Inhibition of Fyn Kinase for Disease-Modifying Therapy of Alzheimer’s Disease

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Disclosure: S.M.S. is a co-founder of Axerion Therapeutics (NgR & PrP$^C$).
Fyn Inhibition by AZD0530 for Alzheimer’s Disease

Fyn kinase couples Aβo synaptotoxicity and Tau pathology in the post-synaptic density.

AZD0530 inhibits Fyn.

UH2/3 Funded Trial: National Center for Advancing Translation Sciences (NCATS) and the NIH Common Fund, through the Office of Strategic Coordination/Office of the NIH Director (PIs: Strittmatter, van Dyck & Nygaard)
Aβ Oligomers Trigger Alzheimer’s Pathophysiology

APP >> Aβ >> Aβ Plaque
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Cell Surface Binding
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Cell Surface Binding

Synapse Impairment
Aβ Oligomers Trigger Alzheimer’s Pathophysiology

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→ Cell Surface Binding

→ Synapse Impairment

→ Morphological Change

→ Cell Loss & Brain Atrophy

Tau Phosphorylation
Neurofibrillary Tangle
Aβ Oligomers Trigger Alzheimer’s Pathophysiology

APP >> Aβ >> Aβ Oligomers >> Aβ Plaque

- Cell Surface Binding
- Synapse Impairment
- Morphological Change
- Cell Loss & Brain Atrophy

- Dementia

- Tau Phosphorylation
- Neurofibrillary Tangle
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APP >> Aβ >> Aβ Oligomers >> Aβ Plaque

Cell Surface Binding

- Synapse Impairment
- Morphological Change
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Dementia

- Tau Phosphorylation
- Neurofibrillary Tangle
Unbiased Genome-Wide Screening for Oligomeric Aβ Binding Sites Identifies PrP<sup>C</sup>
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- Adult mouse brain cDNA library (225,000 clones)
- Expression in COS-7 cells
- Oligomeric biotin-Aβ binding (1 μM)
- Streptavidin-AP detection
- 2 positive clones
- DNA sequence = PrP
Fyn Kinase Is Activated by Aβo via PrPC

- No direct effect of Aβo +/- PrPC on NMDA-R or AMPA-R
- Fyn activated by PrP clustering in rafts
- Fyn required for PrP phenotypes in Zf, worm

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Aβ Oligomer Destabilization of Dendritic Spines Requires PrP<sup>C</sup>

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Fyn in the Post-Synaptic Density Regulates Synaptic Plasticity

- Fyn is concentrated in dendritic spine PSD
- Fyn phosphorylates NMDA-Rs
- Regulates NMDA-R traffic
- Fyn titrates long-term potentiation
- Fyn over-activity causes seizures in mice

Fyn Interacts with Tau & Rescues Transgenic Mouse Models

- Fyn binds Tau
- Fyn phosphorylates Tau
- Tau deletion or truncation prevents Fyn targeting to PSD in dendrite spines
- Uncoupling Fyn from PSD rescues APP/Aβ deficits
- Increased Fyn exacerbates APP/Aβ deficits
- Decreased Fyn reduces APP/Aβ deficits

Aβ Oligomer Signaling Through PrPC
PrP^C Is Required for Aβo Suppression of LTP

PrP<sup>C</sup> Is Required for Aβo Suppression of LTP

Lauren et al. Nature 2009
Spatial Learning Is Normal in AD Mice Lacking PrP<sup>C</sup>
Spatial Learning Is Normal in AD Mice Lacking PrP<sup>C</sup>

Gimbel et al. J Neurosci 2010
Spatial Learning Is Normal in AD Mice Lacking PrP<sup>C</sup>
Screen for Transmembrane PSD Coupling Protein

Um et al Neuron, 2013
Screen for Transmembrane PSD Coupling Protein

A

B

C

D
mGluR5 Antagonist Reverses Learning, Memory and Synaptic Deficits in AD Mouse Models

*Grm5*-/ or high dose MTEP causes memory impairment

Titrate to moderate dose

10 day treatment with 30 mg/kg/d

Narrow therapeutic window

Um et al, Neuron, 2013
mGluR5 Antagonist *Reverses* Learning, Memory and Synaptic Deficits in AD Mouse Models

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Um et al, Neuron, 2013
Fyn Inhibition by AZD0530 for Alzheimer’s

Fyn kinase couples Aβ synaptotoxicity and Tau pathology in the post-synaptic density.

In contrast, PrP<sup>C</sup> has no pharmacological inhibitors and mGluR5 inhibition has narrow therapeutic window with current drugs.

NCATS Repurposing AZD0530 availability from AstraZeneca
AZD0530 (Saracatinib) Inhibits Fyn Kinase

AZD0530 inhibits Src family kinases
For Src family of kinases, Ki = 1-10 nM
ATP competitive mechanism
Inhibition of Abl about 20 fold less potent
~70 other kinases >100 less potent
307 other targets, no activity at 1 µM

Phase 2 studies for solid tumors

97% oral bioavailability
Human plasma half-life is 40 hours
Once daily oral dosing

CSF access?
Preclinical AD efficacy?
Safety in AD?

Kaufman et al, Ann Neurol 2015
AZD0530 Exposure Levels in Mice

- No previous PK data from mouse
- Brain is at least 50% of plasma
- CSF level measurable, and about 1/3 of brain
- Peak levels measured at multiple doses
- Trough levels at 5 mg/kg/d (effective dose)
- Chronic toxicology: no issues over 9 months at doses of 2 and 5 mg/kg/d
Pharmacodynamic Marker:
LOAD, Fyn and Risk Gene PTK2B (Pyk2)

- One confirmed GWAS hit is PTK2B (Pyk2)
- Pyk2 is direct interactor and substrate of Fyn
- Bidirectional and synergistic Fyn/Pyk2 activation
- mGluR and TCR activation induce PTK2B phosphorylation via Fyn

Kaufman et al, Ann Neurol 2015
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Kaufman et al, Ann Neurol 2015
AZD0530 Prevents Pyk2 Activation in Vivo

Oral treatment for 6 weeks started at 11 months after documented memory deficit

Kaufman et al, Ann Neurol 2015
Spatial Memory in Morris Water Maze Short-Term Therapy Not Effective
Spatial Memory in Morris Water Maze Longer-Term Therapy Reverses Deficit

Lower (2 mg/kg/d) dose of AZD0530 not effective
Novel Object Recognition Memory
Longer-Term Therapy Reverses Deficit

6 Weeks of Pre-Treatment

Object Interaction (sec)

Vehicle WT  5 mg/kg/d AZD0530 WT  Vehicle APP/PS1  5 mg/kg/d AZD0530 APP/PS1

**  ***  n.s.  **

Vehicle WT: 5 mg/kg/d AZD0530 WT: Vehicle APP/PS1: 5 mg/kg/d AZD0530 APP/PS1
AZD0530 Reverses Synapse Loss

Kaufman et al, Ann Neurol 2015
AZD0530 Reduces Inflammation in AD Mice
AZD0530 Reduces Tauopathy in 3xTg AD Mice
AZD0530 Reduces Tauopathy in 3xTg AD Mice
Fyn Inhibition by AZD0530 for Alzheimer’s

**Fyn kinase**
Couples Aβ synaptotoxicity and Tau pathology in the post-synaptic density

**AZD0530**
- Inhibits Fyn, and indirectly Pyk2 activation
- Achieves effective CSF concentrations
- Tolerated chronically
- Reverses memory and synaptic deficits in mouse AD model
Phase Ib Design

• Multiple ascending dose study of AZD0530 in 24 subjects with mild to moderate AD (MMSE=16-26), enrolled in three Cohorts of 8 subjects each: 50, 100, and 125 mg of AZD0530, active (n=6), placebo (n=2) in each cohort. 1 month on study medication.

• **Primary Aims:** To assess the safety and tolerability of oral AZD0530 in patients with AD and to determine dose levels that are well tolerated in AD patients and provide CSF concentrations predicted to slow AD.

• **Secondary Aims:** To assess effects of AZD0530 on clinical measures and changes in brain $^{18}$F-FDG PET in patients with AD
AZD0530 in Human CSF at Different Doses

A

B

$R^2=0.97$
AZD0530 Peripheral Target Engagement

- AZD0530 decreases bone resorption by inhibiting osteoclast
- Measured by collagen fragment (sCTX)
## Adverse Events by Treatment Group

### No Laboratory Adverse Events of Special Interest

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Placebo</th>
<th>50 mg</th>
<th>100 mg</th>
<th>125 mg</th>
<th>TOTALS</th>
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<td>Anorexia</td>
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<td>0</td>
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<tr>
<td><strong>TOTALS</strong></td>
<td><strong>7</strong></td>
<td><strong>4</strong></td>
<td><strong>6</strong></td>
<td><strong>16</strong></td>
<td><strong>33</strong></td>
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</table>
Serious Adverse Event, 125-mg Dose

85 WF, baselined 1/27/14.

Hospitalized 2/9/14 with 4 days of fatigue, anorexia, and myalgias and 1 day of shortness of breath (after diuretic held one day).

Diagnoses = congestive heart failure; bronchitis or atypical pneumonia.

Treated with additional diuretic (furosemide) and antibiotics (levofloxacin). Study drug discontinued 2/7/14.

Discharged to short-term rehab on 2/12/14 and home on 3/24/14.

Week 4/early termination visit on 2/26/14. Also seen by Yale ILD expert. Findings most consistent with CHF precipitated by pneumonia. “Drug toxicity is difficult to entirely rule out.”

Possibly related to study drug
Overview of Phase 2a Design

• **Phase 2a Proof of Concept** study to test whether AZD0530 slows, halts or reverses AD over 12-month period

• **Innovation:** $^{18}$F-FDG PET as primary outcome. A biomarker of regional synaptic activity expected to confer greater statistical power than clinical outcomes

• Acquire standard clinical measures of efficacy (ADAS-Cog, ADCS-ADL, CDR-SOB) to power a subsequent Phase 3 pivotal trial

• Randomized, blinded, placebo controlled trial

• 152 subjects (1:1), multicenter design
Twelve-Month FDG PET Decline

Number of patients per group needed to detect a 25% treatment effect with a two-sided $P = 0.05$ and 80% power in a twelve-month placebo-controlled RCT.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Probable AD</th>
<th>MCI</th>
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<tbody>
<tr>
<td>sROI</td>
<td>66</td>
<td>218</td>
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<tr>
<td>CDR-SB</td>
<td>861</td>
<td>1110</td>
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<tr>
<td>ADAS-Cog</td>
<td>353</td>
<td>4219</td>
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<tr>
<td>MMSE</td>
<td>629</td>
<td>5267</td>
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Primary Aims

• **Aim 1:**  
  Effect of AZD0530 on 52-week reduction in $^{18}$F-FDG PET measurements of the cerebral metabolic rate for glucose (CMRgl) in subjects with mild AD.

• **Aim 2:**  
  Assess safety and tolerability of AZD0530 over a 52-week period in subjects with mild AD.
Secondary Aims

- **Aim 3:**
  To assess the effect of AZD0530 on ADAS-cog, MMSE, ADCS-ADL, CDR-sob, and NPI.

- **Aim 4:**
  To assess the effect of AZD0530 on the rate of change in volumetric magnetic resonance imaging (MRI).

- **Aim 5:**
  To assess the effect of treatment with AZD0530 on CSF biomarkers of AD (CSF pTau).

- **Aim 6:**
  To assess the influence of *APOE genotype* on the effects of treatment with AZD0530.
Inclusion Criteria

1. NIA-Alzheimer’s Association core clinical criteria for probable AD
2. $^{18}$F-Florbetapir scan with evidence of elevated $A\beta$ (based on central review)
3. Age between 55-85
4. MMSE score between 18 and 26
Logistics and Status

- Investigator-held IND for AZD0530 in Phase 1 & 2 trials approved by FDA
- Multicenter trial coordinated through Alzheimer Disease Cooperative Study (ADCS)
- IRB filed by 22 sites, approved now at 12 sites
- Subjects screened: 17
- Subjects randomized: 10 of 152
- Enrollment projection: 152 within 12 months
- Completion of clinical portion: 12 months after enrollment complete
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Fyn kinase
Couples Aβ synaptotoxicity and Tau pathology in the post-synaptic density

AZD0530
- Inhibits Fyn
- Reverses memory and synaptic deficits in mouse AD model
- Tolerated in Phase 1b with effective CSF level
- Phase 2a underway
UH2/3 Principal Investigators

Christopher van Dyck
Haakon Nygaard
Stephen M. Strittmatter

Funding
NCATS
NIA
NINDS
Alzheimer’s Association
Falk Trust
BrightFocus

Strittmatter AD Laboratory Studies
- Erik Gunther: Aβ0 Binding Studies
- Jacqueline Heiss: Spine Imaging
- Adam Kaufman: Spatial Memory
- Mikhail Kostylev: Aβ/PrP Structure
- Suho Lee: Signal Transduction
- Laura Haas: mGluR5
- Santiago Salazar: Pyk2
- Hideyuki Takahashi: ApoE & TREM2
- Levi Smith: Receptors and PK
- Zoe Klein: Lysosome and GRN

Collaborators
- Alexander Vortmeyer (Pathology)

Former Lab Members
- Juha Lauren
- Ji Won Um
- Jinhee Yang