ALS

RAC Meeting
November, 2010



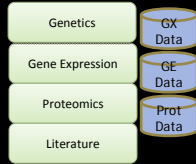
Neurodegeneration.....
A complex process.....





Target Discovery in ALS

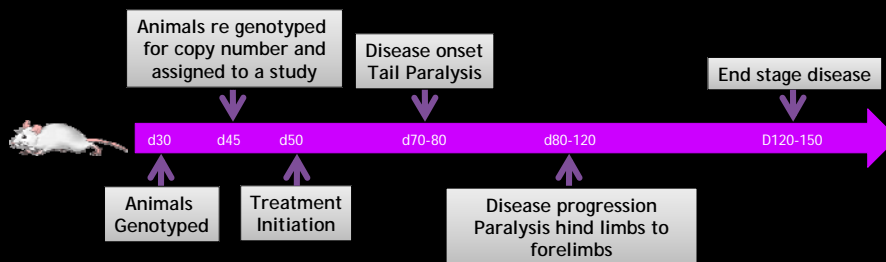
- Create a comprehensive data warehouse



- Gene Expression Studies
 - hSOD1^{G93A} 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, wtSOD transgenic
 - Murine model Traumatic brain injury
 - Murine model Parkinson
 - Murine model Muscular Dystrophy
 - Murine model Spinal Ataxia
 - Murine model Multiple Sclerosis
 - Murine Loa 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 Parkinsons (240 samples)
- Human Alzheimers (52 samples)
- Proteomics Studies (LUMINEX)
 - hSOD1^{G93A} model
 - spinal cord, Blood
 - Human ALS plasma samples



Study Design: Whole Genome Expression Profiling SOD1 Mice



- 5 non transgenics, 5 SOD1^{G93A} 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

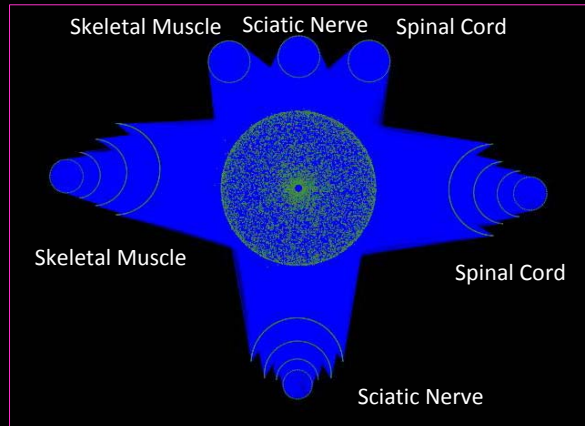


Confidential

SOD1^{G93A} Tissue Interactome

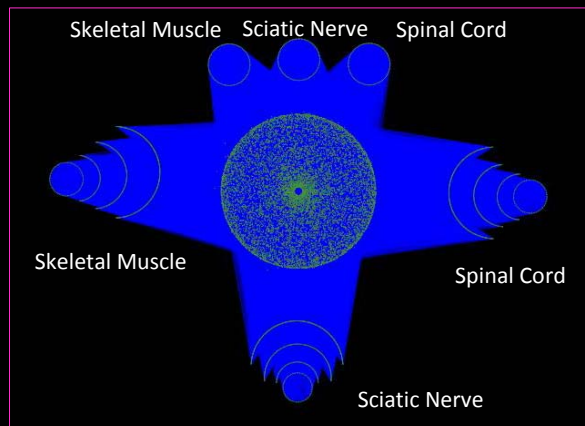
- 3 Tissues:
 - muscle
 - spinal cord
 - sciatic nerve
- 8 time points:
 - days 30, 50, 60
 - 80, 90, 100, 110, 120
- 45,000 genes
 - Affy: MOE430vII
- SOM Clustering of TxP
 - 100 clusters per tissue
- 498 biological pathways
 - kegg, biocarta
- Drug bank interactions
 - 10,500 drugs
- 22,830 nodes
- 52,857 interactions

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



SOD1^{G93A} Tissue Interactome

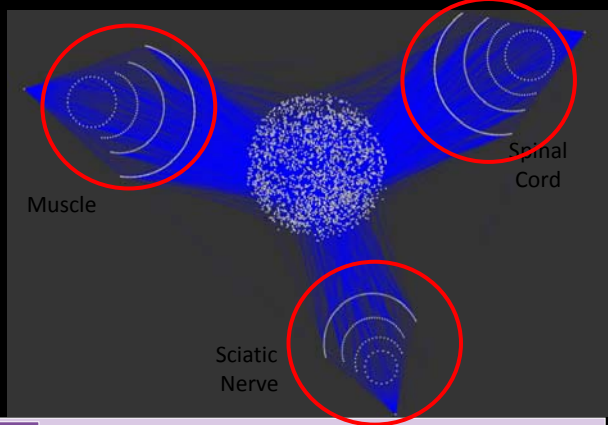
- 3 Tissues:
- 8 time points:
- 45,000 genes
- 100 clusters per tissue
- 498 biological pathways
- 10,500 drugs
- 22,830 nodes
- 52,857 interactions
- Computational Biology Workbench
- Scitegic Pipeline Pilot
 - Data analysis Pipes
 - Gene Expression changes



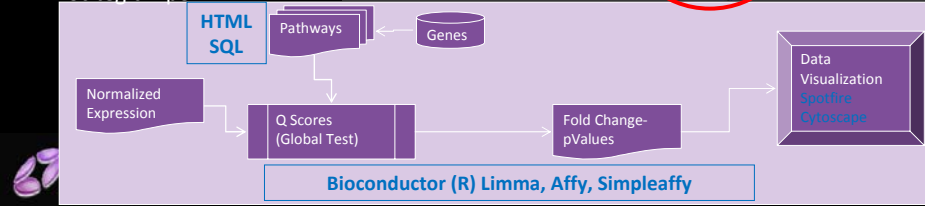
SOD1^{G93A} Tissue Interactome

- 3 Tissues:
- 8 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



Scitegic Pipe



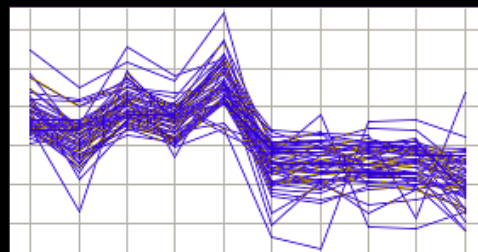
GSEA: Statistical Modeling

- 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

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

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



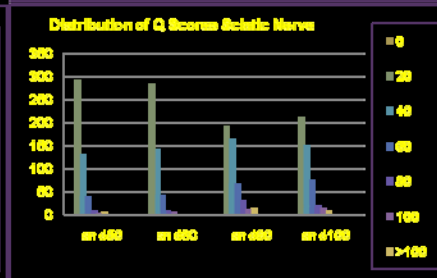
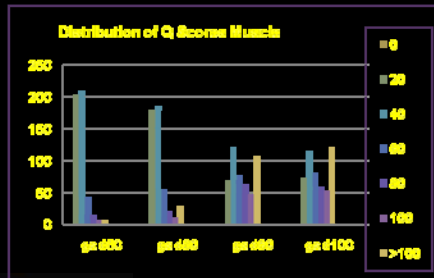
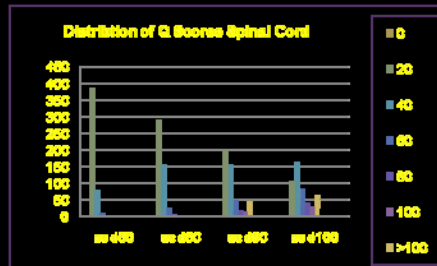
SOD1 d90

Non Tg d90

GSEA: Statistical Modeling

Statistical Analysis

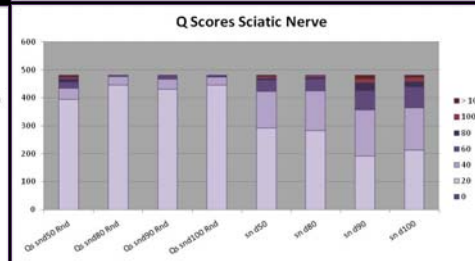
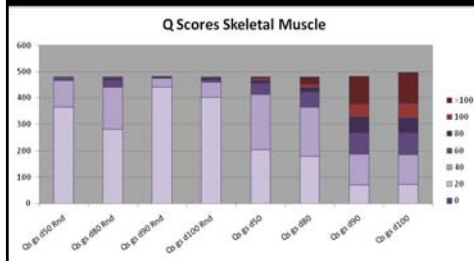
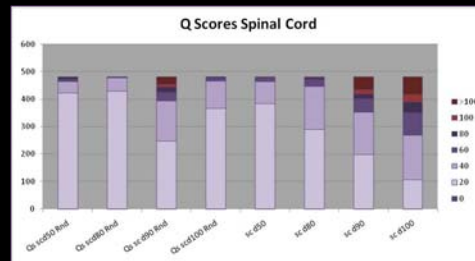
- Goeman et al (Bioinformatics 2004)
- 498 biological pathways
- 3 tissues, 4 time points
- Plotted histograms of Q scores over time



GSEA: Statistical Modeling

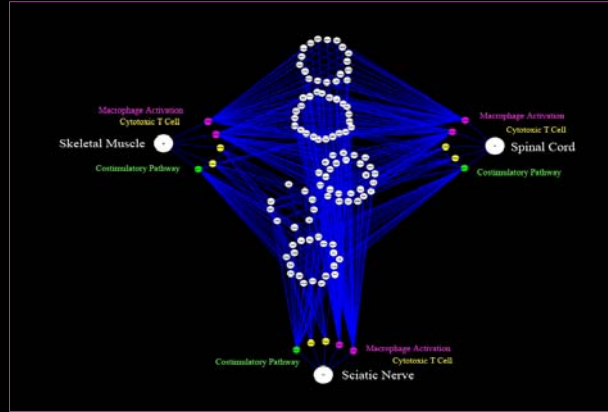
Statistical Analysis

- Goeman et al (Bioinformatics 2004)
- 498 biological pathways
- 3 tissues, 4 time points
- Plotted histograms of Q scores over time
- Randomized genotypes and recalculated

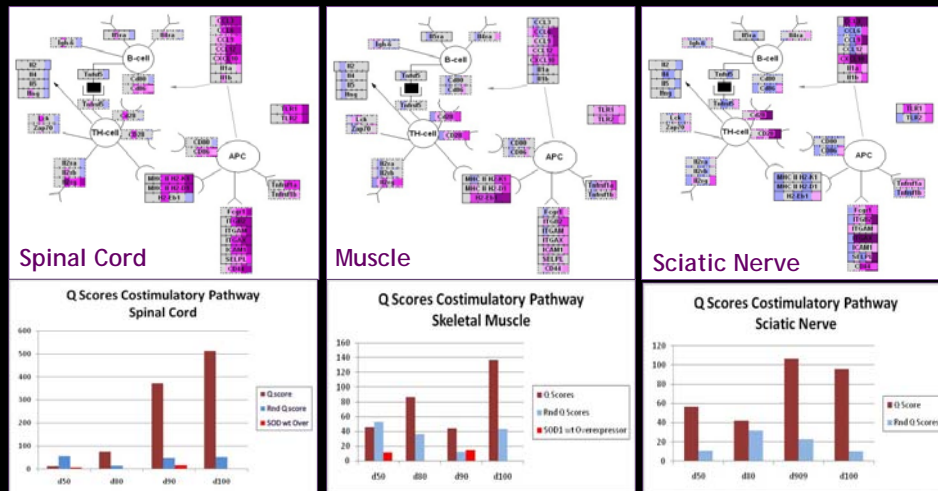


SOD1^{G93A} Tissue Interactome

- 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



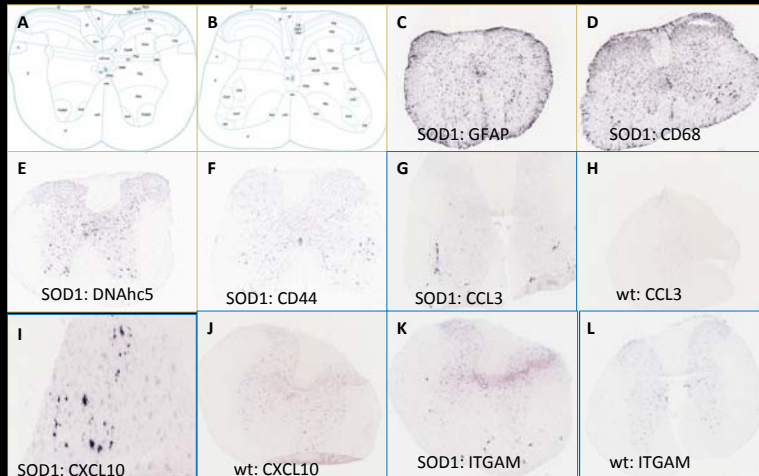
Activation of Costimulatory Pathway



- Drugable pathway present in 3 diseased tissues in the hSOD1 preclinical model

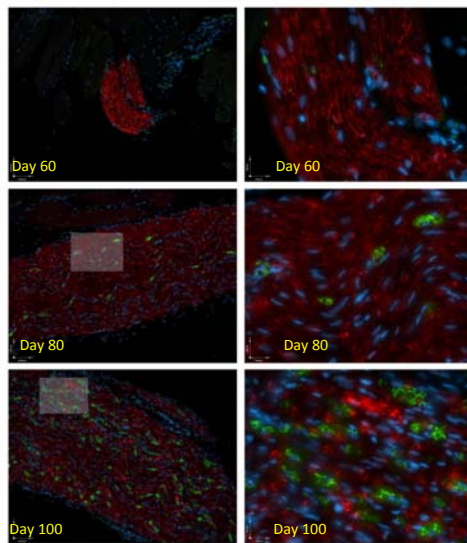
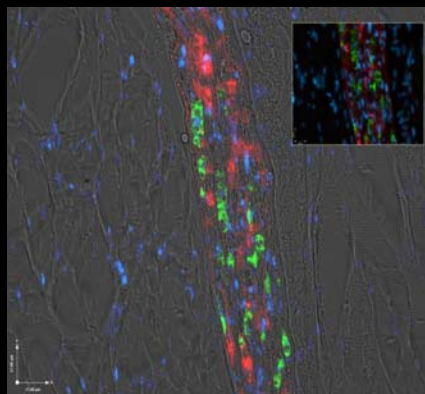


Localization of mRNAs in hSOD1^{G93A} Spinal Cord



Services of the Allen Brain Institute

Macrophage Progressively Accumulate in Nerves



Distal Nerve: SOD1
Red: S100b
Green: CD68
Blue: DAPI



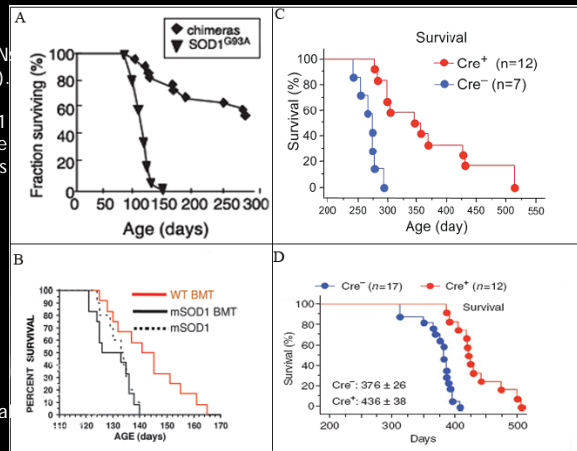
ALS, a non Cell Autonomous Disease...?

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

(B) Transplantation of wt but not mSOD1 bone marrow into mSOD1^{G93A}/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).



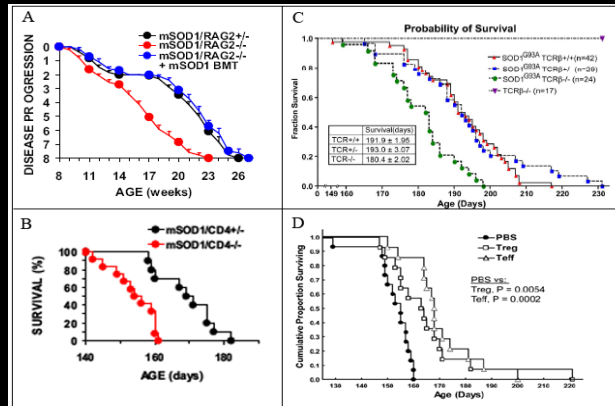
ALS, a non Cell Autonomous Disease...?

(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 TCRβ^{-/-} exacerbates disease progression (Chiu et al., 2008).

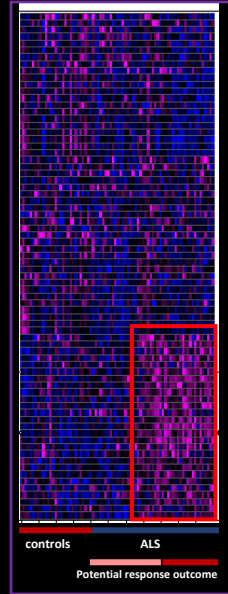
(D) Autologous transplant of wild type Tregs or Teffs into mSOD1 animals slows disease progression and improves survival in mSOD1 mice (Banerjee et al., 2008).



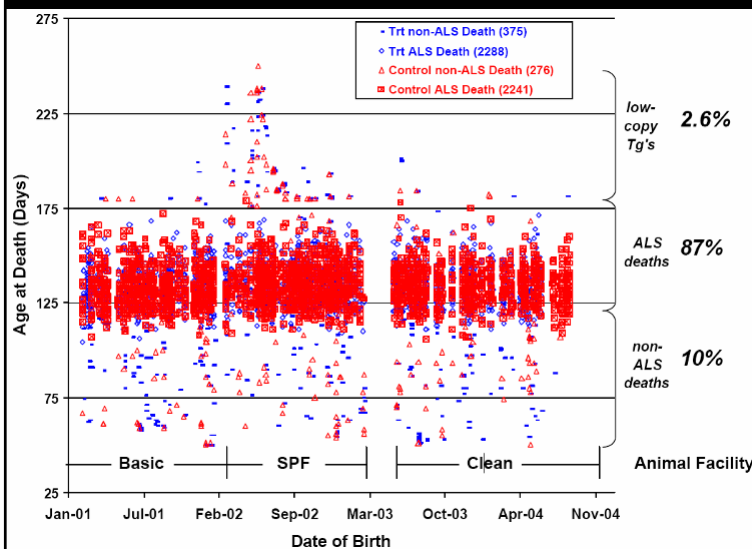
Costimulatory Pathway is Increased in a Subset of ALS

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



- Variable #1: Exclusion Criteria
- Variable #2: Low Copy Animals
- Variable #3: Gender
- Variable #4: Litter

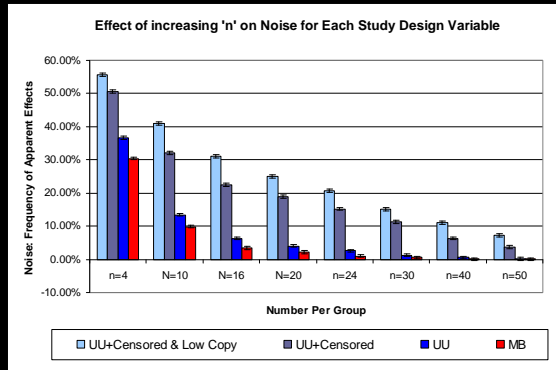


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Control Noise Variables in the Preclinical Model

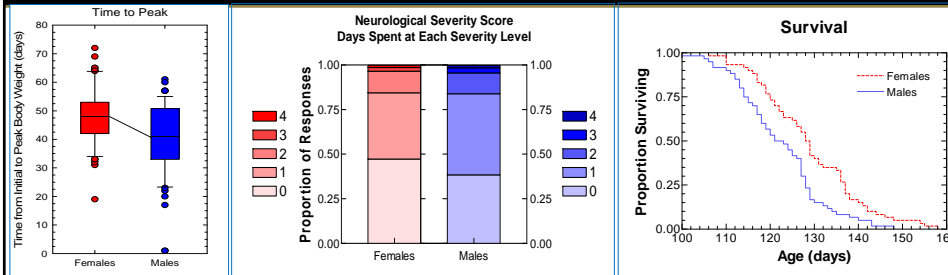
Optimized Study Design

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.

Male SOD1^{G93A} Mice Have Earlier Disease Onset and Shorter Lifespan



Change from Initial BW					
Param	Sex	N	Mean	Std Error	Prob > t
Maximum Increase in Body Weight (g)	F	60	3.0	0.1	0.2989
	M	60	2.8	0.2	
Age at Maximum Body Weight (days)	F	60	96.7	1.3	0.0004
	M	60	89.1	1.6	
Time from Initial to Peak Body Weight (days)	F	60	47.7	1.3	0.0004
	M	60	40.1	1.6	
Time from Peak Body Weight to Death (days)	F	60	32.2	1.7	0.6733
	M	60	33.2	1.6	

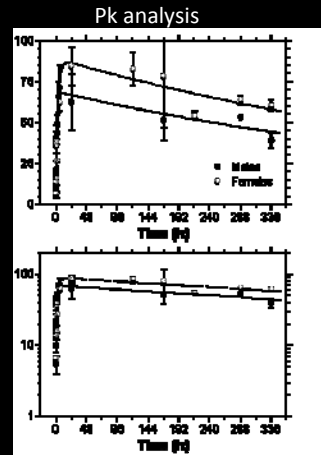
NSave: Categorical Analysis of Score by Sex							
Sex	Parameter	Score0	Score1	Score2	Score3	Score4	
Female	Share of	0.473	0.372	0.121	0.021	0.013	
Male	Responses	0.385	0.455	0.117	0.030	0.014	
		Prob>ChiSq	<.0001	0.0004	0.0370	0.0486	1.0000

Survival (Age, days)									
Sex	N	K-M Product-Limit Survival Fit			Cox Proportional Hazard				
		Median Time	Diff. (M-F)	Log-Rank	Wilcoxon	Median Time	Diff. (M-F)	Hazard Ratio	
F	60	129	-6	P		129	-7	0.49	P
M	60	123	-6	0.0012	0.0013	122	-7	2.02	0.0010



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



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

Females:

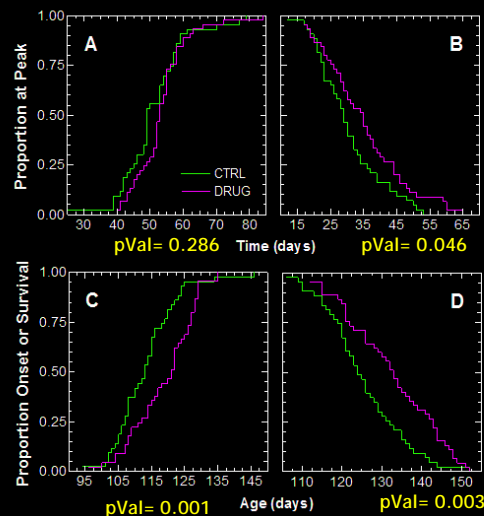
5.22 mg/kg loading dose
 1 mg/kg weekly IP

Males:

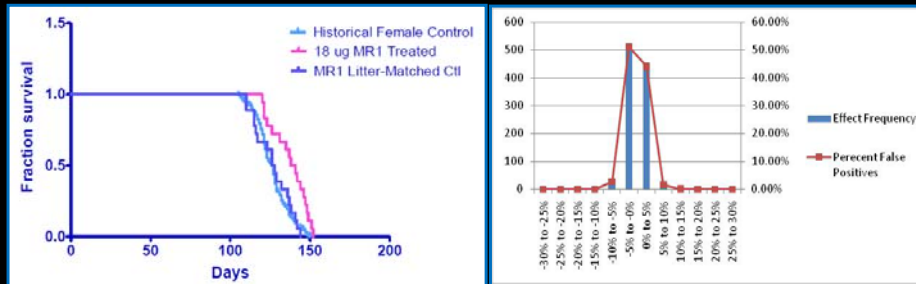
6.75 mg/kg loading dose
 1.34 mg/kg weekly IP

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



Meta Analysis of Anti CD40L Treatment

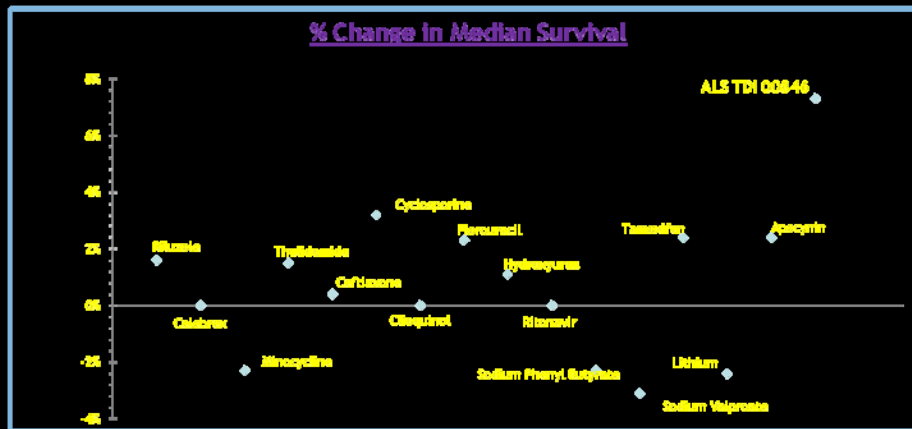


- 30 female MR1 treated mice
- 30 litter matched controls
- 500 historical female controls
- Median Survival
 - Female control: 127 days
 - MR1 treated 139 days
 - pValue: 0.0002

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



ALS TDI Preclinical Drug Screening Pipeline

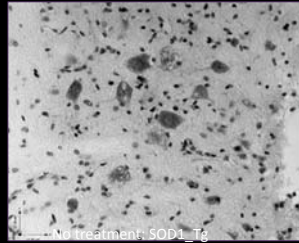


- Screened ~100 compounds
- Repeated preclinical studies

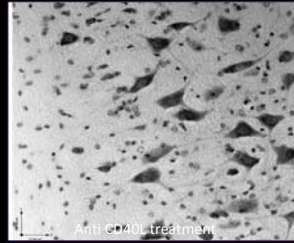
- MR1 is the only compound TDI has tested that:
- Delayed disease onset
 - Delay body weight loss
 - Improve survival



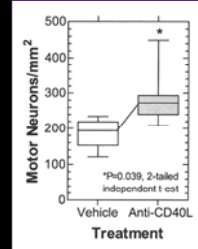
Anti CD40L Treatment Improves Motor Neuron Survival



Control



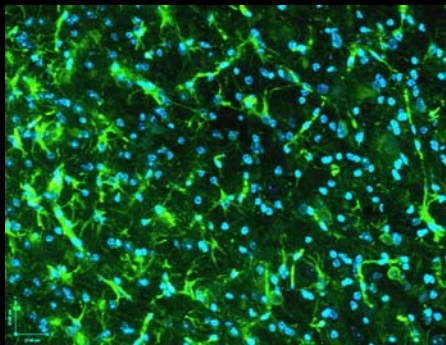
Anti-CD40L



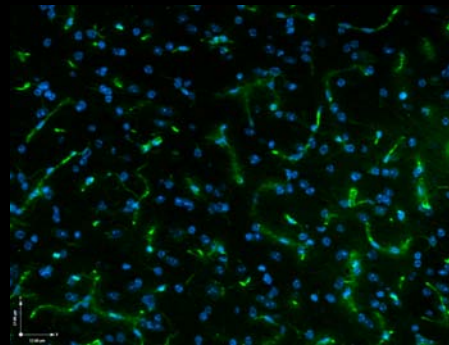
- Lumbar Spinal Cord
- 103 days,
- 53 days treatment 1mg/kg



Anti CD40L Treatment Decreases Astrocytosis in the Lumbar Spinal Cord



Control



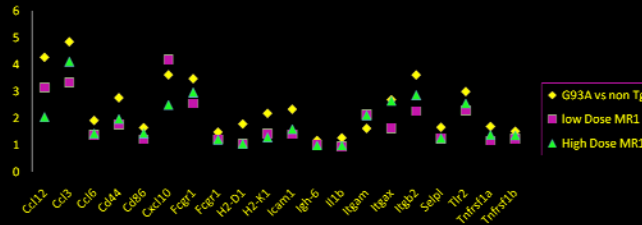
Anti-CD40L

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



Anti CD40L Treatment Modulates Costimulatory Gene Expression in the Spinal Cord

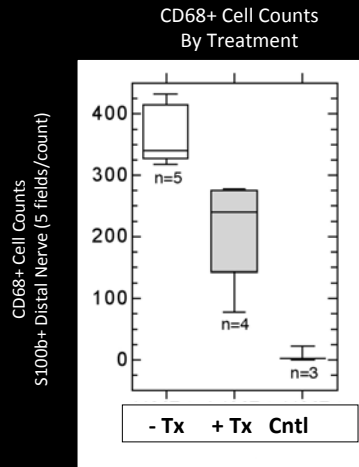
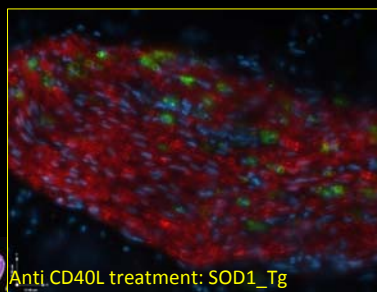
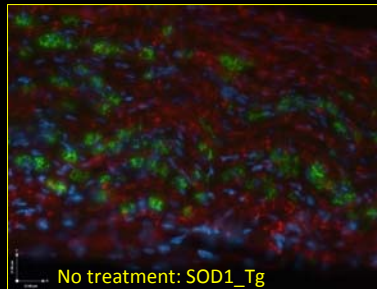
Expression After MR1 Treatment



- 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



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

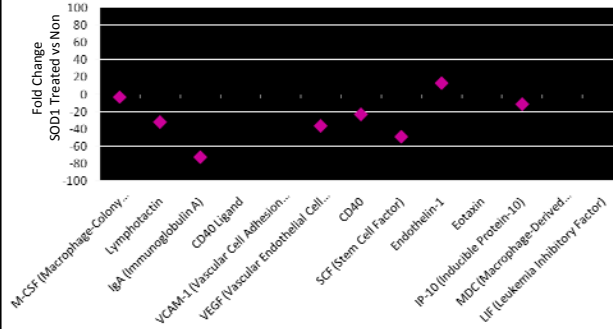
Functional Enrichment Classification

- Cytokine-Cytokine signaling: 7.6E-8
- Immune response: 1.6E-4
- Chemotaxis: 8.7E-2

Analyte pValues (BH corrected)

M-CSF	3.72E-08
Lymphotactin	6.24E-05
IgA	0.000124
CD40 Ligand	0.000196
VCAM-1	0.000332
VEGF	0.000883
CD40	0.001745
SCF	0.00282
Endothelin-1	0.003561
Eotaxin	0.006472
IP-10	0.007369
MDC	0.008028
LIF	0.00851

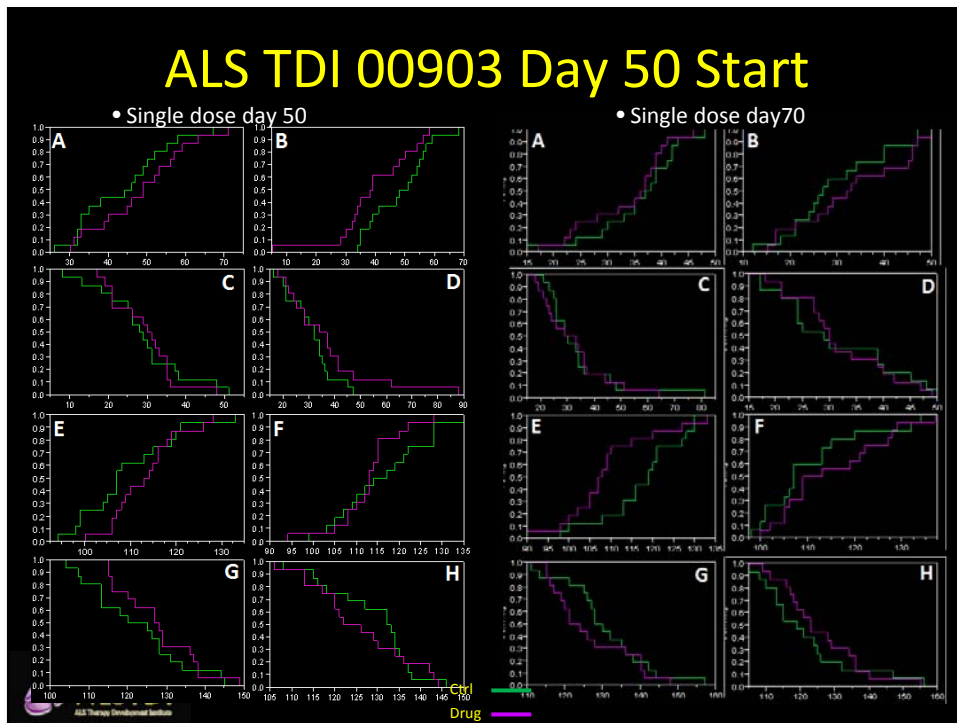
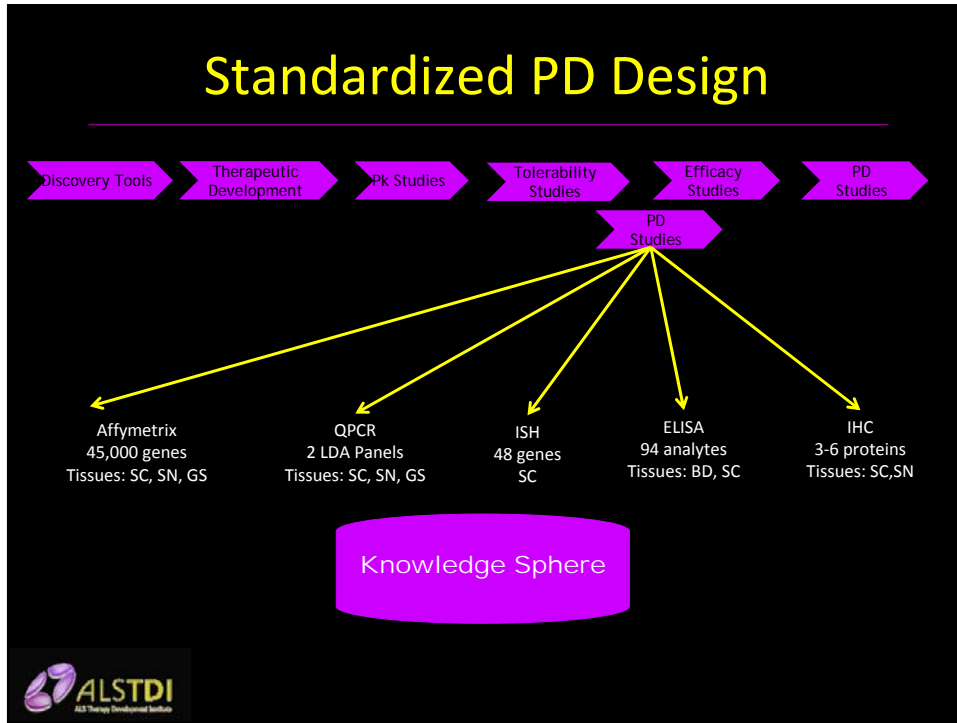
Difference in Median Levels of Analyte



Summary: Preclinical Development of a Blocking Ab to CD40L





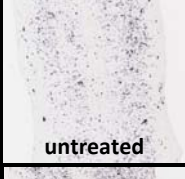
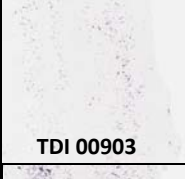


- Comprehensive and unbiased molecular profiling can identify molecular pathways amenable to therapeutic intervention
- Blocking CD40L signaling is efficacious in the hSOD1^{G93A} preclinical model
 - The Costimulatory pathway is activated in spinal cord, muscle and sciatic nerve in hSOD1^{G93A} Mice
 - 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






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

CD68		
Lgals3		
Lys		
C1qA		



Acknowledgements

- Sean Scott
 - John Lincecum
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DOD
RGK Foundation
ALS Patients & Families

