TRANQUIS THERAPEUTICS

The small molecule myeloid cell modulator TQS-168 normalizes the inflammatory phenotype of immune cells from ALS patients and extends survival in the SOD1*G93A ALS mouse model

E. CHAVEZ JUAN[1], D. NESS[1], B. AJAMI[2], N. SCHWARTZ[1], J. HANNESTAD[1], S. KAKKAR[1], H. LANCERO[1]

[1]Tranquis Therapeutics Inc., San Mateo, CA 94404, [2]Department of Molecular Microbiology and Immunology Oregon Health & Science University, Portland, OR 97239

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INTRODUCTION

- Amyotrophic Lateral Sclerosis (ALS) is a rapidly progressing neurodegenerative disease with a median survival of 3-5 years
- While the pathogenesis of ALS is not fully understood, inflammation including activation of myeloid cells plays a key role
- **In these studies, the ability of the small molecule TQS-168 to modulate the immunophenotype of myeloid cells and circulating cytokines was tested in:**
  - Mice:
    - Wild type (WT)+/- the inflammatory stimulus lipopolysaccharide (LPS)
    - SOD1*G93A model of ALS (mouse overexpressing a mutant form of human *SOD1*)
  - Blood samples from ALS patients and healthy subjects (ex-vivo)

MATERIALS AND METHODS

MICE:

Blood samples from wild type mice were collected in EDTA tubes and stimulated with LPS (Sigma Aldrich) .

Mutations in the antioxidant enzyme Cu, Zn superoxide dismutase (SOD1) are observed in familial ALS.

Transgenic SOD1 mice that overexpress the human SOD1*G93A ALS associated mutation progressively develop ALS-like pathologies including motor dysfunction and early death.

Sixty-day old male SOD1*G93A (stock #002726) and wild type (WT; C57BL/6J) mice were obtained from Jackson Labs. At 70 days of age dosing by oral gavage began. Righting reflex was assessed every 3 days when mice were <110 days old and every day >110 days old. Blood samples were collected in EDTA tubes at 2 time points: 90 days of age, and terminal time point (SOD1 mice were euthanized if they could not right in 20sec after being placed onto their back).

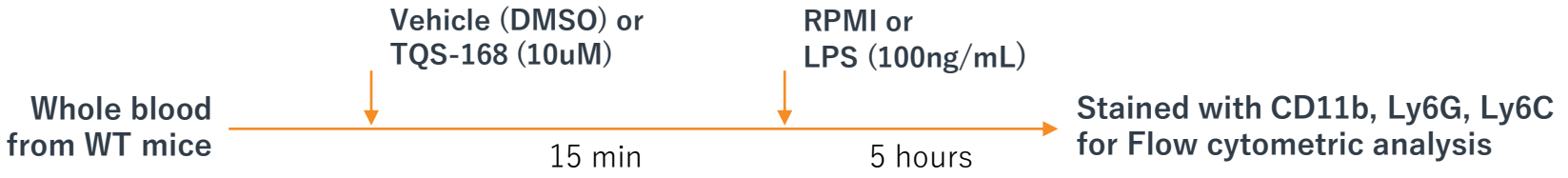
BLOOD FROM ALS PATIENTS: Samples were obtained from iSpecimen (Lexington, MA) and BioIVT (Westbury, NY). Samples were processed within 36 hours of collection and were not frozen.

FLOW CYTOMETRY: Antibodies were purchased from BD Biosciences (San Jose, CA): human: CD16-APC, CD14-BV421; mouse: CD11b-APC Cy7, Ly6G-FITC, Ly6C-APC; Zombie Aqua Live/Dead (Biolegend). Samples were analyzed on either a ZE5 Cell Analyzer (Bio-Rad) or a CytoFLEX S (Beckman Coulter) flow cytometer with FCS Express 7 (De Novo) and CytExpert (Beckman Coulter) software. At least 50,000 cells were counted per analysis.

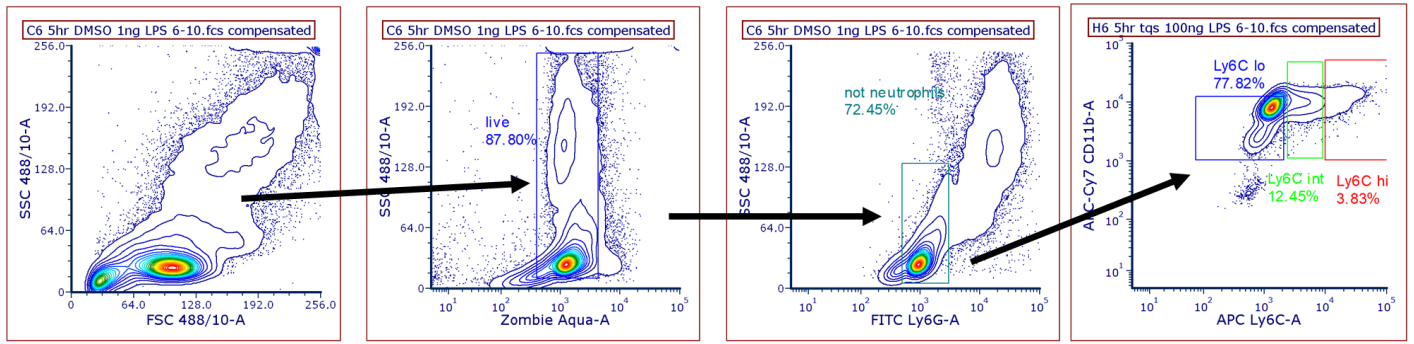
CYTOKINE ANALYSIS: The Stanford Human Immune Monitoring Center measured plasma cytokine levels using the mouse 48-plex Procarta from Thermo Fisher (Santa Clara, CA).

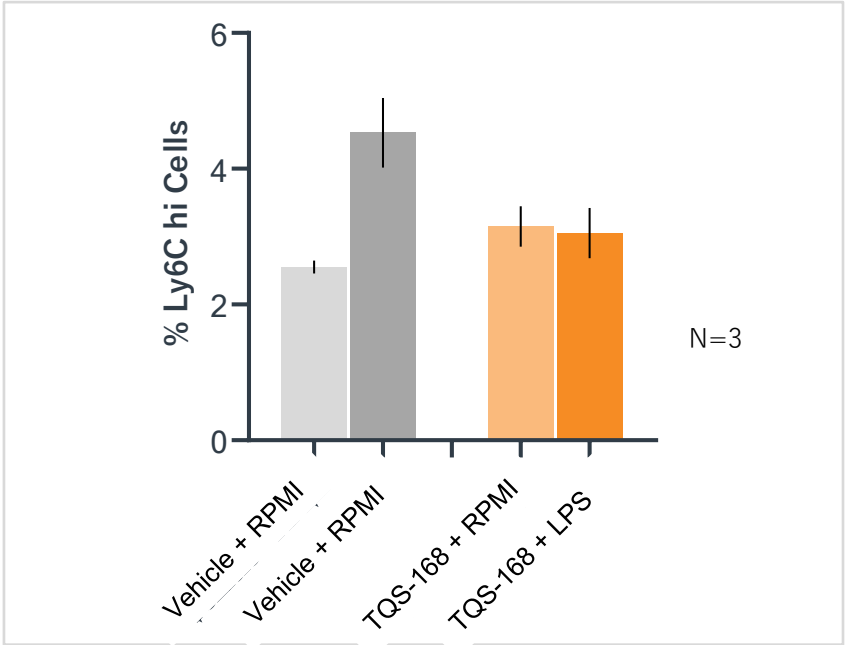
STATISTICAL ANALYSIS and graphing was done in GraphPad (San Diego, CA)

TQS-168 SUPPRESSES THE INFLAMMATORY EFFECTS OF LPS ON MYELOID CELLS FROM WILD TYPE MICE (EX VIVO)



GATING STRATEGY

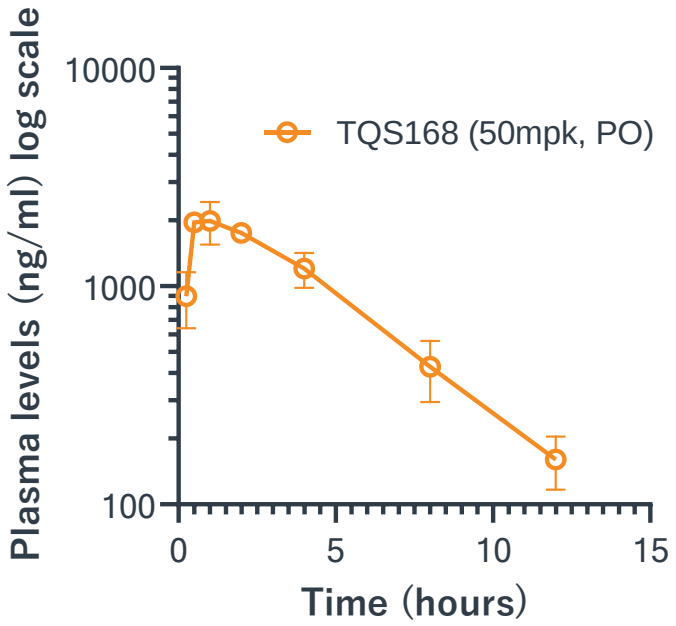




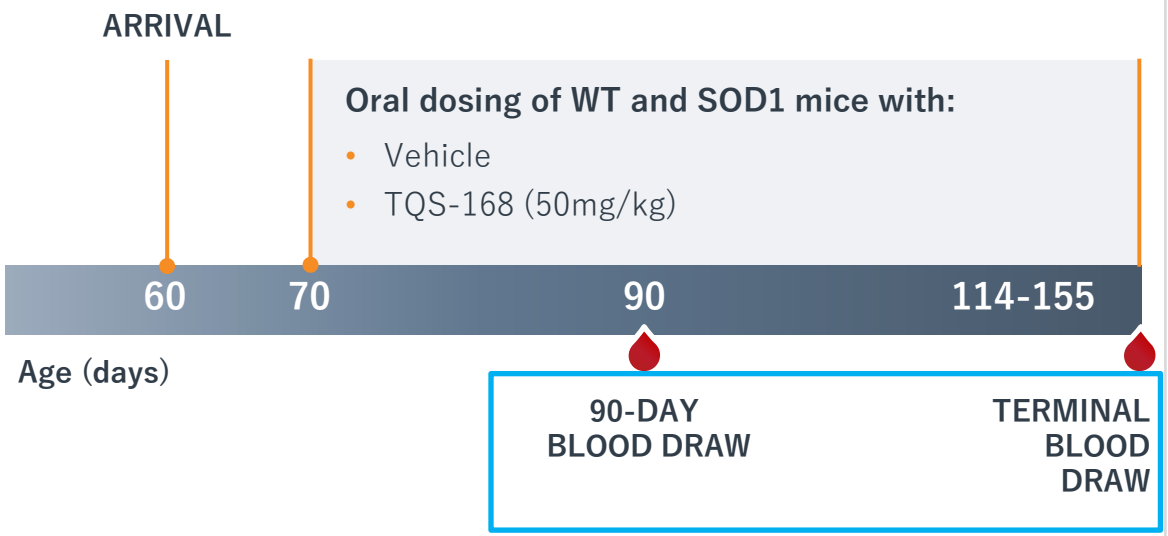
- Ex-vivo incubation of mouse blood with LPS increases the % of inflammatory monocytes (Ly6C^{hi} cells)
- Pre-treatment with TQS-168 inhibits this LPS-induced increase in Ly6C^{hi} cells

MOUSE IN-VIVO STUDY DESIGN AND PK

TQS-168 is orally bioavailable
(WT mice)



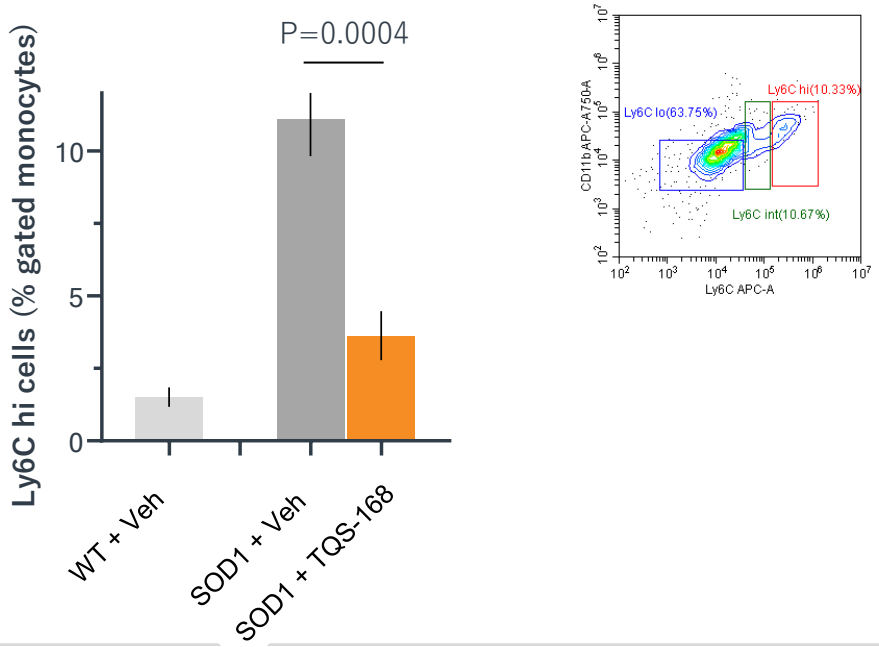
Timeline of SOD1 study



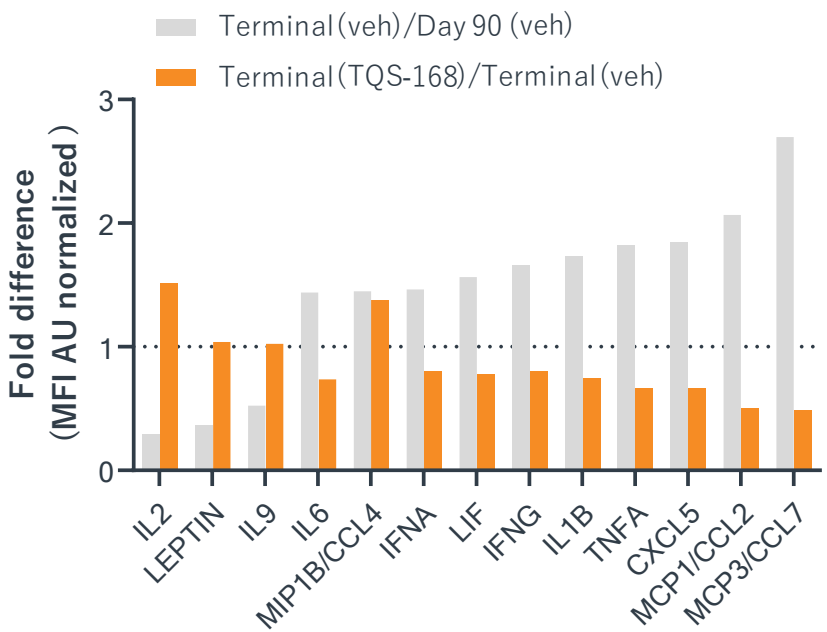
TQS-168 SUPPRESSES INFLAMMATORY MARKERS IN SOD1 MICE

- After 20 days of oral dosing with TQS-168 (90 days of age) the fraction of circulating Ly6C^{hi} cells was measured
- After approximately 20 days of oral dosing (90 days of age) and at the end of life (terminal), plasma cytokines were measured

In SOD1 mice, TQS-168 reduces the fraction of inflammatory monocytes

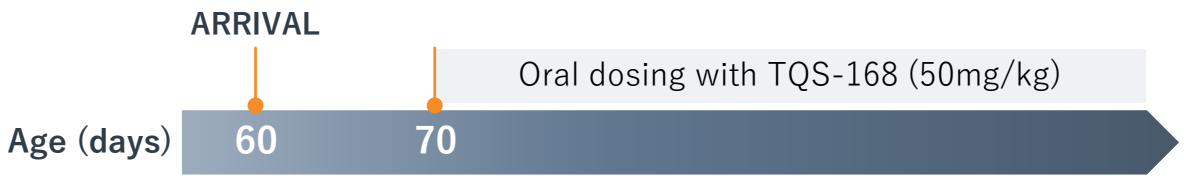


In SOD1 mice, TQS-168 regulates plasma levels of anti and pro-inflammatory cytokines

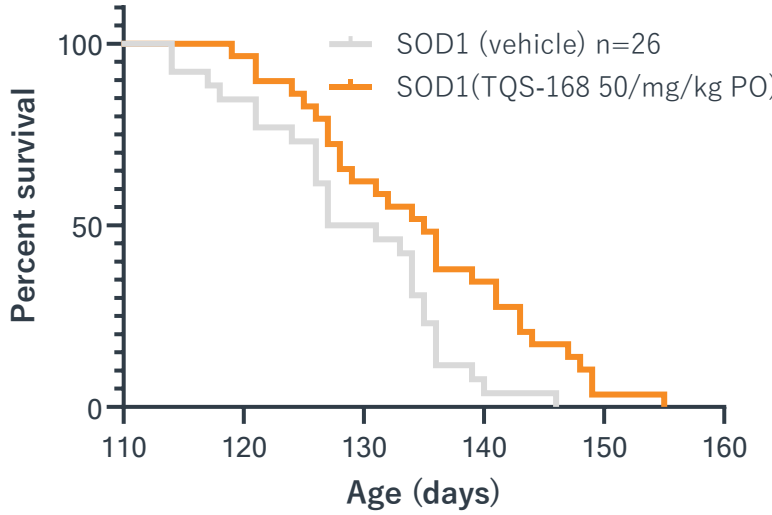


In the SOD1 mouse model of ALS, oral administration of TQS-168 reduces inflammatory markers in blood cells

TQS-168 IMPROVES SURVIVAL IN SOD1 MICE

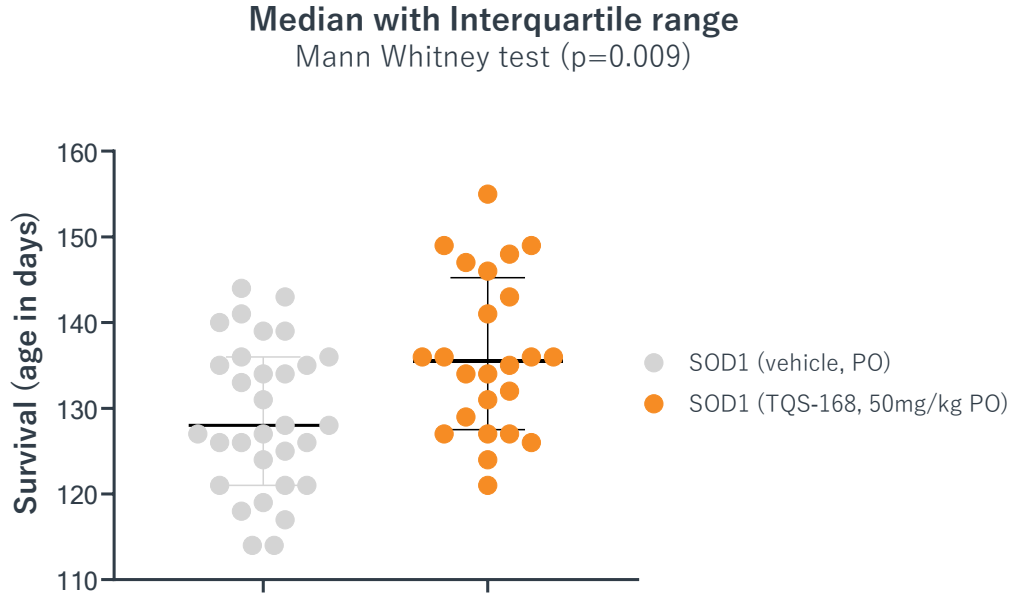


EUTHANIZED:
Unable to right in 20 sec after being placed onto back



Median survival age in days: SOD1 (vehicle): 129; SOD1 (TQS-168): 135

Gehan-Breslow-Wilcoxon comparison of survival curves (χ^2=4.7, df=1, p=0.03)



- SOD1 mice exhibit progressive ALS-like pathology resulting in loss of motor function and early death
- Oral dosing with TQS-168 extended survival of SOD1 mice.
- Oral dosing with TQS-168 did not affect motor function, weight gain or survival of WT animals (data not shown)

TQS-168 SUPPRESSES INFLAMMATORY PHENOTYPE OF CIRCULATING MYELOID CELLS FROM ALS PATIENTS

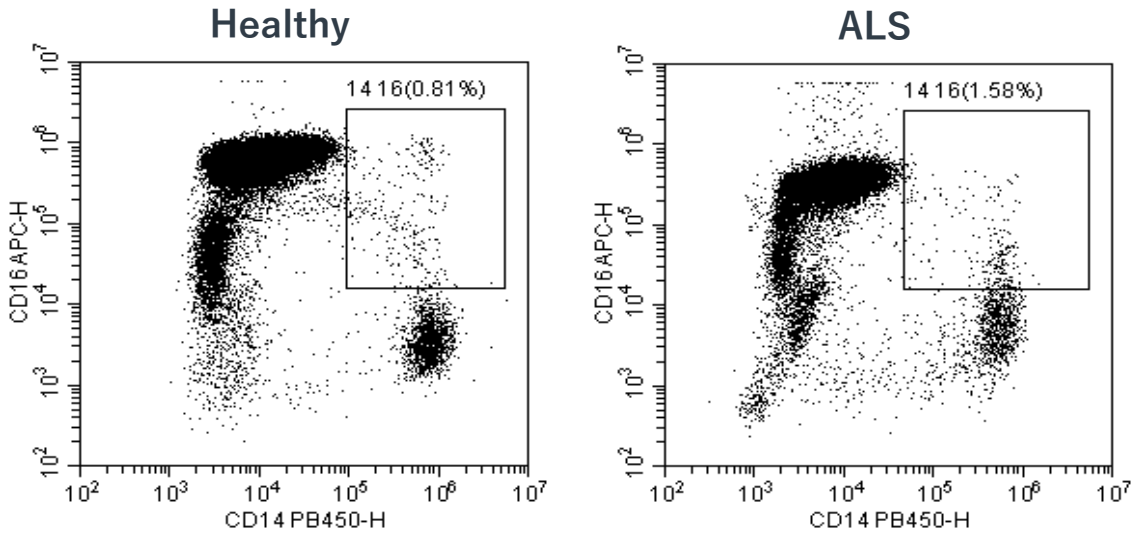
BLOOD SAMPLES
1. Healthy donors (n=2)
2. ALS patients (n=3)

24-36 hours

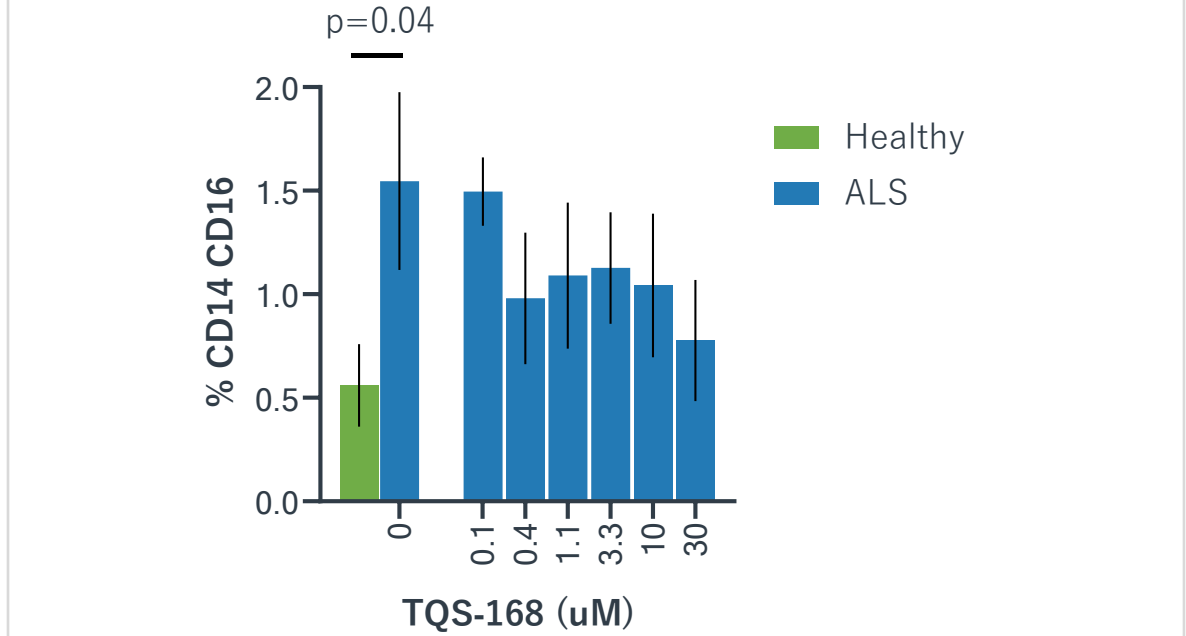
TQS-168 (0 to 30uM)

4hours

Stained with CD14 and CD16 for Flow cytometric analysis



CD14+/CD16+ cells increased in ALS patients



Ex-vivo incubation of ALS blood samples with TQS-168 reduces inflammatory CD14+/CD16+ monocyte expression

CONCLUSIONS

The small molecule TQS-168:

- Regulates the inflammatory response to LPS stimulation (in mouse blood cells)
- Modulates the phenotype of myeloid cells in the SOD1 mouse model of ALS
- Extends survival in the SOD1 model of ALS
- Normalizes the phenotype of myeloid cells from ALS patients

TQS-168 will enter clinical development to evaluate its potential as a treatment for ALS patients.

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THANK YOU

VISIT OUR WEBSITE:

https://www.tranquis.com/

FOR GENERAL INQUIRIES:

info@tranquis.com