Sustained Tau Reduction via Zinc Finger Protein Transcription Factors as a Potential Next-Generation Therapy for Alzheimer’s Disease and Other Tauopathies

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ZFP-TFs are a potent, specific, and efficacious approach to lowering tau

1. Lowering tau is a **validated therapeutic approach** for AD and other tauopathies

2. ZFP-TFs are a **potent, single-gene specific** solution to lowering tau

3. ZFP-TFs are **efficacious in an AD model** and well-tolerated **for at least 11 months** in the brain

4. IV delivery of a ZFP-TF results in **global CNS tau reduction** of up to 70%
Clinical symptoms and neuronal loss closely track with tau but not amyloid

From: Xia C … Dickerson BC. Association of In Vivo [18F]AV-1451 Tau PET Imaging Results With Cortical Atrophy and Symptoms in Typical and Atypical Alzheimer Disease. JAMA Neurol. February 20, 2017

Aggregates of tau, but not amyloid, are found in many other “tauopathy” diseases
Neuronal loss in tauopathies can be blocked by reducing tau expression.

Healthy neuron
Axonal tau

Diseased neuron
Pathological, mislocalized tau
Aggregates and tangles

Dying neuron
Consumed by NFTs
Atrophied

Multiple tau species
Pathogenic form(s) unclear

Preventative
tau KO or tau Het (mice)
H2 haplotype (human)

Reversal
Turn off mutant tau (mice)
ASOs (mice)

Amyloid beta
Alzheimer’s Disease

Mutations in tau
Frontal Temporal Dementia
Progressive Supranuclear Palsy
Corticobasal Degeneration

Brain Injury
Chronic Traumatic Encephalopathy

Phospho-tau
Oligomer
NFT

Tau reduction has been shown to be safe and neuroprotective.
Differentiated platform for tau targeting: ZFP Transcription Factors

ZFP-TFs block all forms of intracellular tau with a single administration
ZFP-Transcription Factors can be engineered to regulate any gene

ZFP-TFs are derived from human proteins and do not edit or cut the DNA
Therapy: Single AAV-ZFP-TF administration that blocks all tau at the DNA level

- **AAV vector encoding a ZFP-TF**
- **Three delivery options:** ITC, IV, or IC
- **AAV traffics to neurons, ZFP-TF is expressed & represses tau**
- **ZFP TF blocks production of all tau forms at the DNA level**

**Option 1** Intrathecal (ITC)

**Option 2** Intravenous (IV)

**Option 3** Intracranial (IC)

ZFP TF repressor is expressed within neurons, but remains separate from neuron’s DNA

- ZFP TF

+ ZFP TF
Robust screening capabilities for rapid lead identification

Activity
1000-3 ng ZFP mRNA
qRT-PCR @ 24 hrs

Specificity
300 ng ZFP mRNA
Genechip @ 24 hrs
• tauG

~30% of tau ZFP-TFs from the initial screen reduced tau by ≥ 50%
Potent, single-gene specific tau repression in primary neurons

~100-fold reduction in tau levels with no detectable off-targets

Tau is the only gene repressed by >2 fold out of 26,491 coding transcripts assayed
ZFP-TFs stably repress endogenous tau for 11 months in the mouse brain.

AAV9 hippocampal coverage using dual stereotaxic injections

Sustained >75% tau reduction with no neuronal loss and minimal glial elevation

N=3-4. Mean +/- SD. * P <0.05; **** P < 0.0001. PBS from 6 wk shown. Normalized to mean of ATP5b, EIF4a2 & GAPDH.
Strong tau reduction \textit{in vivo} driven by neuronal promoters

Wild-type 3 months old

- AAV9 hSYN1-ZFP
- OR
- AAV9 CamKIIα-ZFP
- OR
- AAV9 MeCP2-ZFP

qRT-PCR tau ELISA

8 weeks

Neuronal specific expression confirmed by histology

N=3-6 hemispheres. Mean +/- SD. **** P < 0.0001. Normalized to mean of ATP5b, EIF4a2 & GAPDH.
Can a tau ZFP protect mice against amyloid neurotoxicity found in AD?

- Dystrophic neurites are a pathological hallmark of AD
- Hyperphosphorylated tau surrounds plaques in AD and APP/PS1
- Lowering tau in vitro protects cultured neurons against amyloid
- No tau lowering agent has previously translated this result in vivo

APP/PS1 vs. WT

Plaque
Dystrophic Neurites
APP/PS1 Mouse

AD Human

ZFP T2A Venus
RFP

+ ZFP-TF
- ZFP-TF

4.5 months
10 weeks
7 months
ZFP-TFs reduce neuritic dystrophy in AD mice with established disease

Quantitation: Dystrophies were counted for 121-256 plaques per mouse. Shown are average number of dystrophies per plaque per by mouse (n=4); mean +/- SEM, Two-tailed paired T-test.

First *in vivo* demonstration of a tau lowering agent reducing neuritic dystrophy
Broad CNS coverage may provide greater therapeutic benefit for tauopathies and other neurodegenerative disorders
Global CNS tau reduction following a single IV administration

Wild-type
8 wk old
IV delivery

+ or

AAV9
ZFP-TF

AAV-Sangamo
ZFP-TF

After 4 wks, micro-dissect brain regions, qRT-PCR

~40-70% tau reduction throughout the CNS after one ZFP-TF treatment

N=3-4. Mean +/- SD. * P <0.05; **** P < 0.0001. Normalized to mean of ATP5b, Eif4a2 & GAPDH.

Motor Cortex

Cortex (other)

Cervical SC

Hippocampus

Midbrain

Cerebellum

Thalamus

Hypothalamus

Striatum
ZFP-TFs are a potent, specific, and efficacious approach to lowering tau

**Activity**  
Potent, single-gene specific tau reduction in primary neurons

**Tolerability**  
Sustained tau lowering for 11 mo. in the brain; minimal inflammatory marker elevation

**Efficacy**  
First demonstration of a tau lowering agent reducing neuritic dystrophies

**Delivery**  
AAV-ZFPs can drive global CNS tau reduction via IV delivery

These results support the further development of a tau-targeted ZFP-TF for the treatment of AD and tauopathies
<table>
<thead>
<tr>
<th>Research</th>
<th>Technology</th>
<th>AAV Team</th>
<th>Mass General</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bryan Zeitler*</td>
<td>Sarah Hinkley</td>
<td>Richard Surosky</td>
<td>Sarah DeVos*</td>
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<tr>
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<td>Irina Ankoudinova</td>
<td>Alicia Goodwin</td>
<td>Suzanne Wegmann*</td>
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<td>Qi Yu</td>
<td>Lei Zhang</td>
<td>Hung Tran</td>
<td>Danny Mackenzie</td>
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<td>Jeff Miller</td>
<td>Tim Gabriele</td>
<td>Caitlin Commins</td>
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<td>Rainier Amora</td>
<td>Ed Rebar</td>
<td>Andrea Kang</td>
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We are committed to translating ground-breaking science into genomic therapies that transform patients’ lives