Anti-inflammatory Effects of SM07883, a Novel, Potent, and Selective Oral DYRK1A Inhibitor in Neurodegenerative Mouse Models

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• CME/CE credits will not be awarded for this presentation

• All authors are employees and shareholders of Samumed, LLC

• This presentation is not intended to provide a comprehensive overview of all studies using SM07883

• SM07883 is an investigational compound; SM07883 has not been approved by the U.S. Food and Drug Administration (FDA) or any other pharmaceutical regulatory authority, and no conclusions can or should be drawn regarding the safety or effectiveness of the product candidate

• While the complete mechanism of action (MOA) for SM07883 is unknown, further investigation is being conducted. All of the MOA information is based on nonclinical data and the relationship to clinical benefit is unknown

• This presentation is intended as an exchange of scientific information, is provided for educational purposes only, and is not intended for any promotional purpose or to offer medical advice
AD and other neurodegenerative diseases have underlying inflammatory responses

- Alzheimer’s disease (AD) pathogenesis is associated with microglia and immune function\(^1,2\)
- Incidence of AD may be reduced in patients on immunosuppressive treatment\(^3,4\)
- Immune response is critical in clearing misfolded proteins, but excessive activity can be deleterious\(^1,2\)
  - **In AD, the CNS activates glial (immune) cells**\(^2\)
    - Innate system is engaged by distressed neurons, abnormal microenvironment (plaques), and synaptic impairment sensed by glial cells
  - **In multiple sclerosis (MS), peripheral immune cells are activated**\(^5\)
    - Adaptive immune responses against specific neuro-antigens
- Potential role for SM07883, an oral DYRK1A inhibitor, as an anti-inflammatory agent

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DYRK1A (Dual-Specificity Tyrosine Phosphorylation-Regulated Kinase 1A): A novel target for AD

- Found to be overexpressed in AD, Pick’s disease, and Down syndrome brains
- Regulates phosphorylation of major AD molecular hallmarks such as tau, APP (Aβ), and presenilin
- DYRK1A regulates inflammatory signals STAT3, GFAP, and NFAT

Proposed role of DYRK1A in AD

Proposed mechanism of action of SM07883 in AD: An orally available, potent, and specific DYRK1A inhibitor

AD Pathology

- Tau hyperphosphorylation
- Synaptic impairment, neurofibrillary degeneration
- β-amyloidosis
- Neurodegeneration
- Neuronal death and reduced cognitive function

Inflammation

- Inflammatory pathways
- Proinflammatory mediators

SM07883 inhibits DYRK1A and GSK3β, leading to the following effects:

- Lowered Tau phosphorylation
- Lowered APP phosphorylation
- Lowered inflammatory pathways
- Lowered NFAT phosphorylation
- Studies ongoing

Complementary preclinical mouse models

- **JNPL3**
  - Tau transgenic model
  - Tau P301L

- **3xTg-AD**
  - Amyloid and tau transgenic model
  - APP/PSEN/Tau P301L

- **LPS**
  - Induced inflammation model
  - Acute LPS/IFNγ (IC injection)
  - Chronic, low-dose LPS

- **EAE**
  - Induced inflammation model
  - MOG immunization

EAE: experimental autoimmune encephalomyelitis, MOG: myelin oligodendrocyte glycoprotein, IC: intracranial
SM07883 reduced tau pathology in JNPL3 tau mice

Tau Hyperphosphorylation
(Brainstem, Ser202/Thr205 [AT8] Western blot)

Sarkosyl-insoluble Fraction
(Brainstem, AT8 Western blot)

Tau-positive Inclusions
(Brainstem, AT8 % staining of ROI)
SM07883 reduced tau-induced glial activation in JNPL3 tau mice

**GFAP in the Spinal Cord** (ELISA)

- **GFAP**: WT + Veh. n=9, JNPL3: Veh. n=18 and SM07883 n=19

**Iba1++ Activated Microglial Cells** (Hindbrain staining)

- **3 mos treatment; 3 mg/kg/day**
- Mean ± SEM; ***p<0.001 vs. vehicle

SM07883 reduced amyloid pathology in 3xTg-AD mice

**β-Amyloid Staining in Hippocampal CA1 Region**
(6E10 clone)

- Wild type + Vehicle
- Naive
- Vehicle
- SM07883

26 wks treatment; 5 mg/kg/day

WT + Veh. n=9, 3xTg-AD: Naive n=8, Veh. n=12, and SM07883 n=13; Mean ± SEM; * p<0.05, ** p<0.01 vs. vehicle
SM07883 reduced neurodegeneration-induced glial activation in 3xTg-AD mice

**GFAP in Hippocampal CA1 Region**

- Wild type + Vehicle
- Naive
- Vehicle
- SM07883

**Iba1++ Activated Microglial Cells in Hippocampal CA1 Region**

- Wild type + Vehicle
- Naive
- Vehicle
- SM07883

WT + Veh. n=9, 3xTg-AD: Naive n=8, Veh. n=9, and SM07883 n=11; Mean ± SEM; * p<0.05, *** p<0.001 vs. vehicle
SM07883 reduced neurodegeneration-induced proinflammatory mediators in 3xTg-AD mice

26 wks treatment; 5 mg/kg/day

WT + Veh. n=3, 3xTg-AD: Veh. n=6 and SM07883 n=7; Mean ± SEM; * p<0.05, ** p<0.01 vs. vehicle
SM07883 reduced acute inflammation

Acute intracranial LPS/IFN-\(\gamma\) model\(^1\)

Brain (5 hrs)
(Early microglial response)

Brain (24 hrs)
(Perivascular macrophages and microglia)

Normalized fold to change

\begin{align*}
\text{Normalized fold to change} & \quad \text{Vehicle} & \quad \text{SM07883} \\
\text{TNF-}\alpha & \quad 1.00 \pm 0.25 & \quad 0.50 \pm 0.10 \\
\text{IFN-}\gamma & \quad 1.25 \pm 0.15 & \quad 0.75 \pm 0.10 \\
\text{KC/GRO} & \quad 1.10 \pm 0.10 & \quad 0.80 \pm 0.05 \\
\text{IL-1}\beta & \quad 1.50 \pm 0.20 & \quad 2.00 \pm 0.30 \\
\text{IL-6} & \quad 2.00 \pm 0.25 & \quad 1.00 \pm 0.05
\end{align*}

n=3/treatment group; Mean ± SEM; \(* \ p<0.05, \ ** \ p<0.01\) vs. vehicle


24hrs; 10 mg/kg/day

Vehicle

SM07883

LPS/IFN-\(\gamma\)
SM07883 reduced chronic neuroinflammation

SM07883 and LPS (0.5 mg/kg, IP) for 5 consecutive days; 3 mg/kg/day or 10 mg/kg/day
WT n=2, Veh. n=15, SM07883 + LPS: 3 mg/kg n=15 and 10 mg/kg n=15; Mean ± SEM; ** p<0.01, *** p<0.001 vs. vehicle

Brain CD68 Staining

+ Vehicle

400 μm

+ SM07883

400 μm

Repeat, Chronic, Low-dose LPS IP Injections

Mild chronic brain inflammation

Repeat peripheral immune stimulation

TNF-α in the Brain (ELISA)

Wild type
Vehicle + LPS
SM07883 3 mg/kg + LPS
SM07883 10 mg/kg + LPS

0 5 10 15

TNF-α (pg/ml)
SM07883 reduced microglial cell activation \textit{in vitro}

**BV2 Microglial Cells + LPS**

![Graph showing TNF-\(\alpha\) levels](image)

EC\textsubscript{50} = 71 nM

![Flow cytometry graphs](image)

- **CD45.2**
  - Unstained
  - DMSO
  - SM07883 (0.370 µM, 24 hrs)

- **CD11b**
  - Unstained
  - DMSO
  - SM07883 (0.370 µM, 96 hrs)
SM07883 reduced STAT3 phosphorylation and translocation

**STAT3 Phosphorylation**

**THP-1 Monocytes + LPS (Western blot)**

<table>
<thead>
<tr>
<th>SM07883</th>
<th>1.1 µM</th>
<th>0.3 µM</th>
<th>0.1 µM</th>
<th>LPS only</th>
<th>NT</th>
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<tbody>
<tr>
<td>pSTAT3</td>
<td></td>
<td></td>
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<tr>
<td>totSTAT3</td>
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<tr>
<td>β-actin</td>
<td></td>
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</tbody>
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**Primary Mouse Astrocytes + OSM (Staining)**

0
2
4
6
8
pSTAT3 Y705 (Fold change)

Unstim. LPS OSM

**Mean ± SEM**

*** p<0.001 vs. vehicle

**Human Microglia (HMC-3) + OSM (Staining)**

0
2
4
6
pSTAT3 (Y705)
Nuclear/cytoplasmic ratio

Unstim. OSM

***

**Primary Mouse Astrocytes + OSM (Staining)**

0
2
4
6
pSTAT3 (Y705)
Nuclear/cytoplasmic ratio

Unstim. LPS OSM

***

**OSM: Oncostatin M**

Mean ± SEM

*** p<0.001 vs. vehicle

**STAT3 Translocation**

**Primary Mouse Astrocytes + OSM (Staining)**

0
2
4
6
pSTAT3 (Y705)
Nuclear/cytoplasmic ratio

Unstim. LPS OSM

***

**Primary Mouse Astrocytes + OSM (Staining)**

0
2
4
6
pSTAT3 (Y705)
Nuclear/cytoplasmic ratio

Unstim. LPS OSM

***
SM07883 prevented T cell proliferation and proinflammatory cytokines secretion

CD3/CD28 Stimulated Mouse Splenocytes for 5 Days +/- SM07883

SM07883 (nM)

DMSO | 4.5 | 14 | 41 | ~ EC50 122 | 367 | 1100

CFSE (carboxyfluorescein diacetate succinimidyl ester) staining analyzed by flow cytometry

Reduction at 5 days of: IL-17a: EC50 = 15 nM  IFN-γ: EC50 = 42 nM  TNF-α: EC50 = 46 nM  Meso Scale Discovery
MOG-induced EAE acute symptoms were reduced with SM07883

Vehicle

SM07883

Dosed full length of study; 3 mg/kg/day BID, 5 mg/kg/BID, and 5 mg/kg/day QD

Left: Naive n=2, EAE: Veh. n=15, SM07883 3 mg/kg BID n=15, SM07883 5 mg/kg BID n=15
Right: Naive n=2, EAE: Veh. n=12, SM07883 5 mg/kg BID n=10, SM07883 10 mg/kg QD n=12

Mean ± SEM; ** p<0.01, *** p<0.001 vs. vehicle

1Per Hooke Lab Mouse EAE Scoring Guide (hookelabs.com)
SM07883 reduced EAE-induced proinflammatory mediators in the spinal cord

CD3+ Immunoreactivity

TNF-α

IFN-γ

IL-1β

IL-6

IP-10/CXCL10

RANTES/CCL5

Mean ± SEM; * p<0.05, ** p<0.01, *** p<0.001 vs. vehicle

Also reduced IL-4, MIP-1α, MIP-1β, and GM-CSF compared to vehicle
Conclusion

• SM07883 ameliorated neuroinflammatory responses in preclinical models compared to vehicle
  – Reduced AD-associated neuroinflammation
  – Reduced acute and chronic neuroinflammation in absence of neurodegeneration
  – Not restricted to innate immunity with a potent effect on CNS-related adaptive immune responses

• Potential role of DYRK1A inhibition in both innate and adaptive immunity

• Immune mediators may be useful biomarkers for clinical trials in DYRK1A systemic intervention
Thank you