NOVEL THERAPIES

SMALL MOLECULES TARGETING TAU PROPAGATION DEMONSTRATE EFFICACY IN AN AGGRESSIVE TAUOPATHY MOUSE MODEL
Disclaimer

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Disclosures

Francesca Capotosti is an AC Immune’s employee
No off-label nor investigational use of therapeutic products will be presented
Misfolded proteins are generally recognized as leading causes of neurodegenerative diseases

Introduction

Soluble oligomers

Insoluble fibrils

Normally folded proteins

Misfolded proteins


Drugs targets

Abeta plaques

Tau tangles

α-synuclein
Lewy bodies

Huntingtin inclusion bodies
The Morphormer™ platform
Generation of conformation specific small molecules

- Rational chemical design for small molecules that target CNS diseases
- Protein propagation inhibitors (Kroth et al., 2012)
- Robust library of around 3000 compounds with desirable properties including brain penetration
- Around 1000 compounds screened so far for the Tau SME program

Non-dye compounds with multiple amyloid interaction sites

Hochdörffer et al., 2011
In vitro pharmacology

Biophysical assays

Disaggregation of β-sheet Tau multimers

ThT assay measures effects on pre-formed β-sheet rich full-length Tau multimers

<table>
<thead>
<tr>
<th>Cmp #</th>
<th>EC50</th>
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<tbody>
<tr>
<td>1</td>
<td>9.7 uM</td>
</tr>
<tr>
<td>2</td>
<td>5.5 uM</td>
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Size reduction of Tau aggregates

Dynamic Light Scattering (DLS) assay measures aggregate size of full-length Tau multimers

Hit compounds selected show Tau disaggregation properties and the ability to reduce the size of Tau aggregates
In vitro pharmacology
Biophysical assays

Reduction of Tau aggregation and misfolding

AlphaLISA assay measures effects on pre-formed β-sheet rich and misfolded full-length Tau multimers

Compound 1

Compound 2

<table>
<thead>
<tr>
<th>Cmp #</th>
<th>Reduction of Tau aggregation EC50</th>
<th>Reduction of Tau misfolding EC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>180 nM</td>
<td>170 nM</td>
</tr>
<tr>
<td>2</td>
<td>151 nM</td>
<td>157 nM</td>
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</table>

Two compounds selected as hits decrease Tau aggregation and misfolding with nM potencies
In vitro pharmacology

Cell-based assays – compounds used in the micromolar range

**Rescue of Tau-PHF induced cytoxicity**

MTT assay measures effects on cytoxicity induced by human AD-brain enriched Tau PHF in SH5Y-SY Tau P301L cells

Two compounds selected as hits show the capability to rescue Tau PHF induced toxicity in TauP301L overexpressing cells
In vitro pharmacology
Cell-based assays – Compounds used in the nanomolar range

Reduction of intracellular Tau misfolding in vitro-differentiated SH5Y-SY TauP301L cells

Immuno-cytochemistry assay measures effects on spontaneous Tau misfolding in retinoic acid differentiated SH5Y-SY Tau P301L cells

In vitro differentiated SH5Y-SH TauP301L cells, that acquire neuronal morphology with high level of misfolded Tau expression, treatment with Compound 2 led to a dose-dependent decrease of misfolded Tau in low nM range
In vivo efficacy study – Study 1
Assessment of compound efficacy in an aggressive Tauopathy model

Compounds 1 and 2 were tested for their ability to reduce Tau pathology in rTg4510 mice

The rTg4510 tauopathy model expresses repressible human 0N4R Tau carrying the P301L mutation and displays a rapid onset and progression of NFT pathology, cortical atrophy, and behavior impairments (SantaCruz, 2005)

rTg4510

The Jackson Laboratory

Age 1.5 months

Daily p.o. for 12.5 weeks

- Compound 1 at 7 or 20 mg/kg
- Compound 2 at 20 mg/kg

Age 4.5 months

Water-Maze

Euthanasia

and tissue harvest

Ref.: AC Immune unpublished data
In vivo efficacy study – Study 1
Assessment of compound efficacy in an aggressive Tauopathy model

Behavioral test: Morris Water Maze memory test

Treatment with Compound 1 resulted in a dose-dependent effect. Treatment with Compound 2 exhibited even better potency in spatial learning and memory recall.
**In vivo efficacy study – Study 1**
Assessment of compound efficacy in an aggressive Tauopathy model

**Histology: Analysis of brain atrophy and misfolded Tau**

**Cortical atrophy**

- Treatment with Compound 1 resulted in a tendency for dose-dependent rescue of brain atrophy. Treatment with Compound 2 exhibited a significant effect on brain atrophy rescue as well as reduction of misfolded Tau.

**Misfolded Tau (MC1)**

- Ref: Adolfsson et al., SFN 2015

Mean ± SEM 1-way ANOVA
• *p<0.05
**In vivo efficacy study – Study 1**

Assessment of compound efficacy in an aggressive Tauopathy model – Study 1

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**Biochemistry: Analysis of total and aggregated Tau**

- **Total Tau**
  - WT
  - tTA
  - V
  - Cmp1 at 7 mg/kg
  - Cmp1 at 20 mg/kg
  - Cmp2 at 20 mg/kg
  - rTg4510

- **Aggregated Tau**
  - WT
  - tTA
  - V
  - Cmp1 at 7 mg/kg
  - Cmp1 at 20 mg/kg
  - Cmp2 at 20 mg/kg
  - rTg4510

**Ref.:** Adolfsson et al., SFN 2015

**Mean ± SEM**

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Tau aggregates were not affected after treatment with Compound 1 and only slightly reduced after treatment with Compound 2.
In vivo efficacy study – Study 2
Assessment of compound efficacy in an aggressive Tauopathy model

Compound 2 was tested for its ability to reduce Tau pathology in rTg4510 mice in a Tau ON/OFF paradigm

The rTg4510 tauopathy model expresses repressible human 0N4R Tau carrying the P301L mutation and displays a rapid onset and progression of NFT pathology, cortical atrophy, and behavior impairments (SantaCruz, 2005)

The Jackson Laboratory

rTg4510

Age 1.5 months

rTg4510

Age 4.5 months

Euthanasia and tissue harvest

Doxycycline 3 weeks

Daily p.o. for 15.5 weeks:
Compound 2 at 7 and 20 mg/kg
**In vivo efficacy study – Study 2**

Assessment of compound efficacy in an aggressive Tauopathy model

Biochemistry: Analysis of total, aggregated, and misfolded Tau in Tau ON/OFF rTg4510 mice

**Total Tau**

**Aggregated Tau**

**Misfolded Tau**

Aggregated Tau was dose-dependently reduced after treatment with Compound 2
In vivo efficacy study – Study 2
Assessment of compound efficacy in an aggressive Tauopathy model

Biochemistry: Analysis of sarkosyl soluble and insoluble Tau in Tau ON/OFF rTg4510 mice

Sarkosyl-soluble but not insoluble Tau was significantly reduced with the high dose of Compound 2
In vivo efficacy study – Study 2
Assessment of compound efficacy in an aggressive Tauopathy model

Histology: Analysis of misfolded Tau in Tau ON/OFF rTg4510 mice

Representative pictures from frontal cortex

Vehicle

Compound 2 at 20 mg/kg

Mean ± SEM
1-way ANOVA
* p<0.05

Misfolded Tau was dose-dependently reduced after treatment with Compound 2

Misfolded Tau

50 μm
**In vivo efficacy study – Study 2**
Assessment of compound efficacy in an aggressive Tauopathy model

**Histology: Analysis of Congo Red positive Tau NFTs in Tau ON/OFF rTg4510 mice**

Representative pictures from frontal cortex

Congo Red positive NFTs were dose-dependently reduced after treatment with Compound 2

Mean ± SEM
1-way ANOVA
* p<0.05

Ref.: AC Immune unpublished data
Conclusions

Summary

- Potent Morphomer™ CNS compounds were identified by screening for interaction with β-sheet rich and/or misfolded Tau multimers in biophysical and cell-based assays.

- The rTg4510 mouse model was identified as a robust, although aggressive, Tauopathy model and was selected to test the in vivo efficacy of Morphomers™ using both the Tau ON and the Tau ON/OFF paradigms.

- Compounds selected for in vivo efficacy study had dose-dependent effects on memory performance and showed efficacy as disease modifying agents in multiple read-outs.

Rationally designed small molecules targeting misfolded and aggregated Tau are a promising strategy to reduce Tau pathology.