AMYOTROPHIC LATERAL SCLEROSIS (ALS) DISEASE MODEL

MODEL: **MUTANT SOD-1 EXPRESSING TRANSGENIC MICE**
B6SJL-Tg(SOD1*G93A)1Gur/J

BACKGROUND AND APPLICATION:

Amyotrophic lateral sclerosis (ALS, or "Lou Gehrig's Disease") is a progressive neurodegenerative disorder resulting in progressive weakness and death. There are approximately 10,000 patients diagnosed with ALS in the U.S. every year; the prevalence of this disease is approx. 30,000. The disease strikes patients of all ages. The time from diagnosis to death is typically two years or less.

ALS is manifest by progressive death of motor neurons in the brain and spinal cord. The cause of this selective neuronal death is unknown for most patients. Most cases of ALS are "sporadic"; about 10% of cases cluster within families. Among families with familial ALS (fALS), several mutant genes have been identified. The first and most common of these to be discovered is a mutation in the gene encoding for the free-radical scavenging enzyme superoxide dismutase-1 (SOD-1). Interestingly, this mutation does not result in loss of activity of the enzyme, but rather expression of a functioning mutant enzyme. This mutant protein appears to form microaggregates, which may be related to its toxicity in vulnerable neurons.

The mutant human SOD-1 gene causing one variety of fALS has been inserted into transgenic mice (the B6SJL-Tg(SOD1*G93A)1Gur/J strain). These mice develop a phenotype that is similar to human ALS, with progressive loss of motor neurons, generalized muscle weakness and atrophy, and eventual death. This model has been used widely and productively to screen potential new treatments for human ALS. For example, the one currently marketed drug for ALS, riluzole, increases mean survival in mutant mice by 10-14 days, equivalent to about a 3 month increase in survival in human ALS patients.

This transgenic mouse model is widely used to test putative new treatments for ALS

EXPERIMENTAL OUTLINE:

The timetable for these studies is as follows: Male and female transgenic mice arrive from Jackson Labs at approx. Day 45 of life. Rotarod testing begins at Day 45, twice a week for two weeks, then weekly thereafter. Drug or placebo administration typically begins at around Day 60, usually daily, biweekly, or weekly thereafter. The first signs of animal weakness begins at approx. Day 100-110. Control animals are expected to be euthanized by rules below at approx. Day 135. Treated animals may survive longer, up to Day 160 or longer.

METHODS:

Animals

Male and female B6SJL-Tg(SOD1*G93A)1Gur/J mice are ordered to arrive from Jackson Laboratories at the age of four to five weeks old. Females arrive as 20 per shipping container, and males arrive in containers with their littermates. (This is done due to the tendency of males to fight with non-littermate males.) On arrival, females are randomly assigned to 5 per cage, and males are caged with littermates. Animals are allowed free access to food and water, and separated if any injuries occur, or as otherwise indicated below.

Animals are housed in rooms provided with filtered air at a temperature of 21 ± 2° C and 50% ± 20% relative humidity. The room is on an automatic timer for a light/dark cycle of 12 hours on and 12 hours off with no twilight (e.g. 7am to 7pm). PWI PRO-CHIP 8-16 (Hardwood sawdust) is used for bedding and animals are fed with Global Diet 18% Protein Rodent Diet chow. Water is provided *ad libitum*.

When weakness of animals is first observed (around Day 100 of life), the diet is changed to a nutragel preparation (http://www.bio-serv.com/newcatalog/eeprod/rodent/nutragel.html) that is placed on the floor of each cage. This preparation contains all of the necessary nutrient and fluid requirements of animal, so that animals do not have to feed from food hoppers or drink from water bottles.

Drug Delivery Methods/Dosing Volumes

Depending on the specific individual studies that will be performed, drugs will be delivered at specified time times by several possible delivery methods/routes (generally only one delivery method per protocol). Drug delivery methods include:

Intravenous Injection (IV)

Subcutaneous Injection (SC)

Intraperitoneal Injection (IP)

Oral Gavage (PO)

The ball tip of a feeding needle is directed along the roof of the mouth and toward the right side of the pharynx. The compound is gently passed down into the esophagus.

Behavioral Testing—Rotarod Protocol

Animal strength and endurance will be tested periodically by rotarod testing. Following an initial two week training period, this generally occurs weekly thereafter. The protocol is as follows:

Rotarod Pre-Training Period (only prior to testing period – 2 weeks)

1) Standard Training

-Set rotarod for Fixed Speed 24 RPM

-Test 5 mice at a time, recording fall time or time to rotate 3X on drum for each mouse.

-If mouse rotates 3 or more times, remove mouse from rod and return to home cage.
-Maximum latency: 60 seconds
-Perform 4 trials/mouse, with at least 5-10 minute intertrial interval.
-Mice will be pre-trained a total of 4 times (4 trials/ea) prior to testing period
-If mice do not stay on rotarod for 60s during at least 1 trial, place in remedial training

2) Remedial Training
-Set rotarod for Fixed Speed 20 RPM
-Test 5 mice at a time, recording fall time or time to rotate 3X on drum for each mouse.
-If mouse rotates 3 or more times, remove mouse from rod and return to home cage.
-Maximum latency: 60 seconds
-Perform 4 trials/mouse, with at least 5-10 minute intertrial interval.
-If mice stay on rotarod for 60s during at least 1 trial, place back in standard training

Rotarod Testing Period

1) Fixed Speed Rotarod "warm-up"
-Perform one trial at 16 RPM fixed speed for each mouse at the start of each testing day.
-Test 5 mice at a time, recording fall time or time to rotate 3X on drum for each mouse.
-If mouse rotates 3 or more times, remove mouse from rod and return to home cage until next trial.
-Maximum latency: 60 seconds
-Perform 1 trial/mouse, at start of each testing day, with at least 30 minutes rest before beginning accelerating rotarod training.

2) Accelerating Rotarod
-Set rotarod for acceleration at maximum rate (4 or 5 RPM to 40 RPM over 300 seconds, depending on equipment)
-Test 5 mice at a time, recording fall time or time to rotate 3X on drum for each mouse.
-If mouse rotates 3 or more times, remove mouse from rod and return to home cage until next trial.
-Maximum latency: 300 seconds
-Perform 3 trials/mouse, with approximately 1-hour rest period between trials.

Animal Observations and Survival:
Animals are observed twice per week before 100 days of age, and daily thereafter. Data are recorded using the following scale:

1. Normal healthy animal, displays no signs of the disease.
2. Animal fails to show the ability to fully splay its legs. This is tested by allowing the animal to grab a hold of the grating on top of the cage and then lightly pulling on the animal's tail.
3. Animal displays tremors in one or more of its hind limbs.
4. Animal displays a gait abnormality. This is characterized by squatting or waddling when walking.
5. Animal displayed paralysis in one or more hind limbs.

Euthanasia Rules

As the study progresses, animals become progressively weaker. They are euthanized if either of the following criteria are observed: (a) Animal cannot right itself within 15 seconds when placed on its side, or (b) Animal cannot groom its face (detected by the development of crusting in one or both eyes). At this stage, animals generally can still access nutrition and water, in the form of nutragel placed on the floor of cages. In our experience in such studies, approx. 90% of study animals are euthanized according to the above criteria; 10% are found dead in cages. In this regard, the spontaneous mortality rate is similar to studies in other transgenic mouse strains of CNS disease that we have worked with (e.g., Tg2576+ mouse model of Alzheimer's Disease).

Separation of Animals

Animals are separated into individual cages if: (a) the animal scores a 5 on the observation scale above or, (b) if the animal is injured by another animal in the cage.

Euthanasia/Necropsy

To euthanize animals, they will first be deeply anesthetized by IP injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Blood will then be withdrawn by direct cardiac puncture, CSF will be collected by intracisternal (i.c.) puncture, and animals will be perfusion-fixed with 4% paraformaldehyde before harvesting of brain, spinal cord, and other organs.