Drug discovery for the treatment of Alzheimer’s disease -
Moving away from symptomatic therapies

Menelas N. Pangalos
Executive Vice President & Head of
Discovery Research
Today’s talk

- Introduction
- \(\gamma\) secretase inhibitors
- \(\beta\) secretase inhibitors
- PAI-1 inhibitors
- Summary
Today’s talk

Introduction

γ secretase inhibitors

β secretase inhibitors

PAI-1 inhibitors

Summary
Alzheimer’s disease statistics

- A person develops dementia every three minutes
- AD currently affects more than 24 million people worldwide
  - Expected to reach 80 million by 2040
  - Accounts for >60% of all dementias
  - Prevalence:
    - 1% between the ages of 60-64
    - 50% in people aged 85 and over
  - 1 in 8 baby boomers will get AD
- Through course of the disease patients will require full time care
- Direct and indirect costs in the US are estimated in excess of $150B/yr
  - Could reach $1 trillion by 2050
Wyeth’s AD pipeline uses all three of our technology platforms

- **Small Molecules**
  - GSI-953
  - PAZ-417
  - SAM-531
  - SAM-760
  - NSA-789
  - HTC-867

- **Proteins**
  - Bapineuzumab†
  - AAB-002†
  - AAB-003†

- **Vaccines**
  - ACC-001†
  - ACC-002†

† Alliance with Elan
Today’s talk

Introduction

γ secretase inhibitors

β secretase inhibitors

PAI-1 inhibitors

Summary
Inhibition of the amyloid pathway – Prevention of amyloid plaque formation

Amyloid Protein

Beta Secretase

Gamma Secretase

Aβ

Beta amyloid peptide

Aβ

Beta amyloid aggregates

Aβ

Beta amyloid deposits or plaques

Gamma Secretase or BACE Inhibitors

Neuropathology Leading to Cognitive Dysfunction

AD drug discovery – March 2009
Gamma secretase is a multi-transmembrane protein complex

Cell-based assays demonstrate GSI-953 is a potent inhibitor of Aβ production.

GSI-953: Increases levels of βCTF fragments

### Radiolabeled βCTF Assay

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<thead>
<tr>
<th>WAY-210953 [nM]</th>
<th>% Control</th>
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<tr>
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<td>10</td>
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<table>
<thead>
<tr>
<th>WAY-210953 [nM]</th>
<th>100</th>
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<tbody>
<tr>
<td>% Control</td>
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<td>90</td>
<td>80</td>
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### WAY-210953 [nM] vs % Control

<table>
<thead>
<tr>
<th>WAY-210953 [nM]</th>
<th>% Control</th>
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<tbody>
<tr>
<td>210953</td>
<td>6.5</td>
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<tr>
<td></td>
<td>7.9</td>
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#### EC₅₀ (nM)

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<tr>
<th>WAY-210953 [nM]</th>
<th>EC₅₀ (nM)</th>
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<tbody>
<tr>
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<td>6.5</td>
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</table>

#### Table: Change

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<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>210953</td>
<td>βCTF</td>
</tr>
</tbody>
</table>

### Diagram: Lumen vs Cytoplasm

- **Lumen**: APP, βCTF, βAPPs
- **Cytoplasm**: γCTF, Aβ
γ-Secretase inhibition and potential altered Notch processing

- Notch cleaved by γ-secretase
- NICD translocates to nucleus
- NICD is a transcriptional factor regulating development and cellular differentiation (e.g., gastroendothelium)
GSI-953 selectively inhibits APP processing over Notch

<table>
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<tr>
<th>Compound</th>
<th>EC50 (nM)</th>
<th>Ratio Notch/Aβ</th>
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<tr>
<td></td>
<td>Notch</td>
<td>Aβ42</td>
</tr>
<tr>
<td>DAPT</td>
<td>14.9 ± 0.9</td>
<td>27.7 ± 4.8</td>
</tr>
<tr>
<td>GSI-953</td>
<td>208.5 ± 27.3</td>
<td>12.4 ± 0.8</td>
</tr>
<tr>
<td>WAY-952</td>
<td>Inactive</td>
<td>&gt;18,000</td>
</tr>
<tr>
<td>LY411575</td>
<td>0.3 ± 0.1</td>
<td>0.36 ± 0.0</td>
</tr>
<tr>
<td>LY450139</td>
<td>62.9 ± 19.5</td>
<td>26.0 ± 1.0</td>
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</table>
APP processing in rat cortical brain slices biolistically transfected with APP
γ-Secretase inhibitors block Aβ formation in brain slices

- APP
- βCTF
- Aβ

Vehicle | GSI-1

kDa

100-

16.0-

3.8-
GSI-953 reduces brain Aβ levels in a time and dose-dependent manner in Tg2576 mice
Contextual fear conditioning (CFC)

- Hippocampal-dependent conditioning in mice
- Learning involves a shock associated with a specific environment (context)
- Memory is expressed by context-dependent freezing in the absence of the shock

**Training (Day 1)**
- 1.5 mAmp
- 120s
- 2s
- 120s
- 2s
- 30s

**Testing (Day 2)**
- 24h
- 300s
GSI-953 reverses hippocampal contextual memory deficits in Tg2576 animals

*sig.diff. from WT vehicle; ^sig.diff. from APP Tg Vehicle

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AD drug discovery – March 2009
γ-Secretase Inhibitors exhibit an Aβ “rebound” effect in plasma but not in brain.

![Graph showing Aβ levels over time with GSI treatment.](image)

- Reduced Aβ
- Increased Aβ

GSI (30mg/kg, po)

* p<0.05, ** p<0.001
GSI-953 reduces plasma $A\beta$ levels in human: target engagement and biomarker strategy
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PAI-1 inhibitors

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Inhibition of the amyloid pathway – Prevention of amyloid plaque formation

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Beta Secretase

Gamma Secretase or BACE Inhibitors

Gamma Secretase

Beta amyloid peptide

Beta amyloid aggregates

Neuropathology Leading to Cognitive Dysfunction

Beta amyloid deposits or plaques
Evolution of a BACE drug discovery program

- Develop a reproducible assay
- Run screen - approximately 1,000,000 compounds tested
- Take identified chemical hits and confirm their activity in a second assay using human hands
- Use X-ray structure to guide chemical drug design
Next step – optimizing “lead” molecules to improve effectiveness

Chemists work with biologists to modify the initial chemical hit to fit “perfectly” on the biological target and have drug-like properties.

Chemical Hit ➔ Chemical Lead
Confirming and understanding an early chemical ‘hit’ for our BACE program

- S1 – S3 forms large hydrophobic binding pocket

Para position provides best opportunity to increase potency via S3 pocket:

![Chemical structure diagram showing S1, S1', S2', S3, and FLAP regions with polar/charged and hydrophobic labels.](image-url)
Drug discovery – optimizing a chemical ‘hit’

- Start with lead compound

BACE1 IC50
3700 nM
Drug discovery – optimizing a chemical ‘hit’

- Start with lead compound
  - Examine each pocket

BACE1 IC50
3700 nM

600 nM
--unstable
Drug discovery – optimizing a chemical ‘hit’

- Start with lead compound
  - Examine each pocket
  - Combine results

BACE1 IC50
3700 nM
800 nM
X = H, O
Good stability
Drug discovery – optimizing a chemical ‘hit’

- Start with lead compound
  - Examine each pocket
  - Continue optimization

BACE1 IC50

S1’ 800 nM

290 nM
X = H, O

AD drug discovery – March 2009
Drug discovery – optimizing a chemical ‘hit’

- Start with lead compound
  - Examine each pocket
  - Optimized tool compound

![Chemical Structure](image)

- **S1’** 3700 nM
- **S2’** 60 nM
- **X = H, O**

BACE1 IC50
Development of more potent BACE inhibitors

- A few thousand compounds synthesized to explore chemistry around initial hits
- Potency improved more than a thousand fold
- Stability and brain penetration maintained and improved

S1, S1', S2', S3, FLAP

Polar/Charged Hydrophobic
Our BACE inhibitors do not lower brain $\text{A}\beta$ acutely in wild type mice

**Brain $\text{A}\beta 40$**

**Brain $\text{A}\beta 42$**
Our BACE inhibitors do lower brain Aβ acutely in PGP KO mice

Brain Aβ40

Brain Aβ42

AD drug discovery – March 2009
Oral dosing of a BACE inhibitor reverses cognitive deficits in an AD mouse model

*sig. diff. from WT vehicle; ^sig. diff. from Tg2576 vehicle
We can see Amyloid deposits increase over a period of a few weeks
... And Our BACE inhibitors can reduce the amyloid load in the brains of these AD mice

2-Photon microscopy in live animals
Plaque load is significantly reduced by chronic administration of a BACE Inhibitor.
Today’s talk

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β secretase inhibitors

PAI-1 inhibitors

Summary
Inhibition of the amyloid pathway – Prevention of amyloid plaque formation

Amyloid Protein → Beta Secretase → Aβ → Gamma Secretase

PAI-1 Inhibitors

Neuropathology Leading to Cognitive Dysfunction

Beta amyloid deposits or plaques

Beta amyloid peptide

Beta amyloid aggregates
Aβ activates and is cleaved by the tPA plasmin cascade

Aβ + Aβ → Aβ(n)

Aβ aggregation

+ mRNA → Plasminogen

Δ tPA affinity for Plgn

Δ tPA → Plasmin

Proteolytic cleavage
Aβ pathology increases PAI-1 levels and inhibits tPA and decreases plasmin formation.
Decreased plasmin activity results in reduced Aβ catabolism and further PAI-1 expression
Aβ is a plasmin substrate plasmin and can cleave it into numerous fragments

HPLC Peptide Cleavage Analysis

Data from Steve Estus lab; Tucker et al. *J. Neurosci.* 2000, 20:3937
tPA and plasminogen are required for Aβ clearance *in vivo*

Data from Sidney Strickland lab; Melchor et al. *J. Neurosci.* 2003, 23:8867

AD drug discovery – March 2009
In Vitro PAI-1 Inhibitor assay: tPA activity cleaves chromogenic substrate

PAI-1 Inhibitor assay: tPA activity cleaves chromogenic substrate

PAI-1 Activity (%) vs. PAI-1 Inhibitor (uM)

<table>
<thead>
<tr>
<th>PAI-1 Inhibitor</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
<th>Avg. ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAI-749</td>
<td>288 ± 41</td>
<td></td>
</tr>
<tr>
<td>PAZ-417</td>
<td>655 ± 29</td>
<td></td>
</tr>
<tr>
<td>PAI-039</td>
<td>1731 ± 203</td>
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**PAI-1 inhibitors activate Aβ and Aβ oligomer degradation *in vitro***

<table>
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<tr>
<th></th>
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<th>4</th>
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<tr>
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<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>tPA</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plgn</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PAZ</td>
<td>+</td>
<td></td>
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<td>+</td>
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</table>

**Western Blot:**
- Tetramer
- Trimer
- Dimer
- Monomer

**Aβ (%)**
- 100
- 86
- 2
- 107
- 63
- 7
Inhibitors of PAI-1 restore deficits of tPA activity in hippocampus of Tg2576 mice

<table>
<thead>
<tr>
<th>IHC (Protein)</th>
<th>Zymograph (tPA Activity)</th>
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<tbody>
<tr>
<td>tPA</td>
<td>PAI-1</td>
</tr>
<tr>
<td>Vehicle</td>
<td>PAZ-417</td>
</tr>
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</table>

**p<0.04
**p<0.004

Vehicle
PAZ

**tPA Activity (%)**

WT
TgAPP

AD drug discovery – March 2009
PAZ-417 reduces plasma levels of Aβ_{40} in Tg2576 mice

MED=10 mg/kg (6 hr)
PAZ-417 reduces plasma and brain levels of Aβ in Tg2576 mice

20 mg/kg, po
6 hrs post-treatment

* p<0.01

% Aβ Levels

Plasma Aβ40

Veh  PAZ

31%

Brain Aβ40

Veh  PAZ

20%

Brain Aβ42

Veh  PAZ

15%
PAZ-417 restores LTP deficits in the dentate gyrus of TgAPP mice

WT

Tg

PAZ-417 Vehicle

< WT level
< deficit
PAZ-417 reverses hippocampal contextual memory deficits

Significant difference from WT (*) or vehicle-treated APP Tg (^)
Today’s talk

Introduction

γ-secretase inhibitors

PAI-1 inhibitors

Summary
## Wyeth CNS pipeline

<table>
<thead>
<tr>
<th>Phase 0 (9)</th>
<th>Phase 1 (4/2)</th>
<th>Phase 2 (3)</th>
<th>Phase 3 (3)</th>
<th>Registration/Approval (1)</th>
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</thead>
<tbody>
<tr>
<td>AAB-002 (AD)</td>
<td>GSI-953 (AD)</td>
<td>Vabicaserin (SCA-136) (Schz/MDD)</td>
<td>Pristiq™ (Neuropathic Pain)</td>
<td>Pristiq™ (Depression)</td>
</tr>
<tr>
<td>SAM-760 (AD)</td>
<td>PAZ-417 (AD)</td>
<td>SAM-531 (AD/Schz)</td>
<td>Pristiq™ (Fibromyalgia)</td>
<td>Pristiq™ (VMS)</td>
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<tr>
<td>AAB-003 (AD)</td>
<td>NSA-789 (AD/Schz)</td>
<td>ACC-001 (AD)</td>
<td>Bapineuzumab (Alzheimer’s Disease)</td>
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<td>NRI-470 (Pain)</td>
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<td>ACC-002 (AD)</td>
<td>Lecozotan (HOLD)</td>
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<tr>
<td>ILS-920 (Stroke)</td>
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<tr>
<td>MMS-255 (ND)</td>
<td>SAX-187 (HOLD)</td>
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<tr>
<td>VRA-175 (Pain)</td>
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<tr>
<td>HTC-867 (AD/Schz)</td>
<td></td>
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Acknowledgements and many thanks....
WAY-210953: Lowers $\text{A}\beta$ levels without alteration of expression or secretion

Radiolabeled Cellular Assay

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<thead>
<tr>
<th>Analyte</th>
<th>Change</th>
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<tbody>
<tr>
<td>APP</td>
<td>no $\Delta$</td>
</tr>
<tr>
<td>$\alpha$APPs</td>
<td>no $\Delta$</td>
</tr>
<tr>
<td>$\text{A}\beta$</td>
<td>$EC_{50}$ ~35 nM</td>
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</tbody>
</table>

WAY-210953 [nM]
GSI-953 reduces Aβ in Tg2576 mouse model (time-course effects, 100 mpk, po)

Brain

CSF

Plasma

Aβ Reduction (%)

Time (hours)

*** p < 0.001

** p < 0.01

*p < 0.05
GSI-953 binds to γ-secretase: There is a clear relationship of binding to inhibitory activity

\[ r^2 = 0.99 \]
Dose dependent cleavage of Aβ and Aβ oligomers by a PAI-1 inhibitor

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<td>1.25</td>
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<td>0.31</td>
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- tetramer
- trimer
- dimer
- monomer