Healthcare Innovations
How practice has changed

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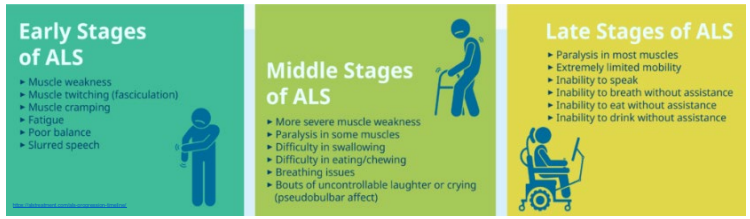
Validating the role of Bruton's Tyrosine Kinase (BTK) in Motor Neuron Disease

Sara Jose[1], Natalie, J. Groves[1], William D. Godfrey[1], Katerina Z. Hanton[1], Nanthini Jayabalan[1], Trent M Woodruff[2], Pamela A. McCombe[1], Robert D. Henderson[1], Fredrick J. Steyn[1,2] Shyuan T. Ngo[3,4], Richard Gordon[1,2]

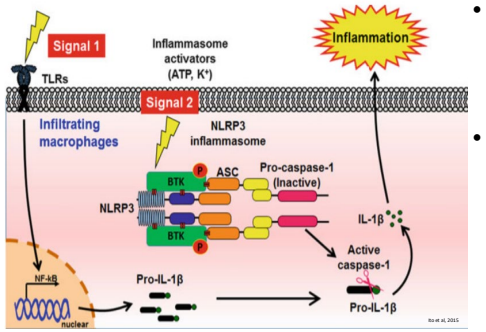
[1]Translational Neuroscience Laboratory, UQ Centre for Clinical Research, The University of Queensland, [2]UQ School of Biomedical Sciences , [3] Australian Institute for Bioengineering and Nanotechnology (AIBN), [4]Queensland Brain Institute, (QBI)

Motor Neurone Disease



- Progressive, terminal neurodegenerative disorder
- Currently no effective treatments available
- 2000 people living with MND in Australia
- 90% Sporadic cases with no known cause

Bruton tyrosine kinase



- Chronic immune activation is a key driver of disease progression in MND.
- BTK has been known to regulate NLRP3 inflammasome activation, which has been implicated in various inflammatory diseases.

Acute and chronic dosing studies in SOD1^{G93A} mouse model of MND

The BTK pathway is highly activated in the SOD1 model of MND

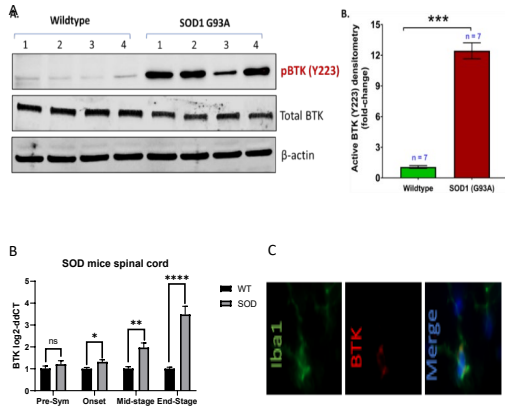


Fig 1. The BTK pathway is highly activated in the SOD1 model of MND **(A) The BTK pathway is highly activated in the SOD1 model of MND** B. Western blots for active pBTK (Y223 activation loop) in spinal cord lysates from wildtype and SOD1 mice C. Densitometric band quantification of active BTK (Y223) from 7 wildtype and SOD1 littermates demonstrating significantly increased (***p<0.001) BTK signaling in the SOD1 MND model. **(B) BTK is highly expressed in SOD1 mice Spinal cord-** qPCR results showing BTK expression in pre-symptomatic, onset stage, Mid-stage and end stage spinal cord samples. Extensive increase is observed in BTK expression in end-stage samples from SOD mice compared to the wildtype. qPCR data was analyzed via the delta-delta CT method, using β-actin as the house keeping gene. Data is represented as mean ± SEM. ****P<0.0001, *P<0.05. **(C)** Immunohistochemistry results showing BTK (red) localised in the microglia (green) (n=1).

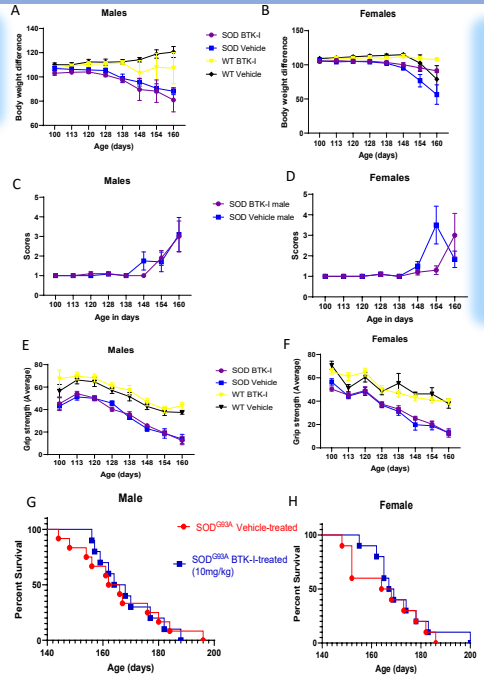


Fig 2:. Body weight variations (A&B), scores (C&D) and grip strength (E&F) in BTK-I-treated mice against vehicle groups. Average mean number of mice analyzed: BTK-I and vehicle treated mice, n = 8-12 for each genotypes; Fig G-H shows percent survival for BTK-I-treated and Vehicle-treated mice: Kaplan-Meier plot of ages (in days) in which SOD1^{G93A} mice treated with BTK-I (10mg/kg) and Vehicle reached end-stage of disease (defined by inability to right itself in 30secs once placed on its back) BTK-I-treated, n = 10 ; Vehicle-treated, n = 8-10

Once daily oral dosing of a BTK-I at 10 mg/kg in SOD1^{G93A} mice did not improve grip strength, motor function or survival in this model

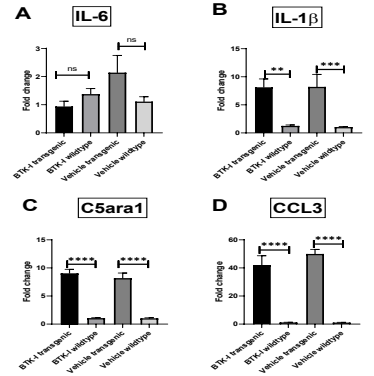
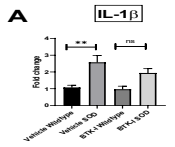


Fig 3 . Treatment of SOD mice with 10 mg/kg of BTK-I by oral gavage until end stage, showed no significant change in neuroinflammatory pathology in the spinal cord n=6-8 per group



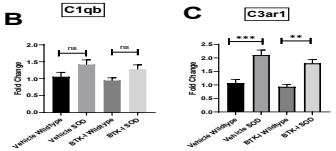


Fig 4 . Treatment of SOD mice with 5 mg/kg of BTK-I by oral gavage for 28 days, showed no significant change in neuroinflammatory pathology in the spinal cord n=6-8 per group

Subset of inflammatory markers did not change significantly in acute and chronic model

Conclusion. Our results confirm a strong upregulation of the BTK pathway in MND mice, which increases with disease progression, this suggests a potential role for BTK driving inflammation in MND. Our ongoing studies are evaluating the efficacy of BTK inhibitors at additional doses to confirm their potential for disease modification in MND.