Increasing Protein O-GlcNAcylation as a Disease Modification Strategy for Tauopathies

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Tauopathies

- A class of neurodegenerative diseases characterized by the deposition of abnormal tau proteins in the human brain

Alzheimer’s disease (AD)

- Aggregation of hyperphosphorylated tau into neurofibrillary tangles is a pathological hallmark of AD
- The degree of tau pathophysiology is correlated with disease severity

Progressive Supranuclear Palsy (PSP)

- PSP is an aggressive neurodegenerative disease for which there are no treatments
- The pathology of PSP is predominately associated with tau aggregation
- Clinical syndrome: gait instability and supranuclear gaze palsy
• O-GlcNAcylation is a common post-translational modification that plays a role in regulating protein stability
  – Destabilization of proteins has been implicated in the etiology of several neurodegenerative disorders including AD, ALS, HD, and PD

• O-GlcNAcylation is regulated by two enzymes
  – O-GlcNAc transferase (OGT)
  – O-GlcNAcase (OGA)

• OGA is highly expressed in the brain and unlike other mechanisms targeting tau is highly amendable to small molecule drug discovery

• Postmortem analysis of brain tissue reveals that protein O-GlcNAcylation is reduced in AD consistent with impaired glucose metabolism in AD brain

• Inhibition of OGA leads to an up-regulation of O-GlcNAc modified proteins both in vitro and in vivo
• In adult brain, tau is O-GlcNAcylated at multiple sites
• Preclinical findings demonstrate that selective OGA inhibitors increase O-GlcNAcylation of tau, reduce the formation of aggregated tau and attenuate neurodegeneration in multiple transgenic models

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Understanding the OGAi MOA:
• Hyperphosphorylated tau (P) aggregates; contains several translational modifications such as acetylation (A), nitration (N) and ubiquitination (U)
• OGA inhibition increases O-GLcNAcylation (G) of soluble tau and prevents pathological tau formation

Directly measure tau modification in human samples
OGA Inhibitors reduced pathological tau conformation in iPSC-derived hu-neurons

- Seeding of tau oligomers induced intracellular tau aggregation
  
- Effect of OGAi, Thiamet G at 3 days post-seeding of tau oligomers
  
iPSC-derived hu-neurons (DIV 7) were pre-treated with Thiamet-G 24h before seeding

Quantification using High Content Image Analysis

* P<0.05 vs. Veh + Tau seeds ANOVA and Dunett's multiple comparison test
The Tg4510 model: A Transgenic Mouse Model of Brain Tauopathy

- Overexpressing human tau with P301L mutation that is linked to FTDP-17
- Mutant tau expression is driven by CaMKII promoter and is regulatable by doxycycline

Brain Atrophy detected in forebrain structures correlates with mutant tau expression

Hyperphosphorylated tau in Tg4510 brain (bottom) and Neurofibrillary tangles (top)

Mutant tau expression

<table>
<thead>
<tr>
<th>Tau Pathology</th>
<th>Brain Atrophy</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhF8 AlphaSA (RLU)</td>
<td>Mutant tau expression</td>
</tr>
<tr>
<td>2 mo</td>
<td>n=26</td>
</tr>
<tr>
<td>3 mo</td>
<td>n=26</td>
</tr>
<tr>
<td>4 mo</td>
<td>n=26</td>
</tr>
<tr>
<td>5 mo</td>
<td>n=27</td>
</tr>
<tr>
<td>6 mo</td>
<td>n=23</td>
</tr>
<tr>
<td>7 mo</td>
<td>n=28</td>
</tr>
</tbody>
</table>

nY29 (NFT)

4-5 mo

TT

WW

10 mo
Efficacy of OGA Inhibitors in Tg4510 Mice

- Tg4510 mice were treated (n=25/group) from age 8 week to 16 week with L-184 (30 and 500 mpk, QD, PO)
- L-184 (500 mpk) significantly increased brain o-protein levels, reduced brain PHF6 tau and CSF total tau, prevented brain atrophy and ameliorated NFT pathology
OGA Inhibitors are Efficacious in Tg4510 Mice with Existing Tau Pathology

**Age:**
- 8 wks
- 12 wks
- 16 wks
- 20 wks
- 24 wks

- **Veh**
- **L-184**
  - 16 wks treatment
  - 12 wks treatment
  - 8 wks treatment
  - 4 wks Tx

- **n = 20/group**

**PHF6 AlphaLisa**
- Veh
- L-184 (500mpk)
- 8-24 wks
- 12-24 wks
- 16-24 wks
- 20-24 wks

- 19%
- 20%
- 17%
- 13%

**Total Brain Weight**
- Veh
- L-184 (500mpk)
- 8-24 wks
- 12-24 wks
- 16-24 wks
- 20-24 wks

- ***
- **
- ***

**Brain O-protein (RL2)**
- Veh
- L-184 (500mpk)
- 8-24 wks
- 12-24 wks
- 16-24 wks
- 20-24 wks

- 30%
- 15%
- 19%
- 10%

**NFT Histology**
- Veh
- L-184 (500mpk)
- 8-24 wks
- 12-24 wks
- 16-24 wks
- 20-24 wks

- 30%
- **

**Total Brain Weight**
- Veh
- L-184 (500mpk)
- 8-24 wks
- 12-24 wks
- 16-24 wks
- 20-24 wks

- 175
- 150
- 125
- 100

**NFTs/section, entorhinal cortex**
- Veh
- L-184 (500mpk)
- 8-24 wks
- 12-24 wks
- 16-24 wks
- 20-24 wks

- 30%
- **
**Inflammatory Gene Expression is Reduced in Tg4510 Mice Treated with OGA Inhibitor**

**Inflammatory Marker mRNA**

<table>
<thead>
<tr>
<th>Inflammatory Marker mRNA</th>
<th>Fold Change (+ upper limit CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFAP baseline</td>
<td>#</td>
</tr>
<tr>
<td>GFAP vehicle</td>
<td>***</td>
</tr>
<tr>
<td>GFAP L-184</td>
<td>#</td>
</tr>
<tr>
<td>AIF1 baseline</td>
<td>#</td>
</tr>
<tr>
<td>AIF1 vehicle</td>
<td>#</td>
</tr>
<tr>
<td>AIF1 L-184</td>
<td>#</td>
</tr>
<tr>
<td>CD33 baseline</td>
<td>#</td>
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<tr>
<td>CD33 vehicle</td>
<td>#</td>
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<tr>
<td>CD33 L-184</td>
<td>#</td>
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<tr>
<td>CD68 baseline</td>
<td>#</td>
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<tr>
<td>CD68 vehicle</td>
<td>#</td>
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<tr>
<td>CD68 L-184</td>
<td>#</td>
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<tr>
<td>TREM2 baseline</td>
<td>**</td>
</tr>
<tr>
<td>TREM2 vehicle</td>
<td>#</td>
</tr>
<tr>
<td>TREM2 L-184</td>
<td>#</td>
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</tbody>
</table>

# - significant elevation above baseline  
** - significant reduction from vehicle  
n=10/group

GFAP (astrocytosis) and TREM2 (phagocytic microglia) are significantly reduced in 16wk old Tg4510 mice treated with L-184 (500mpk) by single gene qPCR

**Tg4510 RNAseq**

Induction (red) of human brain inflammatory geneset is ameliorated in Tg4510 mice treated with OGA inhibitor

Full transcriptome effects of OGAi in Tg4510 are under analysis.
OGA Inhibitor Efficacy has Been Reported in Additional Transgenic Mouse Models

<table>
<thead>
<tr>
<th>Mouse Model</th>
<th>Promoter</th>
<th>Transgene</th>
<th>OGAi Reduces</th>
<th>Tangle / Plaque Pathology</th>
<th>Neuro-degeneration</th>
<th>Behavior deficit</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Tg4510</td>
<td>Tet / CamKII</td>
<td>Tau P301L</td>
<td>Yes (tau tangle)</td>
<td>Yes (CSF t-tau)</td>
<td>Yes</td>
<td>Yes</td>
<td>Internal Data</td>
</tr>
<tr>
<td>JNPL3</td>
<td>Prion</td>
<td>Tau P301L</td>
<td>Yes (tau tangle)</td>
<td>Yes (motor neuron)</td>
<td>No, trend</td>
<td></td>
<td>Yuzwa et al. 2012, Nature Chemical Biology Vol. 8, 393–399</td>
</tr>
<tr>
<td>Tau.P-301L</td>
<td>Thy-1</td>
<td>Tau P301L</td>
<td>Not determined</td>
<td>Yes (survival)</td>
<td>Yes</td>
<td></td>
<td>Borghgraef et al., 2013, PLOS One Vol. 8(12) e84442</td>
</tr>
<tr>
<td>5XFAD</td>
<td>Thy-1</td>
<td>APPSw iLon PSEN* 146L*L 86V</td>
<td>Yes, amyloid plaque</td>
<td>Not determined</td>
<td>Yes</td>
<td></td>
<td>Kim et al. 2013, Neurobiology of Aging Vol. 34 (1), 275 - 285</td>
</tr>
</tbody>
</table>
• Inhibition of O-GlcNAcase (OGA) enzyme reduced pathological tau aggregation in iPSC-derived human neurons
• Oral administration of OGA inhibitor produced a sustained elevation in O-Protein levels in the brain
• OGA inhibition significantly reduced pathological tau, brain inflammation, and brain atrophy in Tg4510 transgenic mice
• Increasing protein O-GlcNAcylation is a promising disease modification strategy for tauopathies
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