SM07883, A NOVEL, POTENT, AND SELECTIVE ORAL DYRK1A INHIBITOR, REDUCES NEUROINFLAMMATORY RESPONSES IN MOUSE MODELS

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Poster #205

Background

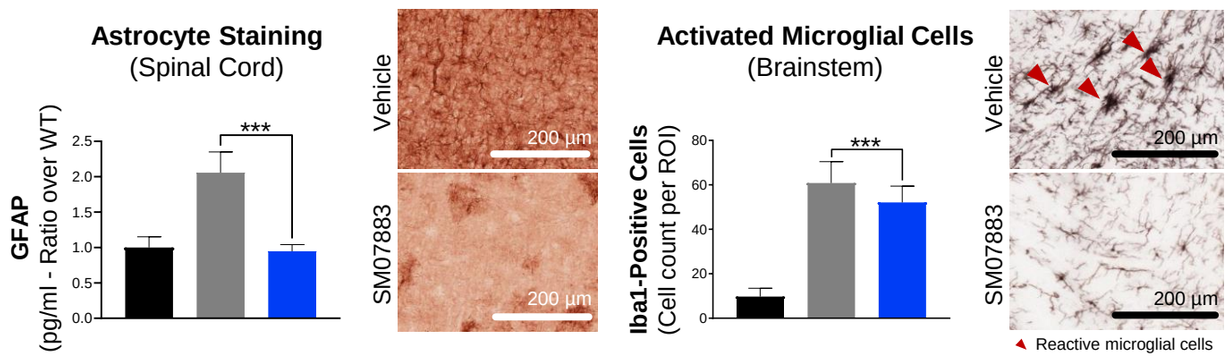
- Neuroinflammation is a hallmark of many central nervous system disorders, including Alzheimer's disease
- SM07883 is an oral, brain-penetrant DYRK1A inhibitor that has been shown to reduce tau and amyloid pathology and gliosis in neurodegenerative mouse models[1,2]
- This study assessed the potential of SM07883 to modulate innate immunity *in vitro* and *in vivo* using tau transgenic mice and inflammatory mouse models

Conclusions

- **SM07883 reduced glial activation, pro-inflammatory cytokine expression, and nitric oxide production compared with vehicle in preclinical models**
- **SM07883 represents a potential treatment for neuroinflammation in neurodegenerative disorders**
- **Phase 1 human studies are ongoing**

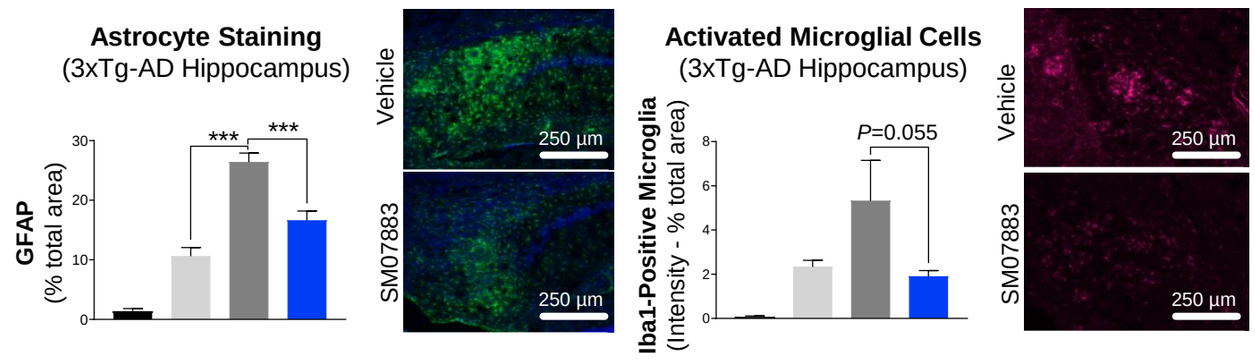
Results

Figure 1. SM07883 reduced tau-induced glial activation in JNPL3 mice[1]



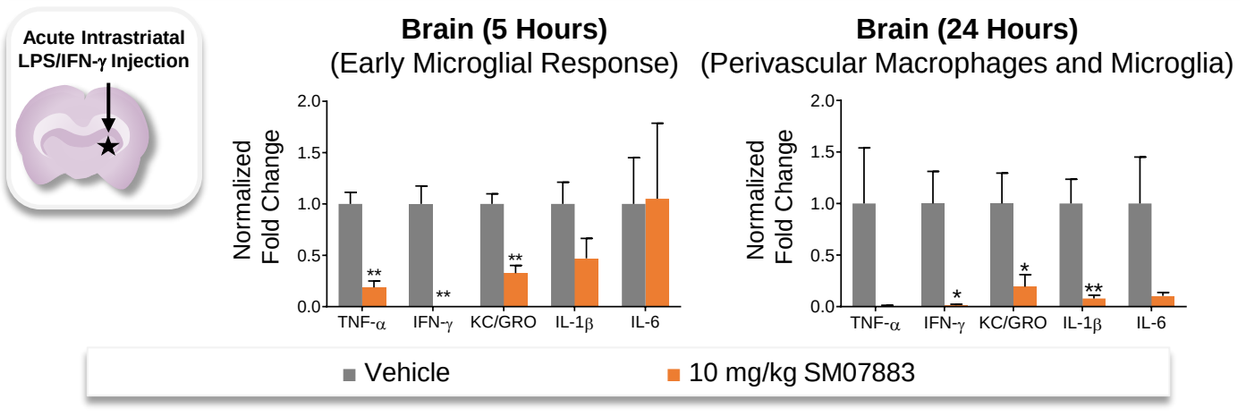
Left: GFAP; WT + Veh. n=9, JNPL3: Veh. n=18, SM07883 n=19; Images depict brainstem staining
Right: Iba1; WT + Veh. n=11, JNPL3: Veh. n=32, SM07883 n=19; mean ± SEM; ****P*<0.001 vs. vehicle

Figure 2. SM07883 reduced neurodegeneration-induced glial activation in 3xTg-AD mice



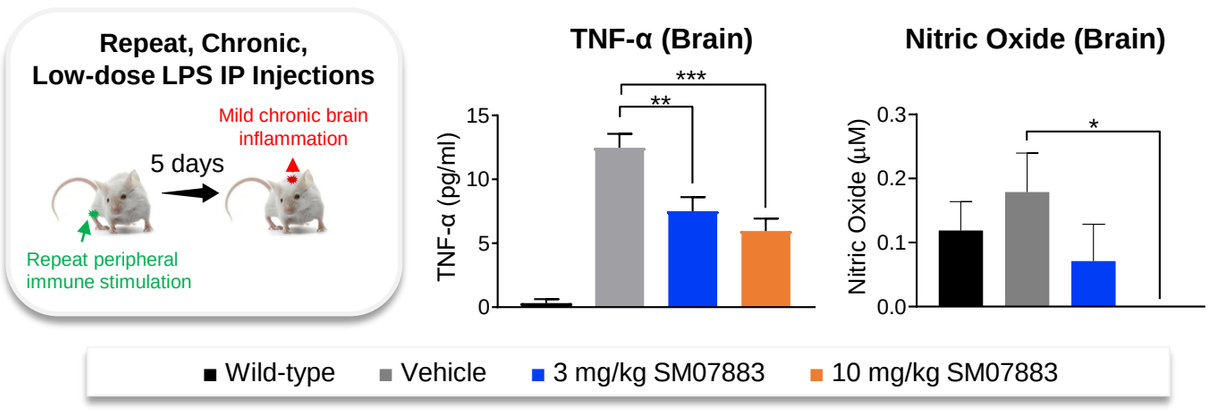
Hippocampal CA1 stained with GFAP (green) or Iba1 (magenta); Blue is a nuclear DAPI stain
Quantification of staining in WT + Veh. n=9, 3xTg-AD: Veh. n=9 and SM07883 n=11; mean ± SEM; ****P*<0.001 vs. vehicle

Figure 3. SM07883 reduced acute neuroinflammation *in vivo*



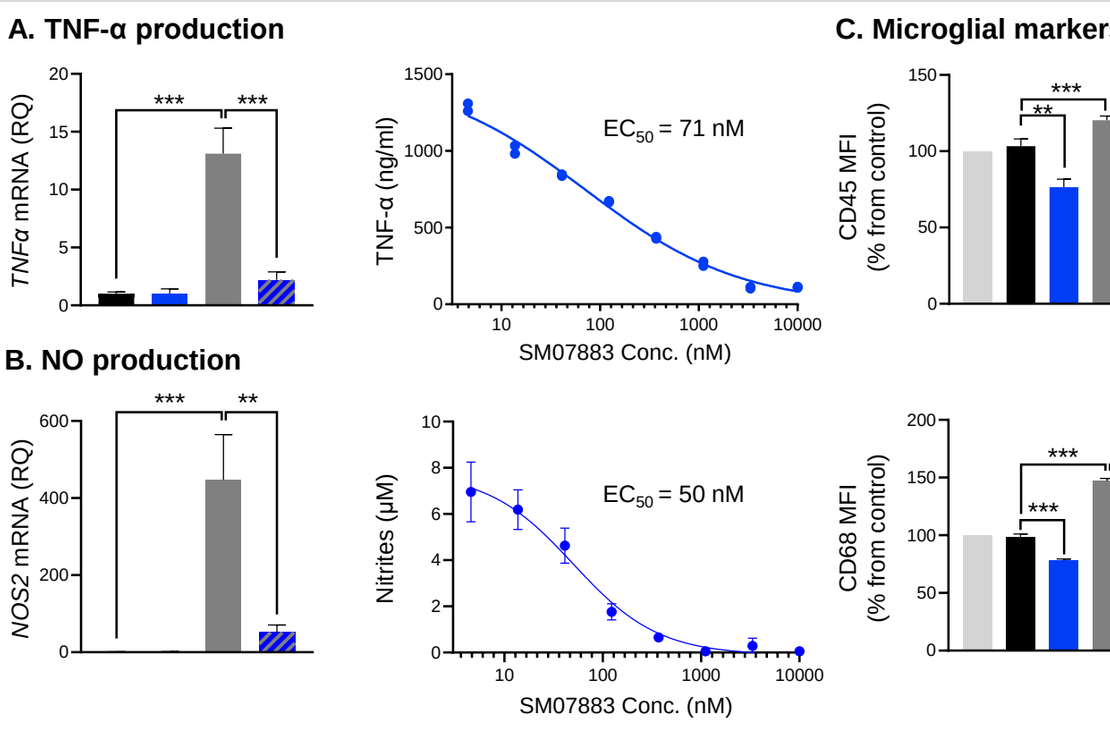
n=3 mice/treatment group; mean ± SEM; **P*<0.05, ***P*<0.01 vs. vehicle

Figure 4. SM07883 reduced chronic neuroinflammation *in vivo*



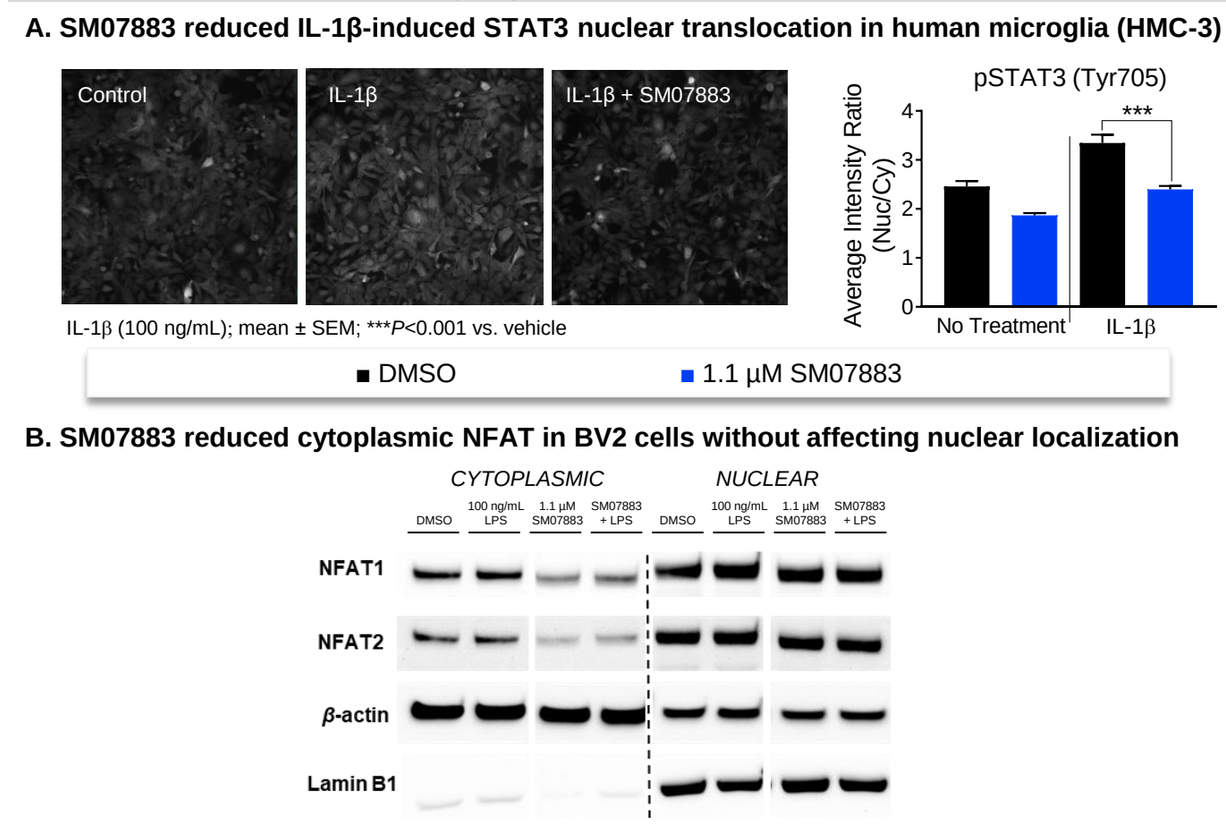
WT n=2, Veh. n=15, SM07883 + LPS: 3 mg/kg n=15 and 10 mg/kg n=15; mean ± SEM; **P*<0.05, ***P*<0.01, ****P*<0.001 vs. vehicle

Figure 5. SM07883 reduced microglial cell activation *in vitro*



mean ± SEM; ***P*<0.01, ****P*<0.001 vs. vehicle

Figure 6. SM07883 reduced STAT3 translocation and NFAT cytoplasmic localization *in vitro*



IL-1β (100 ng/mL); mean ± SEM; ****P*<0.001 vs. vehicle

Methods

In vivo

- Ten-month-old JNPL3 mice (P301L human tau overexpression mutation) or eleven-month-old 3xTg-AD mice (APP, PSEN, P301L tau) were orally administered vehicle or SM07883 (JNPL3: 3 mg/kg, QD, 3 months; 3xTG-AD: 5 mg/kg, QD, 6 months). Glial activation was assessed using either a glial fibrillary acidic protein (GFAP) ELISA quantification assay in spinal cords of JNPL3 mice or by measuring GFAP staining in hippocampal regions of 3xTg-AD mice. Activated microglial cells were identified and quantified by Iba1-positive staining (**Figs. 1** and **2**)
- *Acute inflammation:* Balb/c mice were administered SM07883 (PO, 10 mg/kg QD) 3 days prior to injection of 5 µL of LPS (100 ng/ml) and IFN-γ (10 U/ml) into the left striatum. Left hemispheres were collected at 5 and 24 hours post injection and assayed for cytokines (Meso Scale Discovery [MSD] V-PLEX)[3] (**Fig. 3**)
- *Chronic inflammation:* Balb/c mice were administered SM07883 (PO, 3 or 10 mg/kg) and LPS (IP, 0.5 mg/kg) for 5 consecutive days. Brains were collected and analyzed for TNF-α (Millipore Milliplex Magnetic Bead Panel) and NO_2^- (Griess reaction) (**Fig. 4**)

In vitro

- Mouse BV2 microglial cells were cultured overnight with SM07883 (1.1 µM) and challenged with LPS (100 ng/ml) for 6 hours. *TNFα* and *NOS2* expression was measured by qRT-PCR using TaqMan® primers and normalized to GAPDH via $^{\Delta\Delta}$Ct. BV2 cells were also cultured with serial dilutions of SM07883 and challenged with LPS (250 ng/ml) for 5 to 24 hours. Supernatants were tested for TNF-α (MSD) and NO_2^- (Griess reaction) (**Figs. 5A** and **B**)
- BV2 cells were exposed to SM07883 (1.1 µM) for 1 hour prior to overnight exposure to LPS (100 ng/ml). Cells were collected and stained with fluorescently labeled anti-murine CD45 or CD68 and analyzed by flow cytometry (**Fig. 5C**)
- BV2 or human microglia HMC-3 cells were incubated overnight with SM07883 (1.1 µM) and then treated with IL-1β or LPS (100 ng/mL) the next day for 15 minutes. Nuclear and cytoplasmic pSTAT3 was assessed in HMC-3 cells by immunostaining (CellInsight CX5, Thermo Fisher) (**Fig. 6A**). In BV2 cells, nuclear and cytoplasmic fractions were prepared using the NE-PER™ kit (Thermo Fisher) and NFAT protein levels were qualitatively assessed by Western blot (**Fig. 6B**)

References: 1) Melchior B, et al. *Aging Cell*. 2019. 2) Melchior B, et al. *Alzheimer's & Dementia*. 2019. 15(7 Suppl). 3) Schmid CD, et al. *J. Neurochem*. 2009.
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