SM07883, a novel oral DYRK1A kinase inhibitor, reduced tau, amyloid pathology, and related inflammation in preclinical models

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Background

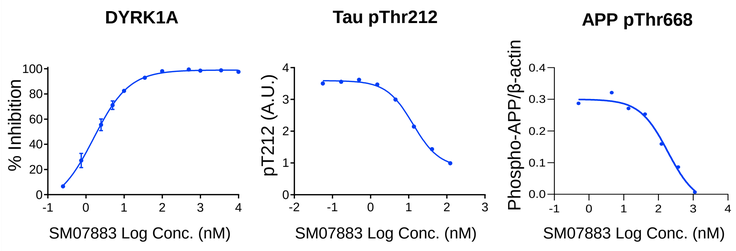
- Dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) regulates amyloid precursor protein (APP) and tau phosphorylation (pTau). It is overexpressed in Alzheimer's disease (AD) brains and correlates with pathology[1-4]
- DYRK1A inhibition reduces phospho-APP (pAPP) and amyloid pathology[5,6]
- A potential therapeutic for AD, SM07883 is an oral, small-molecule DYRK1A inhibitor
- This study assessed the effects of SM07883 *in vitro* and *in vivo* on amyloid, tau, and neuroinflammation pathology together with function and cognition in two mouse AD models: human tau transgenic (JNPL3) and triple transgenic (3xTG-AD)

Conclusions

- **SM07883, a small-molecule DYRK1A inhibitor, may have therapeutic potential in neurodegenerative diseases**
  - **SM07883 reduced tau pathology (pTau, aggregation, NFTs), reduced neuroinflammation, and improved functional deficits/health in tau transgenic mice (JNPL3)**
  - **SM07883 reduced AD hallmarks (amyloid and tau), hippocampal neuroinflammatory markers, and protected against cognitive deficits in behavioral tests in triple transgenic mice (3xTG-AD)**
- **Phase 1 human study is ongoing**
  - **ANZCTR.org.au registration #ACTRN12619000327189**

Results

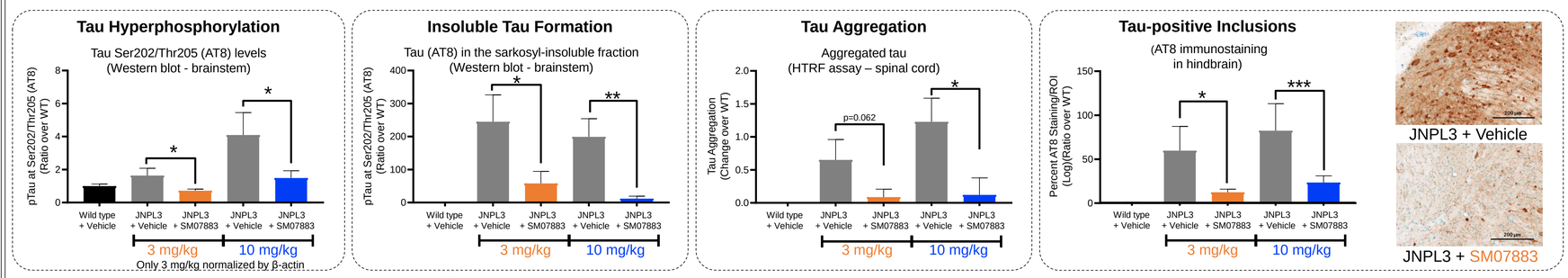
Figure 1. SM07883 potently inhibited DYRK1A and reduced pTau, pAPP, $A\beta_{40}$, and TNF-α production *in vitro*



SM07883 *in vitro*	Cell Lines	IC_{50} / EC_{50}
Potently inhibited DYRK1A kinase activity[3]	N/A (Kinase inhibition assay)	IC_{50} 1.6 nM
Tau phosphorylation at pThr212	Tau/DYRK1A in HEK293	EC_{50} 16 nM ‡
Tau phosphorylation at pSer202/Thr205 (AT8)	Tau/DYRK1A in HEK293	EC_{50} 69 nM †
Tau phosphorylation at pThr181	Tau/DYRK1A in HEK293	EC_{50} 127 nM †
Tau phosphorylation at pSer396	Human neuroblastoma cells (SH-SY5Y)	EC_{50} 200 nM ‡
Reduced DYRK1A-mediated pAPP at Thr668	Human neuroblastoma cells (SH-SY5Y)	EC_{50} 187 nM †
Reduced $A\beta_{40}$ secretion	SH-SY5Y cells overexpressing hAPP(wt)	EC_{50} 798 nM ‡
Reduced TNF-α secretion	LPS-challenged BV2 microglial cells	EC_{50} 71 nM ‡

†Measured by Western blot, ‡Measured by immunoassays
Note: EC_{50} = average across multiple assays

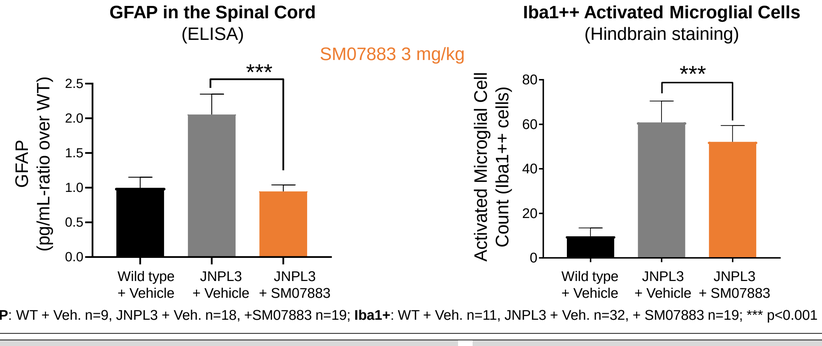
Figure 2. SM07883 inhibited tau pathology in JNPL3 tau mouse brains



3 mg/kg study: WT + Veh. n=9, JNPL3 + Veh. n=19, + SM07883 n=19 **10 mg/kg study**: WT + Veh. n=10, JNPL3 + Veh. n=15, + SM07883 n=13

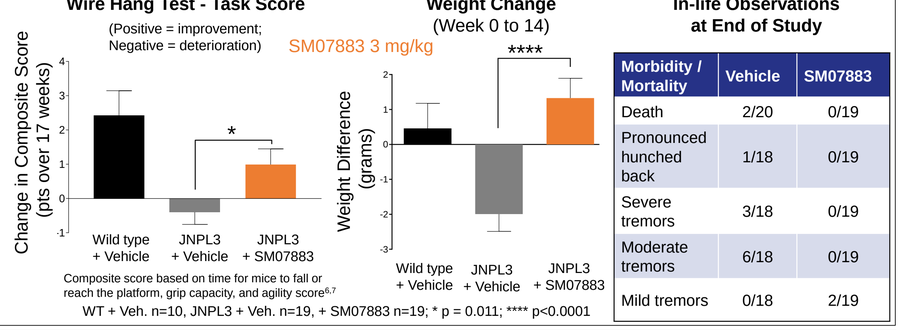
* p<0.05, ** p<0.01, *** p<0.001 JNPL3 SM07883 vs. Vehicle

Figure 3. SM07883 reduced tau-induced glial activation (neuroinflammation) in JNPL3 tau mouse CNS



GFAP: WT + Veh. n=9, JNPL3 + Veh. n=18, +SM07883 n=19; **Iba1+**: WT + Veh. n=11, JNPL3 + Veh. n=32, + SM07883 n=19; *** p<0.001

Figure 4. SM07883 improved motor function, weight, and general health of JNPL3 tau mice

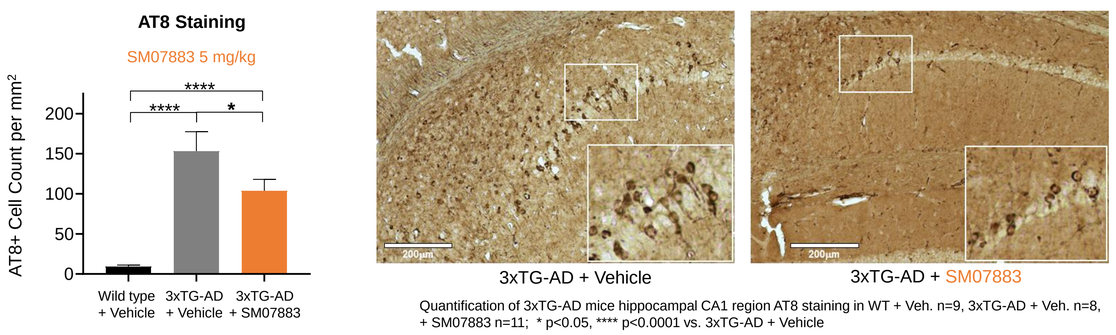


Composite score based on time for mice to fall or reach the platform, grip capacity, and agility score[6,7]

In-life Observations at End of Study		
Morbidity / Mortality	**Vehicle**	**SM07883**
Death	2/20	0/19
Pronounced hunched back	1/18	0/19
Severe tremors	3/18	0/19
Moderate tremors	6/18	0/19
Mild tremors	0/18	2/19

WT + Veh. n=10, JNPL3 + Veh. n=19, + SM07883 n=19; * p = 0.011; **** p<0.0001

Figure 5. SM07883 reduced tau pathology in 3xTG-AD mouse brains



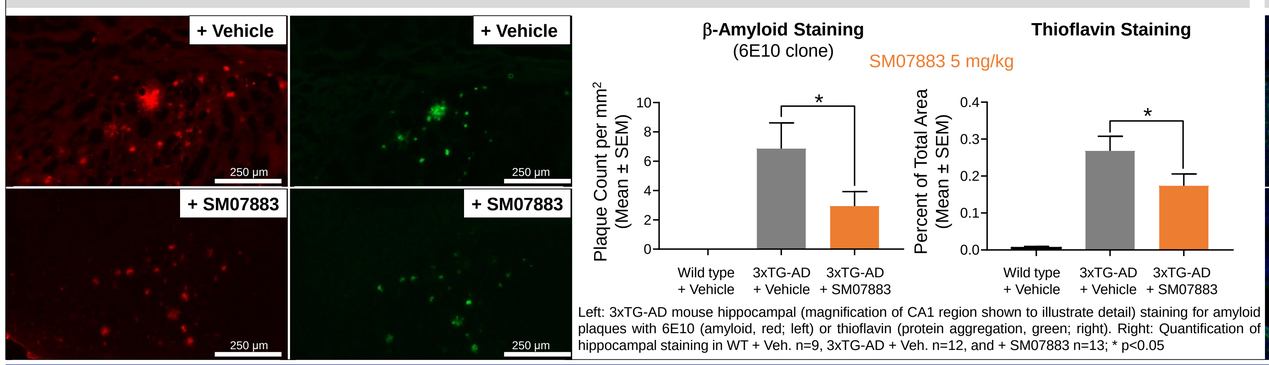
Quantification of 3xTG-AD mice hippocampal CA1 region AT8 staining in WT + Veh. n=9, 3xTG-AD + Veh. n=8, + SM07883 n=11; * p<0.05, **** p<0.0001 vs. 3xTG-AD + Vehicle

Figure 6. SM07883 prevented cognitive deficit in NOR and in the Y Maze in 3xTG-AD mice

Novel Object Recognition at Week 21; WT + Veh. n=9, 3xTG-AD + Veh. n=10, + SM07883 n=11; * p<0.05, ** p<0.01

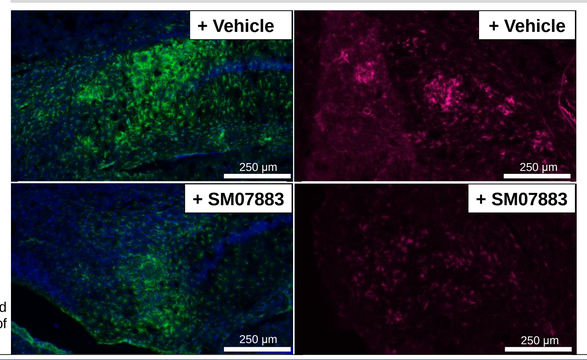
Y Maze alternation at Week 25; WT + Veh. n=9, 3xTG-AD + Veh. n=7, + SM07883 n=8; * p<0.05 vs. 3xTG-AD + Vehicle

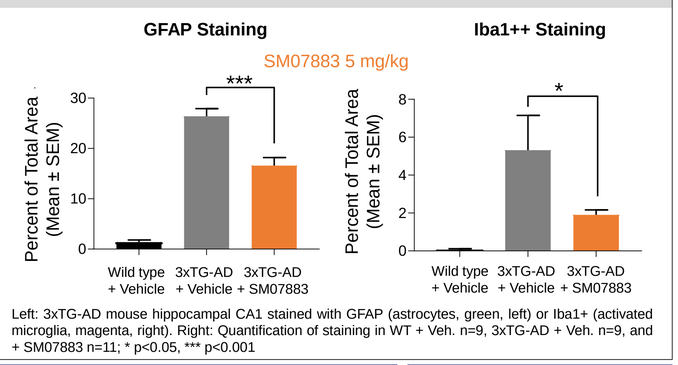
Figure 7. SM07883 reduced amyloid burden in 3xTG-AD mouse brains



Left: 3xTG-AD mouse hippocampal (magnification of CA1 region shown to illustrate detail) staining for amyloid plaques with 6E10 (amyloid, red; left) or thioflavin (protein aggregation, green; right). Right: Quantification of hippocampal staining in WT + Veh. n=9, 3xTG-AD + Veh. n=12, and + SM07883 n=13; * p<0.05

Figure 8. SM07883 reduced neuroinflammation (gliosis) in 3xTG-AD mouse brains





Left: 3xTG-AD mouse hippocampal CA1 stained with GFAP (astrocytes, green, left) or Iba1+ (activated microglia, magenta, right). Right: Quantification of staining in WT + Veh. n=9, 3xTG-AD + Veh. n=9, and + SM07883 n=11; * p<0.05, *** p<0.001

Methods

- SM07883 inhibition of tau phosphorylation (pTau) was measured in human tau/DYRK1A-transfected HEK-293T cells and human neuroblastoma cells. pAPP dose-response curves were measured in Western blots from unstimulated SH-SY5Y human neuroblastoma cells (densitometry, ImageJ). $A\beta_{40}$ secretion was measured by MesoScale Discovery (MSD) in stably transfected SH-SY5Y cells overexpressing wild type human APP (hAPP(wt)). SM07883 potency was evaluated in a DYRK1A kinase inhibition assay (**Fig. 1**)
- Ten-month-old JNPL3 mice (P301L human tau overexpression mutation) were orally administered vehicle or SM07883 (3 or 10 mg/kg, QD, 3 months) (**Fig. 2-4**)
  - pTau, sarkosyl-insoluble fraction, and aggregated tau were biochemically quantified in brainstems and spinal cords. Tau-positive inclusions were detected and quantified by immunostaining with a Ser202/Thr205 tau (AT8 clone) antibody at termination (13 months)
  - Glial activation was assessed in the brainstems using glial fibrillary associated protein (GFAP) staining and ELISA quantification, and activated microglia were identified by Iba1++ staining at 13 months
  - General tolerability was assessed by monitoring weight, morbidity, and mortality
  - Motor coordination was evaluated biweekly after treatment initiation using a wire hang test[7,8]
- Ten-month-old and twelve-month-old female 3xTG-AD mice (APP/PSEN/tau P301L) were orally administered SM07883 (5 mg/kg) or vehicle daily for 26 weeks. Wild type (WT) controls were age matched and administered vehicle (Veh.)
- Cognitive behavior was assessed by 1) Novel Object Recognition (NOR) discrimination index and time spent near novel object (10-min trial) and 2) Y Maze spontaneous and percent alternations (5-min trials) (**Fig. 6**)
- At termination, brains were analyzed for amyloid, tau, and inflammation
  - Hippocampal and surrounding cortical area lysates from one hemisphere were analyzed for amyloid (MSD) and tau fragments (**Fig. 5**; HTRF assay)
  - The other hemisphere was collected in formalin, sectioned, and stained for amyloid, tau, and gliosis markers. Immunoreactivity in the hippocampus was quantified (stain intensity; ImageJ) (**Figs. 7 and 8**)

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