# A transgenic rat expressing the SOD1-G93A mutation exhibits ALS-like phenotypes, rendering it an effective translational model for drug development

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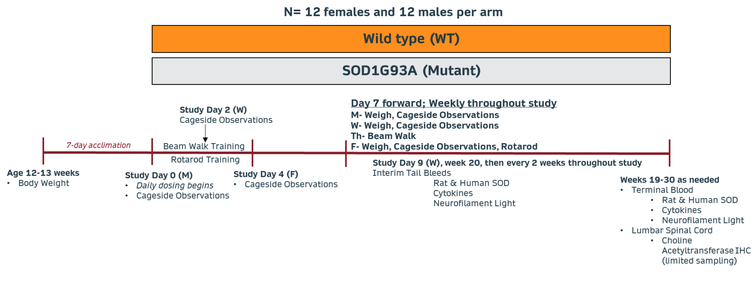
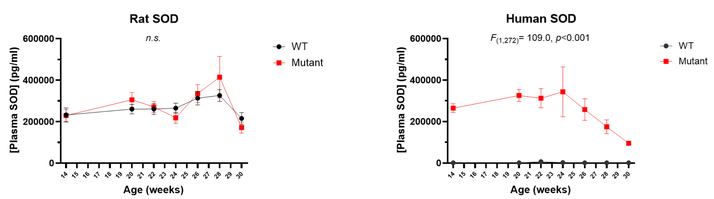
## Introduction

Drug discovery for Amyotrophic Lateral Sclerosis (ALS) is crucial due to the absence of effective treatments for this debilitating neurodegenerative disease. ALS is characterized by the progressive degeneration of motor neurons, resulting in muscle weakness and eventual paralysis. Among the genetic factors in ALS, mutations in the gene for superoxide dismutase 1 (SOD1) have been associated with the familial form of the disease. As the encoded enzyme is critical for managing oxidative stress, mutated SOD1 has been linked to toxic effects on motor neurons. The validation work reported here was a collaborative effort between Inotiv and Taconic Biosciences, which provided the SOD1 rat carrying a transgene for human SOD1 with the G93A mutation. The 16-week, longitudinal study correlated motor phenotypes with a clinically relevant biomarker, neurofilament light (NfL). The findings underscore the relevance of the SOD1-G93A rat to major aspects of ALS, making it an invaluable tool in the development of potential therapeutics for the disease.

## Study Design

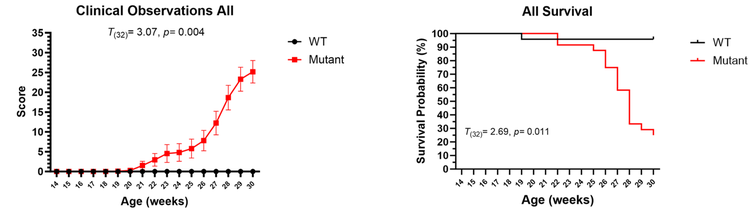



## 1 Mutated human superoxide dismutase was overexpressed in transgenic SOD1 rats



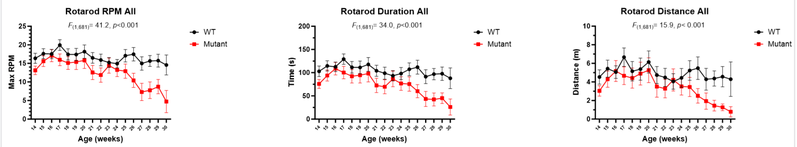

No genotypic differences existed in the levels of normal, rat SOD *(left)*. However, only the transgenic rats had detectable levels of mutated human SOD (*right*)

## 2 Over time, SOD1 rats had increased clinical observations and reduced survival probability



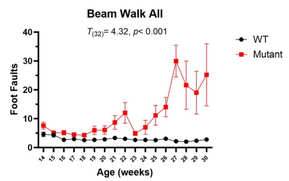

An 11-point clinical observation system was used for overall health assessments. Each element was scored on a scale of 0-3, and then all parameters were summed. Animals that reached criteria scores of 6 or more over the course of a week were removed from the study and assigned the maximum score of 33 for the remainder of the study. As expected, transgenics had progressively higher clinical scores, primarily associated with walking , posture, and body condition (*left*), and they had progressively lower chances of survival (*right*).

## 3 SOD1 rats exhibited reduced performance on the rotarod test



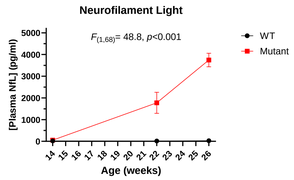

Motor function as measured by maximum RPM (*left*), maximum duration (*middle*), and total distance (*right*) on the rotarod was progressively worse in the mutants.

## 4 Over time, SOD1 rats made more mistakes on the beam walk



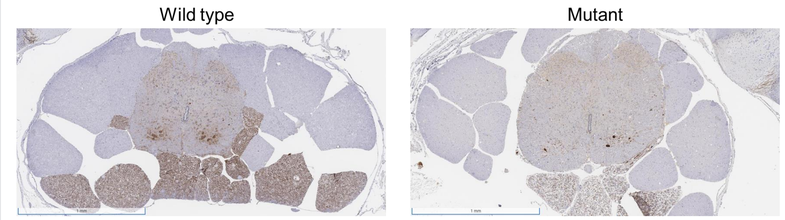

Transgenics performed significantly worse than the wild types on the beam walk test.

## 5 Neurofilament Light (NfL) increased in SOD1 rats over the course of the study



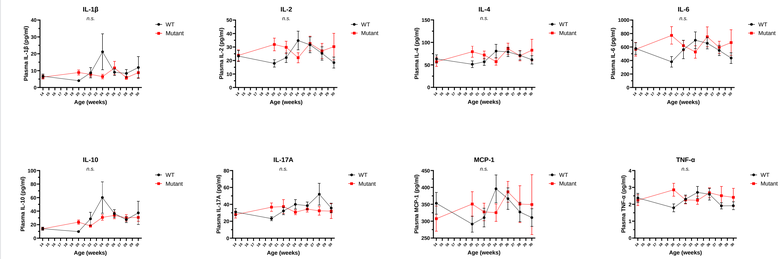

Transgenics had progressively more NfL in their blood, while the wildtypes had none.

## 6 Loss of motor neurons in the lumbar spinal cord




Choline acetyltransferase (ChAT) immunohistochemistry in the lumbar spinal cord indicated that wildtypes had strong staining of motor neuron cell bodies in the ventral horn of the spinal cord and in the axons of the spinal nerves. However, ChAT staining in the transgenics was markedly reduced in comparison.

## 7 Unexpected lack of SOD1 effects on plasma cytokines




No genotype-related differences were detected in any of the 8 cytokines that were measured by multiplexed Luminex® over the course of the study.

## Conclusions

- The SOD1-G93A rat models important aspects of ALS such as:
  - >Higher expression of mutated human SOD1
  - >Progressive neuron damage as measured by increased NfL in the plasma
  - >Loss of motor neurons in the spinal cord
  - >Progressive loss of voluntary muscle function
  - >Progressive clinical issues with walking, posture, and body condition
  - >Reduced survival
- Thus, the SOD1-G93A rat can be used to test new therapies for ALS and related diseases