TAU PATHOLOGY REDUCTION WITH SM07883, A NOVEL, POTENT, AND SELECTIVE ORAL DYRK1A INHIBITOR - A POTENTIAL THERAPEUTIC FOR ALZHEIMER’S DISEASE


AAT AD/PD Focus Meeting
Turin March 15th, 2018
<table>
<thead>
<tr>
<th>Company Name</th>
<th>Honoraria/Expenses</th>
<th>Consulting/Advisory Board</th>
<th>Funded Research</th>
<th>Royalties/Patent</th>
<th>Stock Options</th>
<th>Ownership/Equity Position</th>
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<td>Samummed, LLC</td>
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Disclaimer

• 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 US 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 non-clinical data and the relationship to clinical benefit is unknown.

• This presentation is intended as a scientific exchange of medical information, is provided for educational purposes only, and is not intended for any promotional purpose or to offer medical advice; the information contained in this presentation is confidential and proprietary and is not available for further distribution in any form whatsoever.
AD pathophysiology and potential therapeutic targets

- Tau phosphorylation
- Tau aggregation
- Neurofibrillary Tangles
- Inflammation
- Synaptic impairment & Neurodegeneration
- Alzheimer's Disease
- Memory Loss
- Agitation
- Dementia
- Amyloid Precursor Protein
- Aβ fragments
- Amyloid Plaques

References:
- Selkoe DJ. Nat Cell Biol. 2004
- Heneka MT et al. Lancet Neurol. 2015
- Crews L and Masliah E. Hum Mol Genet. 2010
Tau hyperphosphorylation and pathology

- Tau hyperphosphorylation causes oligomerization and aggregation leading to neurofibrillary tangles (NFTs)\(^1\)
- Tau oligomers are believed to spread across the synapse to unaffected neurons\(^2\)
- SM07883 blocked hyperphosphorylation in rodents and inhibited downstream effects leading to NFTs and neuronal degeneration


NFT illustration adapted from Alzheimer’s Disease Research, a program of the BrightFocus Foundation.
Dual specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A) contributes to AD pathology

**DYRK1A:**

- **Elevated expression in AD brains** and in Down Syndrome patients (on chromosome 21)\(^1,2\)
- **Regulates phosphorylation of Tau,\(^1,2\) APP,\(^3\) Presenilin\(^4\)
- **Regulates inflammation** (suppresses Th17\(^5\), triggers T-regulatory cell differentiation\(^5\), regulates STAT3 pathway\(^6\))
- **Primes and regulates GSK3β\(^7\)

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7. Song, WJ. et al. J. Biol. Chem. 2015

Image adapted from Hume, C. http://www.pharmacy.arizona.edu/directory/christopher-hume-phd accessed 12/18/17
SM07883 Drug Discovery
SM07883, a potent DYRK1A kinase inhibitor with a unique selectivity profile

In kinase inhibition screen assays of 414 kinases, SM07883 showed relatively specific and potent inhibition of DYRK1A – 6 kinases within the 15-fold range of DYRK1A IC$_{50}$

IC$_{50}$ = 1.6 nM
SM07883 inhibited Tau phosphorylation \textit{in vitro}.

DYRK1A induced Tau hyperphosphorylation in Tau/DYRK1A HEK293T cells.

Tau phosphorylation in a human neuroblastoma cell line (SH-SY5Y).

SM07883 potently inhibited DYKR1A mediated Tau hyperphosphorylation in two human cell lines.
SM07883 is bioavailable, brain penetrant and has sustained therapeutic exposure in animals

- **Good bioavailability** across species 35% – 100%
- **High permeability** and **limited efflux** (Caco-2 Efflux ratio: 0.283)
- **Brain / Plasma ratio:** >3 in mice ($F_{ub}/F_{up}$=0.64); ~30% plasma free fraction across species; ~6% brain free fraction (rodent)
- **High correlation of PK between brain, CSF and plasma:** half life was consistent between plasma and brain
- **Allometric projection:** >11hrs half life in human and potentially amenable to once a day dosing in human
- **Plasma levels may be a surrogate for CNS exposure**
SM07883 Additional drug properties

- MW < 400 kDa, solubility >50 mg/mL
- No brain or tissue accumulation over 3 months daily testing in mice
- Low intrinsic clearance; metabolic stability >960 min in human hepatocytes
- Low CYP inhibition (IC$_{50}$ >20 µM with CYP2C19 = 6.8 µM)
- Limited transporter inhibition (OCT2, MATE 1/2) with low potential for drug-drug interactions
- Limited off-target binding in panel with channels and receptors IC$_{50}$ >15 µM
  - hERG channel inhibition IC$_{50}$ of 0.6 µM
Animal toxicology and safety findings

• No cardiac abnormalities (e.g. QT prolongation, arrhythmia) were detected up to 50 mg/kg in monkey

• *In vitro* and *in vivo* studies demonstrated that SM07883 had low potential for genotoxicity in human

• ‘No Observed Adverse Effect Level’ was 30x higher in AUC than the minimum efficacious dose (1.25 mg/kg/day) in mice
  – 8x total exposure in monkeys (GI intolerance was the dose limiting factor, reversible)

• Suggested a broad therapeutic window for human dosing
Preclinical Tau efficacy studies
SM07883 reduced Tau hyperphosphorylation in the mouse brain

- **Single oral dose** of SM07883 in wild type mice, followed (3 hr) by anesthesia-induced transient Tau hyperphosphorylation\(^1\) with brain collection at 4h and western blot for pTau

- SM07883 produced a **dose dependent inhibition** of pTau

- Significant reduction of pTau after a single dose as low as **1.25 mg/kg** compared to vehicle

- SM07883 follows “**free drug principle**”

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**Induced Tau hyperphosphorylation**

<table>
<thead>
<tr>
<th>SM07883 Concentration</th>
<th>60</th>
<th>145</th>
<th>256</th>
<th>360</th>
<th>1283</th>
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<tbody>
<tr>
<td>Plasma (ng/ml)</td>
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<td>Brain (ng/ml)</td>
<td>92</td>
<td>226</td>
<td>451</td>
<td>655</td>
<td>2353</td>
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*** p<0.001 compared with vehicle

1. Bretteville et al., *Scientific reports*, 2012
Tau transgenic mouse model for preclinical efficacy

- JNPL3 mice carry a mutated form of **human Tau** from autosomal dominant Tau FTD patients
- In this model, Tau spreads from the **brain stem and spinal cord**
  - Decreased motor coordination, no cognitive deficit
- The P301L mutation in JNPL3 mice results in **Tau hyperphosphorylation at sites similar to AD brains** (Thr181, Ser202/Thr205 [AT8 epitope], Thr212, Thr231, Ser396)
- JNPL3 mice (10 months old) treated with QD SM07883 for 14 weeks and evaluated for:
  - Tau hyperphosphorylation
  - Formation of Tau oligomers, aggregation, and NFTs
  - Neuroinflammation
  - Health and motor deficits

Pathological Tau staining in the brain stem and spinal cord of JNPL3 mice (AT8 antibody, brown)

SM07883 reduced Tau hyperphosphorylation in JNPL3 mice

SM07883 (3 mg/kg or 10 mg/kg SM07883 QD) reduced Tau hyperphosphorylation in the brainstem at the AD pathogenic AT8 epitope (Ser202/Thr205) compared to vehicle

* p<0.05 compared to vehicle
SM07883 prevented formation of insoluble Tau in JNPL3 mice

**AT8 in the sarkosyl-insoluble fraction** *(Western Blot)*

<table>
<thead>
<tr>
<th></th>
<th>Wild type (n=9)</th>
<th>Vehicle (n=20)</th>
<th>SM07883 (3 mg/kg qd) (n=19)</th>
<th>3 mg/kg</th>
<th></th>
<th>Wild type (n=10)</th>
<th>Vehicle (n=15)</th>
<th>SM07883 (10 mg/kg qd) (n=13)</th>
<th>10 mg/kg</th>
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<tr>
<td>Log AT8 staining intensity (A.U.)</td>
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SM07883 (3 mg/kg or 10 mg/kg SM07883 QD) inhibited insoluble Tau formation in the brainstem at the AD pathogenic AT8 epitope (Ser202/Thr205) compared to vehicle (sarkosyl insoluble fraction)

† Only positive values plotted (eight 0 or negative values not shown: 3 WT, 2 Veh, 3 SM07883) * p<0.05, ** p<0.01 compared to vehicle
SM07883 prevented Tau aggregation in JNPL3 mice

Tau aggregates in the spinal cord (HTRF assay)

SM07883 (3 mg/kg or 10 mg/kg SM07883 QD) inhibited Tau aggregation in the spinal cord compared to vehicle in a FRET (HTRF) based assay.

HTRF: Homogeneous Time Resolved Fluorescence
* p<0.05, ** p<0.01 compared to vehicle
SM07883 reduced the formation of NFTs in JNPL3 mice

SM07883 (3 mg/kg QD shown) significantly reduced the formation of brainstem NFTs compared to vehicle

*p<0.05 compared to vehicle*
SM07883 reduced Tau-induced glial activation (neuroinflammation) in JNPL3 mice

SM07883 significantly **reduced GFAP** (astrocytes) and **Iba1** (activated microglia) **expression** compared to vehicle in the brainstems of JNPL3 mice (representative images shown)
SM07883 reduced functional deficits in Tau JNPL3 mice

Motor Coordination
(Wire Hang Test Performance)

SM07883 improved motor coordination in JNPL3 mice compared to Vehicle

* P = 0.011 compared to vehicle
SM07883 improved weight & reduced morbidity / mortality of JNPL3 mice

- JNPL3 mice have low body weight with incidence of morbidities and mortality
- SM07883 treatment **significantly improved body weight** and **empirically improved morbidity / mortality** compared to vehicle

<table>
<thead>
<tr>
<th>Morbidity / Mortality</th>
<th>Vehicle</th>
<th>SM07883</th>
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<tr>
<td>Death</td>
<td>2/20</td>
<td>0/19</td>
</tr>
<tr>
<td>Pronounced hunched back</td>
<td>1/18</td>
<td>0/19</td>
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<tr>
<td>Severe tremors</td>
<td>3/18</td>
<td>0/19</td>
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<td>Moderate tremors</td>
<td>6/18</td>
<td>0/19</td>
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<tr>
<td>Mild tremors</td>
<td>0/18</td>
<td>2/19</td>
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WT n=9, Vehicle n=20, SM07883 n=19; *** p<0.001 vs. JNPL3 + Vehicle
Summary

• SM07883 is a potent DYRK1A inhibitor with a unique selectivity profile that reduces Tau hyperphosphorylation in mice

• In Tau transgenic mice daily SM07883 vs. vehicle control reduced:
  – Tau hyperphosphorylation
  – Formation of Tau oligomers and aggregation
  – Formation of NFTs
  – Glial activation

• SM07883 demonstrated sustained brain and CSF exposures and was stable in all species

• 28 day repeated dose studies in animals demonstrated acceptable tolerability and therapeutic margin

  ➢ SM07883 may provide therapeutic, disease modifying effects in AD

  ➢ IND-enabling studies are completed and a Phase 1 trial in healthy volunteers is planned
Thank you