Alzheimer's Disease Biomarkers And Tau Focused Drug Discovery

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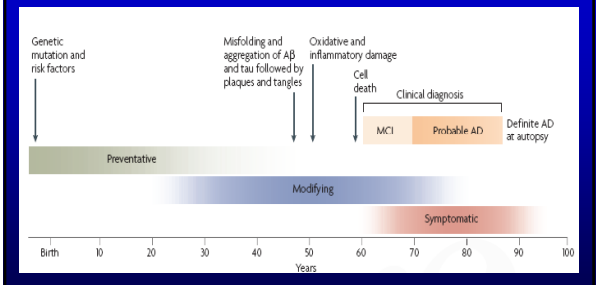
Aging Related Neurodegenerative Diseases Characterized by Filamentous Aggregates of Misfolded Proteins

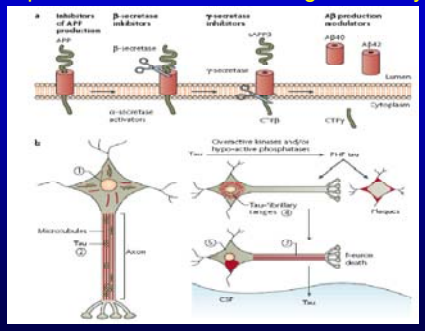
Disease	Lesions	Components
Alzheimer's Disease (A multi-proteinopathy)	**SPs (100%) NFTs (100%) LBs (50%) TDP-43 (50%)**	**Aβ Tau α-Synuclein TDP-43**
Frontotemporal Diseases	Inclusions	Tau, TDP-43, FUS
Amyotrophic Lateral Sclerosis	Inclusions	TDP-43, FUS, Tau
Parkinson's disease +/- Dementia	LBs	α-Synuclein
Multiple System Atrophy	GCIs	α-Synuclein
Prion diseases	SPs	Prions
Trinucleotide repeat diseases	Inclusions	Expanded polyglutamine repeats

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



Shaw LM, Korecka M, Clark CM, Lee VM.-Y, Trojanowski JQ. Nat Rev Drug Discovery, 6:295-303, 2007.

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



Shaw LM, Korecka M, Clark CM, Lee VM.-Y, Trojanowski JQ. Nat Rev Drug Discovery, 6:295-303, 2007.

Enter ADNI October 1st, 2004!

ALZHEIMER'S DISEASE NEUROIMAGING INITIATIVE

FUNDED BY THE NATIONAL INSTITUTE ON AGING

THE TEAM: M. Weiner, P. Aisen, R Peterson, C. Jack, W. Jagust, J Trojanowski, L. Shaw, A. Toga, L. Beckett, D. Harvey, C Mathis, A. Gamst. R. Green. A. Saykin, S. Potkin, J Morris, L Thal (D) Neil Buckholz, David Lee, Holly Soares
AND
The Industry Scientific Advisory Board (ISAB) and Site PIs, Study Coordinators, and
821 subjects enrolled in 58 sites in US and Canada

GOALS OF ADNI-1

($40 M From NIH, $25 M From ISAB, Foundations & FNIH; Funded From 10/1/2004 To 9/30/2009 With 1 Year No Cost Extension To 9/30/2010; Support s Research Conducted By ~75 Co-investigators)

- **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

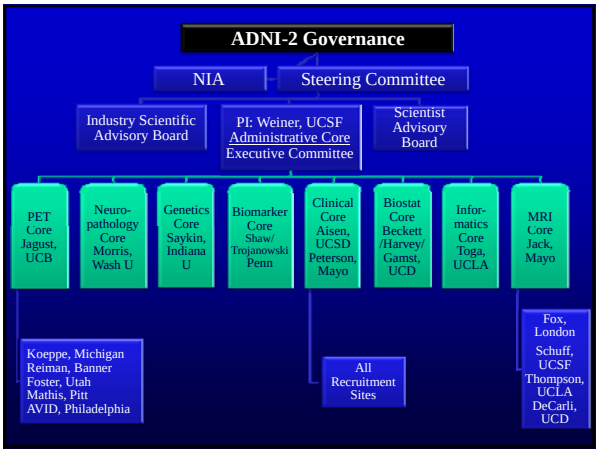
Some also have
- 3.0T MRI (25%)
- FDG-PET (50%)
- PiB-PET (approx 100)

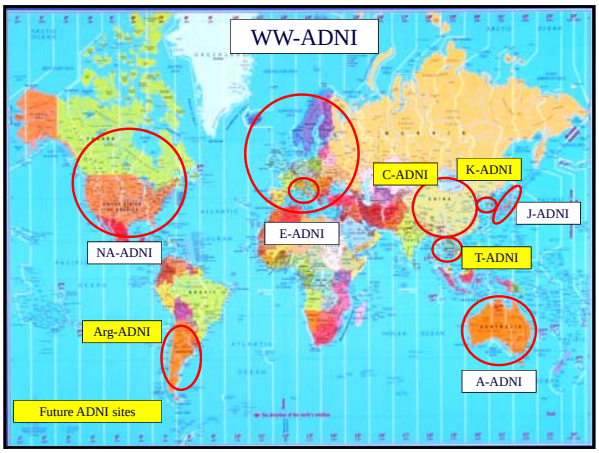
All data in public database:
UCLA/LONI/ADNI
No embargo of data

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**

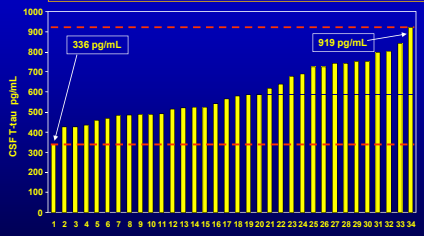




Since there is significant variation in CSF biomarker levels between studies, there is an urgent need to standardize and validate AD biomarkers

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



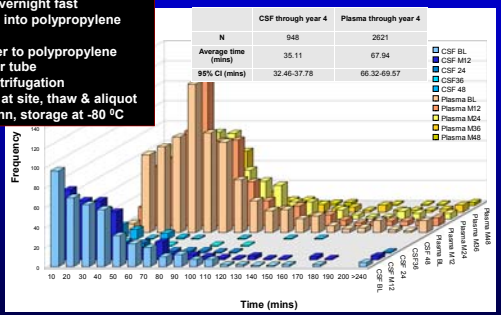
Blennow K, NYAS 2006

→ **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 ºC

	CSF through year 4	Plasma through year 4
N	948	2621
Average time (mins)	35.11	67.94
95% CI (mins)	32.46-37.78	66.32-69.57

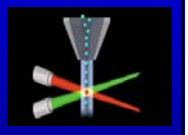


Number of biofluids collected as of 6/30/2010: 13,122
Number of aliquots in biofluid bank: 126,681

Luminex xMAP Technology for Multiparameter Immunoassays

Microspheres coupled with antibody

Each type of microsphere coded with fluorochromes



Microspheres pass 2 lasers:

a) Identification

b) Quantification

Up to 100 proteins can be analyzed at once
Sample volume = 75 µL.

Olsson A, et al, Clin Chem 2005

CSF Biomarker Validation

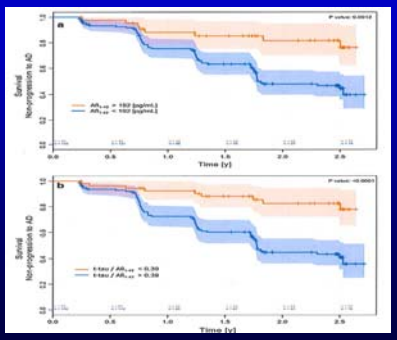
(All Data At ADNI Website And Shaw LM, Vanderstichele H, Knapik-Czajka M, Figurski M, Coart E, Blennow K, Soares H, Simon AJ, Lewczuk P, Dean RA, Siemers E, Potter WZ, Lee VM-Y, Trojanowski JQ, and ADNI. Qualification of the analytical and clinical performance of CSF biomarker analyses in ADNI. Acta Neuropathol., In press, 2011)

- 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

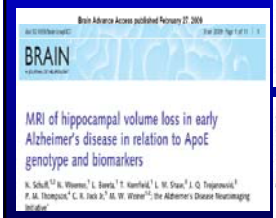
	Tau	$A\beta_{1-42}$	p-Tau_{181p}	Tau/$A\beta_{1-42}$	p-tau_{181p}/$A\beta_{1-42}$	LR TAA
ROC AUC	0.831	0.913	0.753	0.917	0.856	0.938
Threshold values	93 ng/mL	192 ng/mL	23 ng/mL	0.39	0.10	0.22
Sensitivity (%)	69.6	96.4	67.9	85.7	91.1	100
Specificity (%)	92.3	76.9	73.1	84.6	71.2	76.9
Test accuracy (%)	80.6	87.0	73.1	85.2	81.5	88.9
Positive predictive value (%)	90.7	81.8	67.9	85.7	77.3	82.4
Negative predictive value (%)	73.8	95.2	70.4	84.6	88.1	100

Shaw LM, Vanderstichele H, Knapik-Czajka M, Figurski M, Coart E, Blennow K, Soares H, Simon AJ, Lewczuk P, Dean RA, Siemers E, Potter WZ, Lee VM-Y, Trojanowski JQ & ADNI. Qualification of the analytical and clinical performance of CSF biomarker analyses in ADNI. Acta Neuropathol, Online, 2011.



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 $A\beta_{1-42}$ concentrations above or below the threshold value of 192 pg/mL at baseline.

ADNI Data Integration Is Off And Running



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

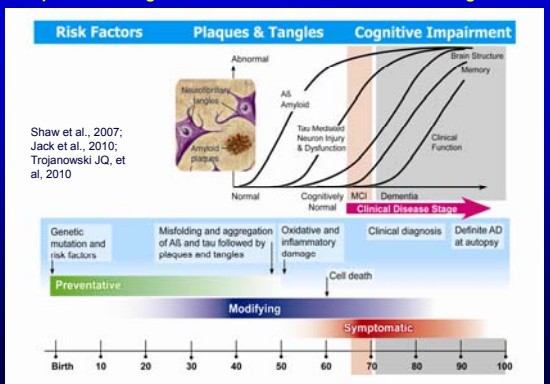
DESIGN: T-tau, p-tau_{181}, and $A\beta_{42}$ assayed in CSF from ADNI participants. Random effects regressions to examine the relationship between CSF abnormalities, cognitive impairment (ADAS-Cog), and functional decline (Pfeffer's FAQ);.

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 $A\beta_{42}$ 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 $A\beta_{42}$ abnormalities are at greatest risk of functional impairment.

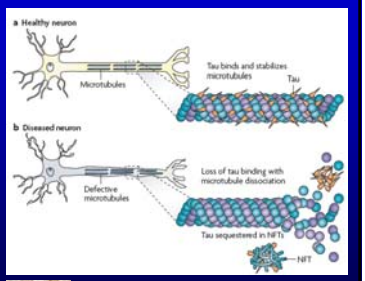
Temporal Ordering of AD Biomarkers Reflects Disease Progression



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

Tau Focused Drug Discovery For AD

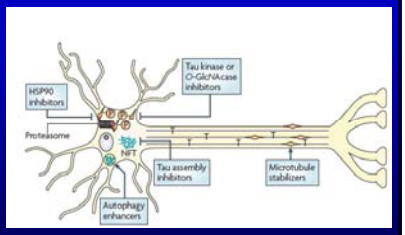
- An abundant low MW MT-associated protein, predominantly in axons
- Binds to MTs, promotes MT polymerization and stabilizes MTs
- Tau phosphorylation negatively regulates the binding of tau to MTs
- Hyperphosphorylated tau accumulates as Neurofibrillary tangles (NFTs) in AD and different FTDs.
- In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



Brunden, et al., Nature review, 8:783-793 2009

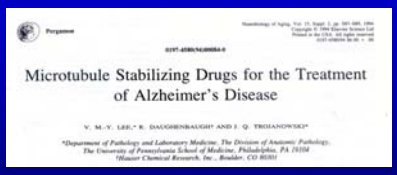
Therapeutic Strategies to Reduce Tauopathy

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



Brunden, et al., Nature review, 8:783-793 2009

Hypothesis



Microtubule Stabilizing Drugs for the Treatment of Alzheimer's Disease

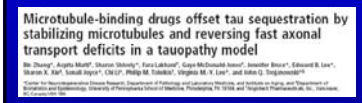
V. M.-Y. LEE,* K. DAUGHENBAUGH† AND J. Q. TROJANOWSKI*

*Department of Pathology and Laboratory Medicine, The Division of Anatomic Pathology, The University of Pennsylvania School of Medicine, Philadelphia, PA 19104 †Hauser Chemical Research, Inc., Boulder, CO 80301

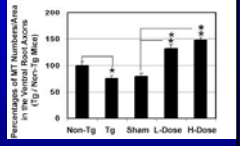
"AD PHFtau does not bind to and stabilize MTs, but these functions are critical for maintaining the network of MTs required for intraneuronal transport. Thus, the loss of function by PHFtau could have deleterious consequences by depolymerizing MTs thereby disrupting axonal transport and compromising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

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

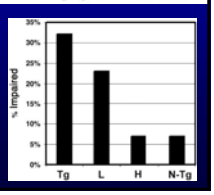
Paclitaxel Improves Motor Neuron Functions in Tau Tg Mice That Model Guam ALS Tauopathy



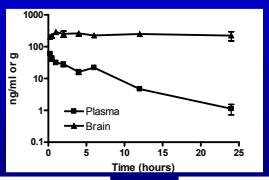
Microtubule-binding drugs offset tau sequestration by stabilizing microtubules and reversing fast axonal transport deficits in a tauopathy model



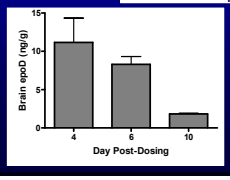
- 3 months i.p. injection of paclitaxel improves peripheral SC nerve function in tau Tg mice.
- Paclitaxel poorly crosses the BBB; not suitable for CNS tauopathies

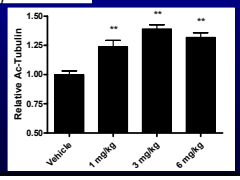


Epothilone D Pharmacokinetics / Pharmacodynamics (PK/PD)



Brunden et al., Pharmacol. Res. 63:341-351



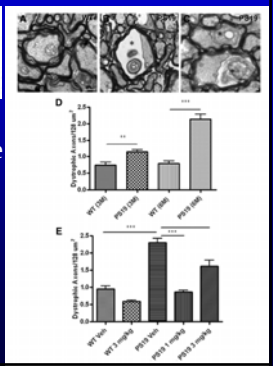


EpoD Improves CNS Nerve Function in Young PS19 Mice

Epothilone D Improves Microtubule Density, Axonal Integrity, and Cognition in a Transgenic Mouse Model of Tauopathy

Kurt R. Brunden,[a] Bin Zhang,[a] Jenna Carroll,[a] Yuemang Yao,[a] Justin S. Potuzak,[a] Anne-Marie L. Hogan,[a] Michiyo Iba,[a] Michael J. James,[a] Sharon X. Xie,[b] Carlo Ballatore,[a] Amos B. Smith III,[a] Virginia M.-Y. Lee,[a] and John Q. Trojanowski[a]

3 to 6-month of Age



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 to12-Month-Old)

- 9M PS-19 (males) n=13 Vehicle Control (DSMO)
- 9M PS-19 (males) n=13 0.3 mg/kg CNDR-66 (EpoD)
- 9M PS-19 (males) n=13 1 mg/kg CNDR-66 (EpoD)
- 9M Non-Tg (males) n=13 Vehicle Control (DMSO)

→ 3 Months Dosing (Weekly i.p. Injections)

Efficacy Testing

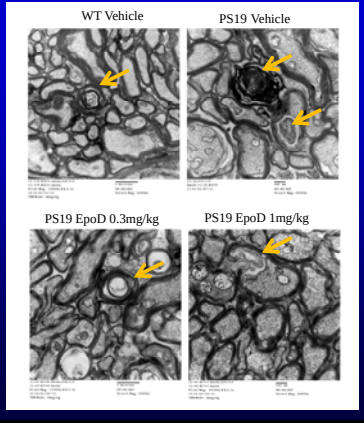
1. Fast Axonal Transport
2. ON Dystrophic Axon Analysis
3. MT Density Analysis
4. CNS IHC and Biochem
5. Behavioral Cognitive Testing

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)

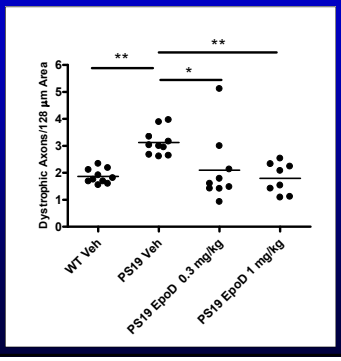
Dystrophic Axons in ON of 12-Month-Old Mice

Degenerated axons observed in all groups of mice



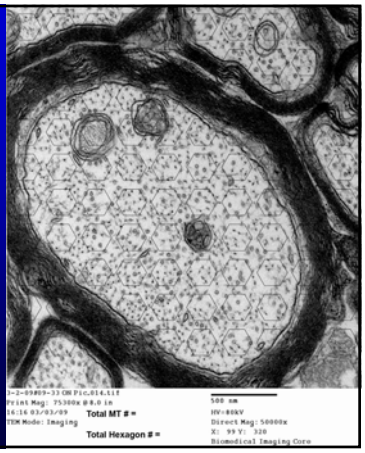
Dystrophic Axon Numbers in 12-Month Old PS19 Mice

One way ANOVA non-parametric statistic test



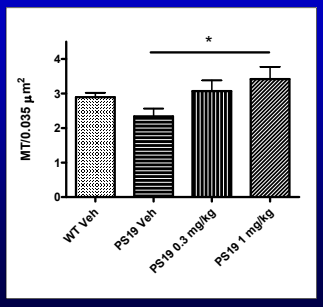
MT Density Analysis

- At 50k 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

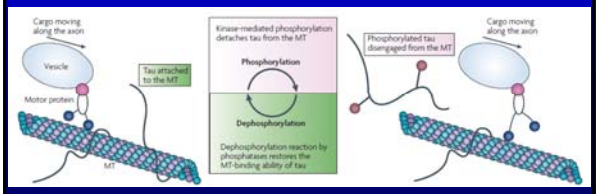


MT Numbers in 12-Month Old PS19 Mice

One way ANOVA non-parametric statistic test



Axonal Transport and Tau MT Binding

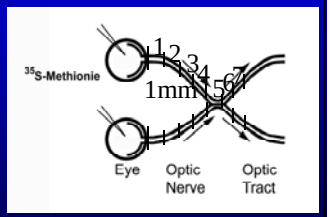


Ballatore et. Al., Review 2007

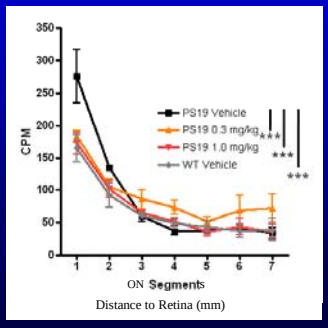
Optic Nerve Fast Axonal Transport (FAT) in EpoD Treated 12-Month Old PS19 Mice

EXP design

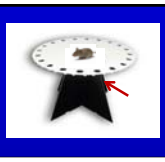
- Treatment for 3 months, n=3/group
  - PS19 Vehicle
  - PS19 EpoD 0.3mg/kg
  - PS19 EpoD 1mg/kg
  - WT Vehicle
- ON FAT
  - Intravitreal injection of ^{35}S-Methionie in both eyes, 0.5mci/eye
- 3 hours after injection
  - Dissect mouse ON and cut into 7 consecutive 1 mm segments from individual mouse (without pooling ON together)
- CPM counts for ^{35}S in each ON segment.
- SDS gels for individual mouse optic nerve segments.

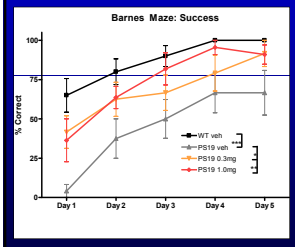


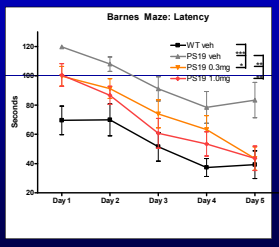
^{35}S-CPM Counts: Mixed Model Parametric Test



Barnes Maze Test for Spatial Memory in 12-Month Old EpoD Treated PS19 Mice

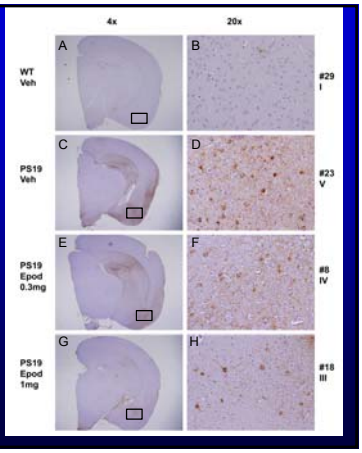




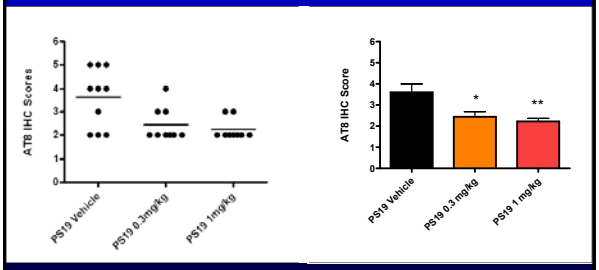


The part of memory responsible for recording information about the environment and its spatial orientation.

Lowest Levels of tangle pathology in EpoD- treated Mice at 12-Months of Age



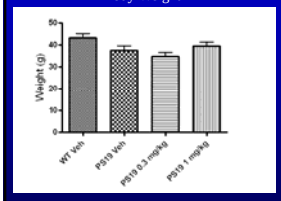
AT8 Tau Immunoactivity in EpoD- or Vehicle-Treated PS19 Mice at 12-Months of Age



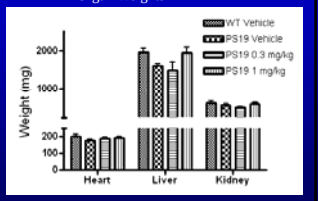
One way ANOVA non parametric Statistic test

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

Body Weight



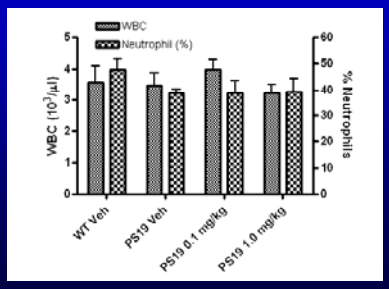
Organ Weights



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 Counts(CBC)

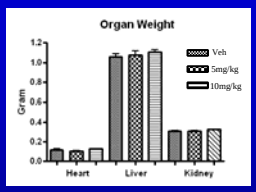


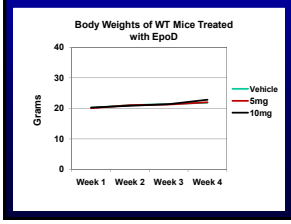
No Adverse Effects on CBC

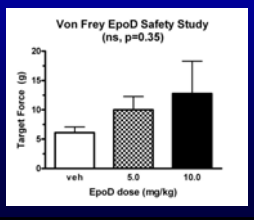
Safety Analyses with Higher Dose of EpoD in WT Mice

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

Societal Cost: Prevalence: 5 M and rising rapidly; $150B- one of the largest single health care burdens

Genomic

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

Symptomology

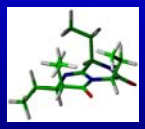
- Patient/family complaints about "forgetfulness"

Biomarkers

- Aβ42/40 ratio in CSF
- CSF phosphotau
- Deviation from normal suggest pathology of an amyloid type or neurodegeneration

Pet/MR Imaging

- Aggregated Aβ42 specific PET measure on PET-CT
- Hippocampal localization; extent and severity of disease confirmed
- Confirm therapy effectiveness



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

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It Takes a Great Team!

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Holly Soares, Eric Siemers, Piotr Lewczuk, William Potter
& many more collaborators

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- ADNI Core leaders and Core members
- ADNI Site PIs
- Our volunteer subjects

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