OBJECTIVES: Amyotrophic Lateral Sclerosis (ALS) is a prevalent neurological disorder characterized by hyperreflexia and inexorable muscle weakness leading to death. The principal pathological finding in ALS is loss of nerve cells in the anterior horns of the spinal cord, the motor nuclei of the brainstem, and the upper motor neurons in the cerebral cortex. Investigations aimed at preventing or limiting progression of ALS have thus focused on identifying the mechanisms by which motor neurons degenerate. A transgenic mouse model has been developed that possesses many of the pathological and clinical features of human familial and sporadic ALS. As nitric oxide (NO) has been shown to mediate neuronal loss in other neurodegenerative conditions, several groups have investigated the role that NO may play in disease progression in this transgenic model. The results have been conflicting likely because currently available inhibitors of NO synthase do not permit optimal control of NO generation in distinct cell types and subcellular compartments. In non-neuronal cells, arginase exists in at least two isoforms and regulates intracellular NO levels by enzymatically regulating availability for arginine in the cytoplasm or mitochondria. We propose that interventions aimed at promoting arginase activities in microglia astrocytes, or motor neurons will limit availability of cellular arginine for NO generation in and thereby diminish cell death and disease progression in ALS but permit NO to mediate survival promoting effects in each of these cell types.

Research Design / Methodology: To: 1) determine the cell types and subcellular compartments where arginase is expressed in the normal central nervous system of mice and humans and how the localization and levels of these arginase isoforms change in ALS as well as a transgenic mouse model of ALS and how this compares to the localization of iNOS, eNOS, nNOS (all forms) in these tissues. 2)determine whether increased arginase activity in microglia, astrocytes or motor neurons from control mice or mice overexpressing copper/zinc superoxide dismutase mutant will abrogate neuronal toxicity of induced by growth factor deprivation, excitotoxins, or LPS/interferon gamma treatment in co-culture experiments; and 3) determine whether decreased arginase activity will accelerate disease progression in a mouse model of ALS and increased arginase activity will decelerate disease progression.

FINDINGS: We have bred G93A transgenic mice (F1) and completed a temporal series at select time points from 30, 70, 90, 110, 120 day of G93A and WT control mice for ICC studies to detect the distribution and cellular localization of arginase and nitric oxide synthase. There was a heterogenous immunostaining of all cell types (neuronal, glial, and endothelial). Arginase 1 and 2 immunoreactivities were increased in all neuronal cell types in comparison to wild type littermate controls in the G93A mice with pathological severity and was most prominent at 120 days. Nitric oxide synthase immunostaining using antibodies directed at iNOS, eNOS, and nNOS has been completed in tissue sections from each time point of G93A and littermate wild type control mice. These specimens have not been analyzed to date. We have also analyzed arginase activity in spinal cord and brain samples at the same select time points from 30, 70, 90, 110, 120 day from G93A and wild type mice. We examined whether administration of minocycline was neuroprotective in G93A transgenic ALS mice. Minocycline delayed disease onset and extended survival in the ALS mice. We have completed immunostaining for arginase and NOS isoforms in human FALS and SALS patients and will complete a comparative analysis with the G93A mice. Arginase immunoreactivity is significantly increased in motor neurons in FALS and SALS patients in comparison to age-matched non-neurological patients. We have recently found that arginase levels lowers n-NOS in "invitro" cell lines of ALS.