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
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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2. Sarlus H and Heneka MT. J Clin Invest. 2017
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\(^1\)
- Regulates phosphorylation of major AD molecular hallmarks such as tau\(^2\), APP (Aβ)\(^3\), and presenilin\(^4\)
- DYRK1A regulates inflammatory signals STAT3\(^7\), GFAP\(^7\), and NFAT\(^8\)

**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

SM07883 suppresses

SM07883 inhibits

DYRK1A

GSK3β

Studies ongoing

Inflammation

- Inflammatory pathways
- Proinflammatory mediators

STAT3: signal transducer and activator of transcription 3, NFAT: nuclear factor of activated T cells, APP: amyloid precursor protein
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)

WT + Veh. n=19, JNPL3: Veh. n=19 and SM07883 n=19; Mean ± SEM; * p<0.05 vs. vehicle
SM07883 reduced tau-induced glial activation in JNPL3 tau mice

GFAP in the Spinal Cord (ELISA)

<table>
<thead>
<tr>
<th></th>
<th>GFAP (pg/ml - ratio over WT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type+Vehicle</td>
<td>2.5 ± 0.5***</td>
</tr>
<tr>
<td>Vehicle</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>SM07883</td>
<td>1.0 ± 0.2</td>
</tr>
</tbody>
</table>

GFAP: WT + Veh. n=9, JNPL3: Veh. n=18 and SM07883 n=19; Iba1++: WT + Veh. n=11, JNPL3: Veh. n=32 and SM07883 n=19

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)

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

26 wks treatment; 5 mg/kg/day
SM07883 reduced neurodegeneration-induced glial activation in 3xTg-AD mice

GFAP in Hippocampal CA1 Region

Iba1++ Activated Microglial Cells in Hippocampal CA1 Region

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

**IL-6**

Wild type + Vehicle

Vehicle

SM07883

**IL-1β**

Wild type + Vehicle

Vehicle

SM07883

**IL-15**

Wild type + Vehicle

Vehicle

SM07883

**MCP-1/CCL2**

Wild type + Vehicle

Vehicle

SM07883

**RANTES/CCL5**

Wild type + Vehicle

Vehicle

SM07883

**MIP-1α/CCL3**

Wild type + Vehicle

Vehicle

SM07883

**MIP-2/CXCL2**

Wild type + Vehicle

Vehicle

SM07883

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-γ model

Brain (5 hrs)
(Early microglial response)

Brain (24 hrs)
(Perivascular macrophages and microglia)

Normalized fold to change

Vehicle  SM07883


24hrs; 10 mg/kg/day

n=3/treatment group; Mean ± SEM; * p<0.05, ** p<0.01 vs. vehicle
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
SM07883 reduced microglial cell activation \textit{in vitro}

\textbf{BV2 Microglial Cells + LPS}

\textbf{TNF-\alpha}

EC$_{50} = 71$ nM

\begin{itemize}
  \item \textbf{CD45.2}
    \begin{itemize}
      \item Unstained
      \item DMSO
      \item SM07883 (0.370 $\mu$M, 24 hrs)
    \end{itemize}
  \item \textbf{CD11b}
    \begin{itemize}
      \item Unstained
      \item DMSO
      \item SM07883 (0.370 $\mu$M, 96 hrs)
    \end{itemize}
\end{itemize}
SM07883 reduced STAT3 phosphorylation and translocation

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

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

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

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

**STAT3 Phosphorylation**

**STAT3 Translocation**

**OSM: Oncostatin M**
Mean ± SEM
*** p<0.001 vs. vehicle
SM07883 prevented T cell proliferation and proinflammatory cytokines secretion

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

SM07883 (nM)

DMSO  4.5  14  41 122 367 1100

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

Reduction at 5 days of:  
IL-17α: 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

Clinical Score

Clinical Score

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

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

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

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