Alzheimer’s Disease Biomarkers And Tau Focused Drug Discovery

John Q. Trojanowski, M.D., Ph.D.

NIA Alzheimer’s Disease Core Center, NINDS Udall Center of Excellence For Parkinson’s Disease Research, Center for Neurodegenerative Disease Research, Marin S. Ware Alzheimer Program, Institute on Aging, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA

Aging Related Neurodegenerative Diseases Characterized by Filamentous Aggregates of Misfolded Proteins

<table>
<thead>
<tr>
<th>Disease</th>
<th>Lesions</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alzheimer’s Disease (multi-proteinopathy)</td>
<td>SPs (100%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NFTs (100%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LBDs (90%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TDP-43 (50%)</td>
<td></td>
</tr>
</tbody>
</table>

Frontotemporal Diseases

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inclusions</td>
<td>Tau, TDP-43, FUS</td>
</tr>
</tbody>
</table>

Amyotrophic Lateral Sclerosis

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inclusions</td>
<td>Tau, TDP-43, FUS</td>
</tr>
</tbody>
</table>

Parkinson’s disease +/- Dementia

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBDs</td>
<td>α-Synuclein</td>
</tr>
</tbody>
</table>

Multiple System Atrophy

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCIIs</td>
<td>α-Synuclein</td>
</tr>
</tbody>
</table>

Prion diseases

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPs</td>
<td>Prions</td>
</tr>
</tbody>
</table>

Trinucleotide repeat diseases

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inclusions</td>
<td>Expanded polyglutamine repeats</td>
</tr>
</tbody>
</table>

Hypothetical Timeline for the Onset and Progression of AD Neurodegeneration and Dementia: The Need For AD Biomarkers Is Urgent


Georgia Institute of Technology

For Example, Biomarkers Will Accelerate The Pace Of Aβ and Tau Focused AD Drug Discovery


GOALS OF ADNI-1

Optimize and standardize biomarkers for clinical trials
Validate biomarkers as measures of change
Validate biomarkers as diagnostics or predictors
Establish world-wide network for clinical AD studies and treatment trials
ADNI-1: Naturalistic study of AD progression

- 200 NORMAL 3 yrs
- 400 MCI 3 yrs
- 200 AD 2 yrs
- Visits every 6 mo
- 57 sites
- Clinical, blood, LP
- Cognitive Tests
- 1.5T MRI

Some also have
- 3.0T MRI (25%)
- FDG-PET (50%)
- PiB-PET (approx 100)

All data in public database:
UCLA/LONI/ADNI
No embargo of data

SCOPE OF ADNI-2

($40 M From NIH & $29 M From ISAB, Foundations & FNIH; Funded From 10/1/2010 To 9/30/2015)

- Goal to continue to follow >400 controls and MCI from ADNI-1 for 5 more years and enroll:
  - 100 additional EMCI (supplements 200 from GO)
  - 150 new controls, LMCI, and AD
- MRI at 3, 6, months and annually
- F18 amyloid (AV-45)/FDG baseline and Yr 2
- LP on 100% of subjects at enrollment
- Genetics

ADNI-2 Governance

NIA | Steering Committee

Industry Scientific Advisory Board
PET Core: C. Jack, Mayo
Neuroimaging Core: M. Mathis, U. Pennsylvania
Genetics Core: J. Shaw, Penn
Reactivation Core: A. Trojanowski, Penn
Clinical Core: J. Fox, N. London
Biomarker Core: R. Knopman, Mayo
Informatics Core: C. Toga, UCLA
MRI Core: R. Buckner, UCLA
All Recruitment Sites

PI: Weiner, UCSF
Administrative Core
Executive Committee

PET Scientist Advisory Board

NIA Steering Committee
Koeppe, Michigan
Kwong, Harvard
Koeppe, Indiana
Mathis, Penn
Shaw, Philadelphia
Since there is significant variation in CSF biomarker levels between studies, there is an urgent need to standardize and validate AD biomarkers.

Study design: All studies on CSF T-tau with >25 AD cases

Innogenetics T-tau ELISA
34 studies, 2600 AD cases

Comparison of: mean level of CSF T-tau

<table>
<thead>
<tr>
<th>Study</th>
<th>0</th>
<th>100</th>
<th>200</th>
<th>300</th>
<th>400</th>
<th>500</th>
<th>600</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>336 pg/mL</td>
<td>919 pg/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

→ Need for standardization: CSF sampling / handling procedures
Laboratory procedures
External control program

Pre-Analytical Issues are Critical:
CSF & Plasma Collections For ADNI

- After overnight fast
- Collect into polypropylene tube
- Transfer to polypropylene transfer tube
- No centrifugation
- Freeze at site, thaw & aliquot at UPenn, storage at -80 °C

Number of biofluids collected as of 6/30/2010: 13,122
Number of aliquots in biofluid bank: 126,681
Luminex xMAP Technology for Multiparameter Immunoassays

Microspheres coupled with antibody
Each type of microsphere coded with fluorochromes
Up to 100 proteins can be analyzed at once
Sample volume = 75 μL


CSF Biomarker Validation


- Calibration curve stability
- Aliquot reproducibility
- Short- & long-term within- and between-day reproducibility
- Stability of biomarkers in CSF
  - Freeze-thaw
  - Room temp
  - +40C

CSF Biomarker Cutpoints Established Using CSFs Collected from ADNI-Independent Autopsy-Based AD and Age-Matched Cognitively Normal Subjects

<table>
<thead>
<tr>
<th></th>
<th>Tau</th>
<th>Aβ1-42</th>
<th>p-Tau181p</th>
<th>Tau/Aβ1-42</th>
<th>p-Tau181p/Aβ1-42</th>
<th>LR TAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROC AUC</td>
<td>0.631</td>
<td>0.913</td>
<td>0.763</td>
<td>0.917</td>
<td>0.856</td>
<td>0.928</td>
</tr>
<tr>
<td>Threshold</td>
<td>93 ng/mL</td>
<td>192 ng/mL</td>
<td>73 ng/mL</td>
<td>0.39</td>
<td>0.10</td>
<td>0.22</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>89.6</td>
<td>96.4</td>
<td>85.6</td>
<td>91.1</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>92.3</td>
<td>76.9</td>
<td>73.1</td>
<td>84.6</td>
<td>71.2</td>
<td>76.9</td>
</tr>
<tr>
<td>Test accuracy</td>
<td>80.6</td>
<td>87.0</td>
<td>73.1</td>
<td>85.2</td>
<td>81.5</td>
<td>84.9</td>
</tr>
<tr>
<td>Positive</td>
<td>90.7</td>
<td>81.8</td>
<td>67.9</td>
<td>85.7</td>
<td>77.3</td>
<td>82.4</td>
</tr>
<tr>
<td>Negative</td>
<td>73.8</td>
<td>95.2</td>
<td>70.4</td>
<td>84.6</td>
<td>88.1</td>
<td>100</td>
</tr>
<tr>
<td>predictive</td>
<td>value (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>value (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Kaplan-Meier time to conversion to AD for ADNI subjects with MCI at their baseline visit. For example, A shows the survival curves for MCI subjects with CSF Aβ42 concentrations above or below the threshold value of 192 pg/mL at baseline.

OBJECTIVES: Investigate effect of CSF abnormalities on rate of functional decline in NC, MCI, and mild AD.

DESIGN: T-tau, p-tau181, and Aβ42 assayed in CSF from ADNI participants. Random effects regressions to examine the relationship between CSF abnormalities, cognitive impairment (ADAS-Cog), and functional decline (Pfeffer’s FAQ);

SETTING: ADNI.

PARTICIPANTS: 114 NC, 195 MCI, 100 mild AD.

OUTCOME MEASURE: Decline in Pfeffer’s FAQ.

RESULTS: Across all groups, persons with a combination of tau and Aβ42 abnormalities exhibited the steepest rate of functional decline.

CONCLUSIONS: CSF abnormalities are associated with functional decline, and the development of AD in NC and MCI subjects, and those persons with tau and Aβ42 abnormalities are at greatest risk of functional impairment.
**Temporal Ordering of AD Biomarkers Reflects Disease Progression**

Shaw et al., 2007; Jack et al., 2010; Trojanowski JQ, et al, 2010

**Tau Focused Drug Discovery For AD**

- An abundant low MW MT-associated protein, predominantly in axons
- binds to MTs, promotes MT polymerization and stability
- tau phosphorylation negatively regulates the binding of tau to MTs
- hyperphosphorylated tau accumulates as Neurofibrillary tangles (NFTs) in AD and different FTDs.
- In tauopathy, hyperphosphorylated tau reduces binding to MTs and acquires a toxic tau.
- The loss of tau normal function results in MT instability and damages axonal transport
- Further accumulated free tau in cell body decreases tau solubility and further form NFTs

**Therapeutic Strategies to Reduce Tauopathy**

- HSP90 inhibitors might increase proteasome-mediated clearance of misfolded hyperphosphorylated tau monomers
- Tau kinases or b-N-acetylglucosaminidase inhibitors might inhibit tau hyperphosphorylation
- Tau assembly inhibitors might decrease tau assembly and increase tau solubility
- Autophagy enhancers might increase removal of tau aggregates
Hypothesis

“AD PHFtau does not bind to and stabilize MTs, but these functions are critical for maintaining the network of MTs required for intraneuronal transport. Thus, the loss of function by PHFtau could have deleterious consequences by depolymerizing MTs thereby disrupting axonal transport and compromising the function and viability of affected neurons in AD.”

Hypothesis: MT stabilizing drugs have therapeutic benefits in mouse models of tauopathies.

Paclitaxel Improves Motor Neuron Functions in Tau Tg Mice That Model Guam ALS Tauopathy

- 3 months i.p. injection of paclitaxel improves peripheral SC nerve function in tau Tg mice.
- Paclitaxel poorly crosses the BBB; not suitable for CNS tauopathies

Epothilone D Pharmacokinetics / Pharmacodynamics (PK/PD)

Brundin et al., Pharmacol Res. 62:341-351
EpoD Improves CNS Nerve Function in Young PS19 Mice

Questions

- Is EpoD efficacious in tau Tg mice with established NFT-like pathology (9 to 12-months of age)?
  - Axonal integrity and MT density?
  - Fast axonal transport?
  - Tau pathology?
  - Tau solubility and phosphorylation?
  - Cognitive impairment?

EpoD Efficacy Testing in Old PS19 Mice
(9 to 12-Month-Old)

<table>
<thead>
<tr>
<th>9M Non-Tg (males)</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle Control (DMSO)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>9M PS-19 (males)</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3 mg/kg CNDR-66 (EpoD)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>9M PS-19 (males)</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg/kg CNDR-66 (EpoD)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>9M PS-19 (males)</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle Control (DMSO)</td>
<td></td>
</tr>
</tbody>
</table>

Efficacy Testing
1. Fast Axonal Transport
2. ON Dystrophic Axon Analysis
3. MT Density Analysis
4. CNS IHC and Biochem
5. Behavioral Cognitive Testing

Safety Testing
1. Behavioral Observations
2. Motor Function
3. Body and Organ Weights
4. Examine of Peripheral Organs
5. Complete Blood Counts
6. High Dose of EpoD Safety Test in additional 15 WT mice (5 or 10mg/kg)
Dystrophic Axons in ON of 12-Month-Old Mice

Degenerated axons observed in all groups of mice.

Dystrophic Axon Numbers in 12-Month Old PS19 Mice

One way ANOVA non-parametric statistic test

MT Density Analysis

- At 50x mag, find the coordinates and systematically take axon EM images at vertical cut level to visualize MTs and NFs.
- Put 10x10 hexagons on the images.
MT Numbers in 12-Month Old PS19 Mice

One way ANOVA non-parametric statistic test

Axonal Transport and Tau MT Binding

Optic Nerve Fast Axonal Transport (FAT) in EpoD Treated 12-Month Old PS19 Mice

ESP Design

- Treatments for 3 months, 5 groups:
  - PS19 Vehicle
  - PS19 EpoD 0.3 mg/kg
  - PS19 EpoD 1 mg/kg
  - WT Vehicle

- ON FAT
  - Intravitreal injection of 35S-Methionine in both eyes, 6 mice/eye
  - 3 hours after injection:
    - Dye was ON and cut into 7 consecutive 1 mm segments from individual mouse
      (without pooling ON together)
  - CPM counts for % in each ON segment
  - SDS gels for individual mouse optic nerve segments
The part of memory responsible for recording information about the environment and its spatial orientation.
AT8 Tau Immunoactivity in EpoD- or Vehicle-Treated PS19 Mice at 12-Months of Age

Safety Analysis for EpoD-Treated PS19 Mice at 12-Months of Age

Body Weight

Organ Weights

No Significant Difference in Body and Organ Weights between Vehicle- and EpoD-Treated Mice at 12-months of age.

Safety Analysis for EpoD-Treated PS19 Mice at 12-Months of Age

Complete Blood Count (CBC)

No Adverse Effects on CBC
Safety Analyses with Higher Dose of EpoD in WT Mice

5 or 10 mg/kg, N=5/group
Weekly i.p. injection for 4 weeks

Summary
EpoD
- Attenuates dystrophic axons in mouse model of tauopathy at 12 months
- Recovers MT density in PS19 Tau Tg mice
- Improves FAT in PS19 Tau Tg mice
- Reduces tau pathology in PS19 Mice at 12m of Age
- Improves working and spatial learning and memory in aged tau Tg mice
- No detectable toxicities observed in the Tau or WT mice treated with EpoD

EpoD might be a therapeutic drug candidate for the treatment of tauopathies such as AD or FTLD-Tau

Risk, Biomarker, Disease and Therapeutic Evaluation

Integrating AD Diagnosis and Therapy/Prevention

Genomic
- ApoE allele assessment: homozygous 4/4 increase risk
- APP/PS1, familial AD risk

Symptomology
- Patient/family complaints about “forgetfulness”

Biomarkers
- Aβ42/40 ratios in CSF
- C9orf72 expansion
- Deviation from normal AD biomarkers: ApoE, Tau, P-Tau

PET Imaging
- Hypometabolic patterns in preclinical AD

Therapy
- Beta-secretase inhibitor (APP processing to Aβ)
- Passive/active immunization

Therapy Evaluation
When Disease Modifying Therapies Arrive For AD, The Penn Biomarker Core Will Be Ready With LP Bistros!

Please visit LP Bistro next time you are at Philadelphia International Airport.

Acknowledgements For EpoD Studies

Bin Zhang, MD, PhD & Kurt Brunke, Ph. D
Jenna Carroll, Ph. D
Misty Tay
Michael Jones
Sharon Yu, Ph. D
Anna Basler
Mickey Ba, Ph. D
Jennifer Makino
Hadas Smoroditsky
Clx Li, MS
Nabil Darwich
Akio Wada
Gina mo
Jenny Chee
CNDR Drug Discovery (Biology)
Edward Nagle, Ph. D
Hai Li, Ph. D
Alice Green, MS
James Beyer, Ph. D
Andrea Kline, MS
Virginia M.-Y. Lee, MBA, PhD

It Takes a Great Team!

Supported by the NIN/NIA/NINDS, Michael J. Fox Foundation, Marian S. Ware Alzheimer Program, William Maul Measey–Truman G. Schnabel, Jr. Chair of Geriatric Medicine & Gerontology and the Families of our Patients.