ADNI Biomarker Core

Progress Report

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ADNI 2: Biomarker Core, study year 1

- Biofluids received, aliquoted, stored at -80 ^{0}C
  - BASELINE: 452 serum, 452 plasma, 406 CSF
  - 6 month visit: 190 serum, 190 plasma
  - 12 month visit: 123 serum, 123 plasma
- NIA RARC-approved studies
  - PPSB-sponsored ADNI/PPSB/FNIH add-on proteomic study using Rules Based Medicine xMAP Luminex multiplexed immunoassay for 159 analytes in 327 blinded ADNI 1 CSF samples:
    - 2 AAIC posters* and subsequent manuscripts to follow
    - Dataset and study Primer uploaded on ADNI LONI website following data qc
  - PPSB-sponsored add-on study to measure beta secretase 1(BACE 1) enzyme activity & sAPPβ (the N-terminal secreted fragment of APP generated by BACE 1), in 402 BASELINE blinded CSF samples. Dataset and study Primer uploaded on ADNI LONI webstite.
  - Retrieved, shipped 372 BASELINE blinded CSF samples & matching BASELINE blinded plasma samples to an investigator whose study was reviewed and approved by RARC

*__Cerebrospinal fluid (CSF) biomarkers in Alzheimer's disease (AD), mild cognitively impaired (MCI) and age-matched healthy controls (HC) from the ADNI cohort.__ Judith A. Siuciak1, William Z. Potter2, Eve Pickering3, Fred Immermann3, Max Kuhn3, Leslie M. Shaw4, the Alzheimer's Disease Neuroimaging Initiative (ADNI) and Foundation for NIH (FNIH) Biomarkers Consortium CSF Proteomics Project Team **Poster #P1-319**

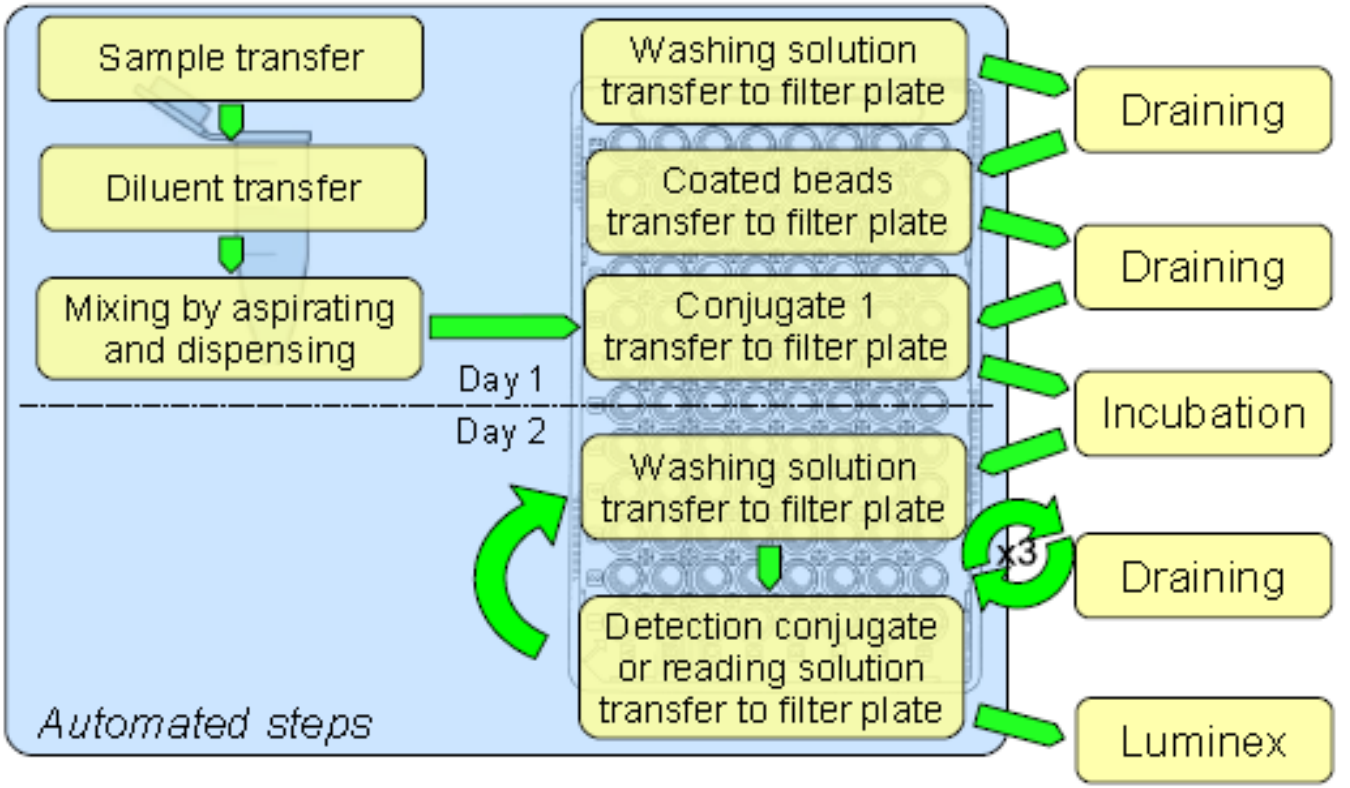
*__Cerebrospinal fluid (CSF) vs Plasma based biomarkers in Alzheimer's disease (AD), mild cognitive impaired (MCI) and age-matched healthy controls (HC) from the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort.__ William Z. Potter1, Eve Pickering2, Fred Immermann2, Max Kuhn2, Judith A. Siuciak3, Leslie M. Shaw4, the Alzheimer's Disease Neuroimaging Initiative (ADNI) and Foundation for NIH (FNIH) Biomarkers Consortium CSF Proteomics Project Team **Poster # P1-320**

Cross-Validation Of A Plasma Multi-Analyte Panel (RBM) Across Three Independent Cohorts, William T. Hu, MD, PhD,David M. Holtzman, MD,Anne M. Fagan, PhD,Leslie M. Shaw, PhD,Richard Perrin, MD, PhD,Steven E. Arnold, MD, PhD, Murray Grossman, MD,Chengjie Xiong, PhD,Rebecca Craig-Schapiro, PhD,Christopher M. Clark, MD,Eve Pickering, PhD,Max Kuhn, PhD, Yu Chen, PhD,Vivianna M. Van Deerlin, MD, PhD,Leo McCluskey, MD, MBE,Lauren Elman, MD,Jason Karlawish, MD,Alice Chen-Plotkin, MD, Howard I. Hurtig, MD,Andrew Siderowf, MD,Frank Swenson, PhD,Virginia M.-Y. Lee, PhD, MBA, John C. Morris, MD,John Q. Trojanowski, MD,PhD,and Holly Soares, PhD; for the Alzheimer's Disease Neuroimaging Initiative.

Plasma biomarkers associated with apolipoprotein E genotype and Alzheimer disease. Holly D. Soares, PhD; William Z. Potter, MD, PhD; Eve Pickering, PhD; Max Kuhn, PhD;Frederick W. Immermann, MStat; David M. Shera, ScD; Mats Ferm, PhD; Robert A. Dean, MD, PhD;Adam J. Simon, PhD; Frank Swenson, OD, PhD; Judith A. Siuciak, PhD; June Kaplow, PhD;Madhav Thambisetty, MD, PhD; Panayiotis Zagouras, PhD; Walter J. Koroshetz, PhD; Hong I. Wan, PhD;John Q. Trojanowski, MD, PhD; Leslie M. Shaw, PhD; for the Biomarkers Consortium Alzheimer's Disease Plasma Proteomics Project. Arch Neurol. Published online July 16, 2012.doi:10.1001/archneurol.2012.1070

ADNI 2: Biomarker Core

- Automated sample processing for plasma $A\beta_{1\text{-}42/1\text{-}40}$ INNO-BIA Plasma $A\beta$ forms immunoassay



- Although did not reach clinical significance for AD detection in the ADNI 1 population earlier Changes may be important and significant, and this test is useful as a PD marker

Figurski M, et al, Alz & Dem, 2012;8:250-260; Toledo J, et al, Acta Neuropath 2011;122:401-413

ADNI 2: Biomarker Core

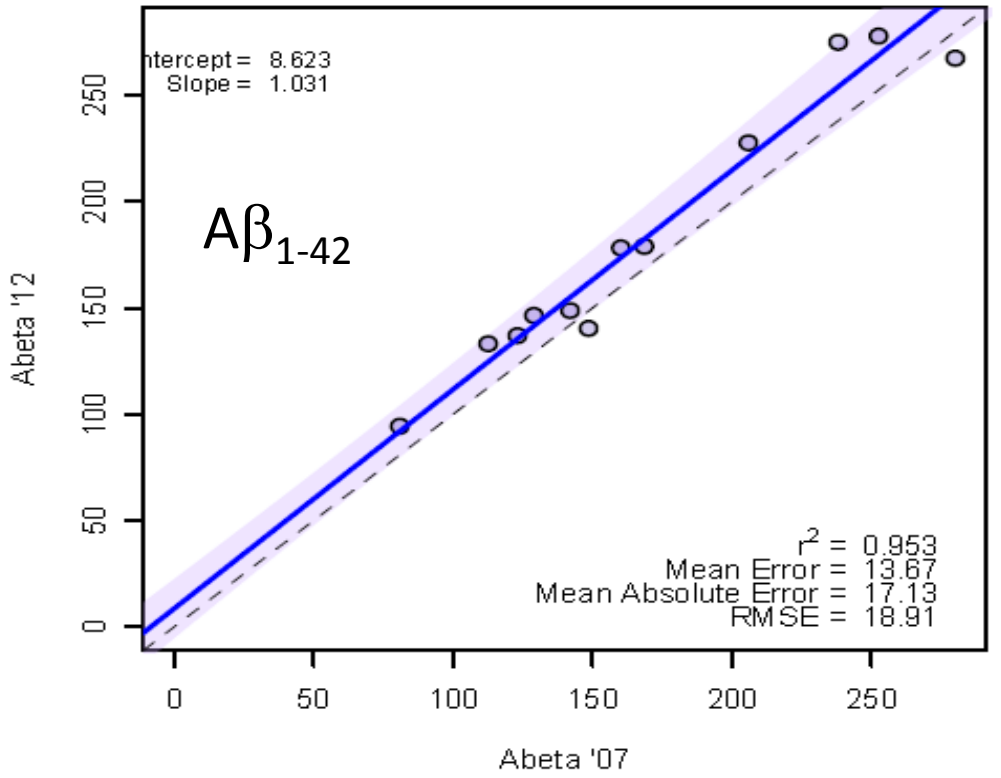
- Batch analyses of ADNI GO + ADNI 2 CSFs

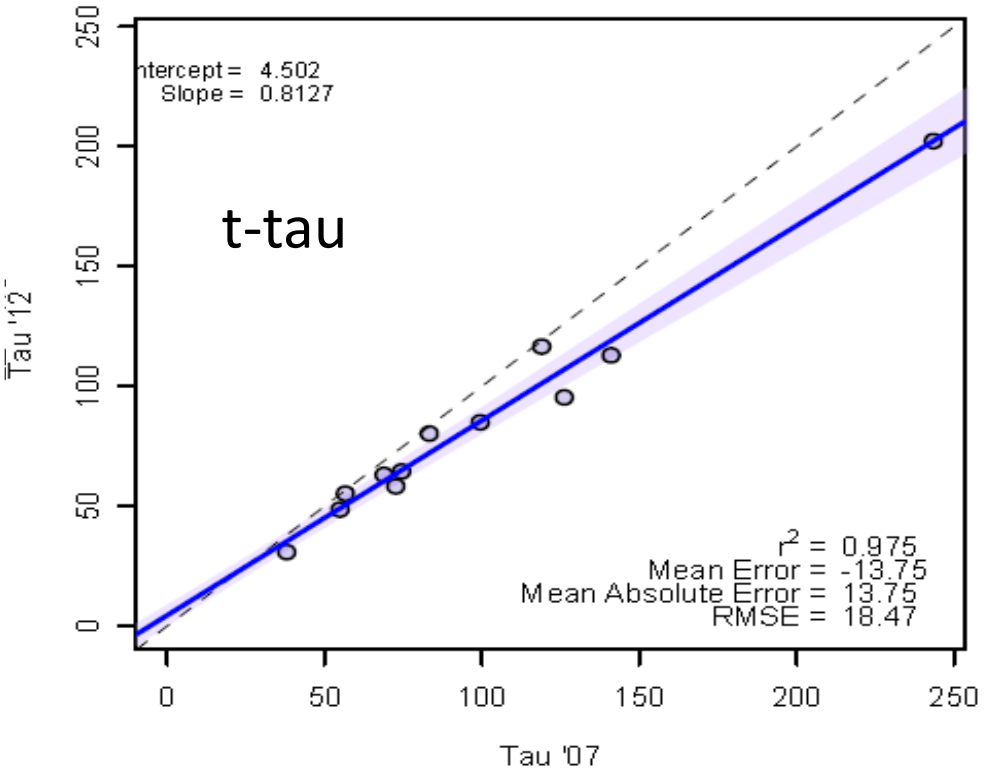
— All ADNI GO + ADNI 2 CSFs (through 2/21/2012)
— N=467 [390 BASELINE + 77 follow-up]; in addition, 28 replicates
— This set of CSFs is enriched with EMCI subject samples, smaller numbers of new LMCI, early AD and cognitively normal subjects
— AlzBio 3 immunoassay (Fujirebio/Innogenetics) for $A\beta_{1\text{-}42}$, t-tau, p-tau_{181}
— Assessed 2012 vs 2007 using 12 CSFs(ADNI 1 BASELINE) across conc. range for lot to lot performance assessment prior to use and bridging of 2012 dataset to 2007 BASELINE dataset
— ~5% random samples re-tested
— Inclusion of 2 new CSF pools, 1 (cog normal), 1 (clin AD) & 2 aqueous qc's
— Detailed quality control review completed and included in a report, "ADNI GO and ADNI 2 CSF report"; blinded to diagnoses until data lock
— Reported on analytical performance in Melbourne
— Reported preliminary BASELINE analyses at the ADNI meeting in New Orleans

ADNI 2: Biomarker Core

- Continued assessments of analytical performance of the AlzBio3 xMAP immunoassay system:
  - Precision performance
    - Calibrator reproducibility
    - Run-to-run precision of new abnormal and normal CSF pools
    - Test/re-test precision for randomly selected ~5%
  - Concentration accuracy with ADNI 2007 as basis for bridging 2012 CSF data.
- Clinical utility analyses include:
  - Cross sectional comparisons of the EMCI cohort with each of the following cohorts: LMCI, AD, NC biomarker data. ***Do EMCI CSF profiles differ from LMCI subject profiles?***
  - characteristics of the data distributions, eg, **presence of bimodal distribution for $A\beta_{1\text{-}42}$;**
  - assess **predictive performance of CSF Baseline biomarkers for cognitive decline (eg ADAScog) in each ADNI cohort. Comparison with AV45**
  - **Using AV45 as a clinical endpoint, perform ROC analyses: assess CSF biomarker cutpoints**
  - Estimate variance around cutpoint for $A\beta_{1\text{-}42}$, t-tau, p-tau_{181} and ratios

Performance assessment for AlzBio3 reagents: 2012 vs 2007

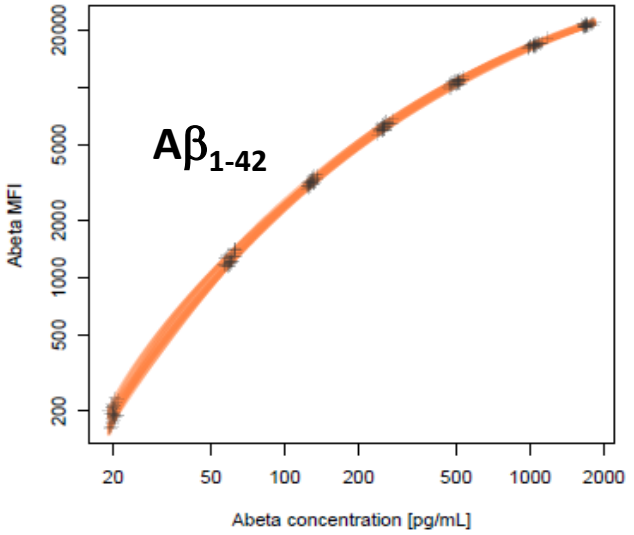


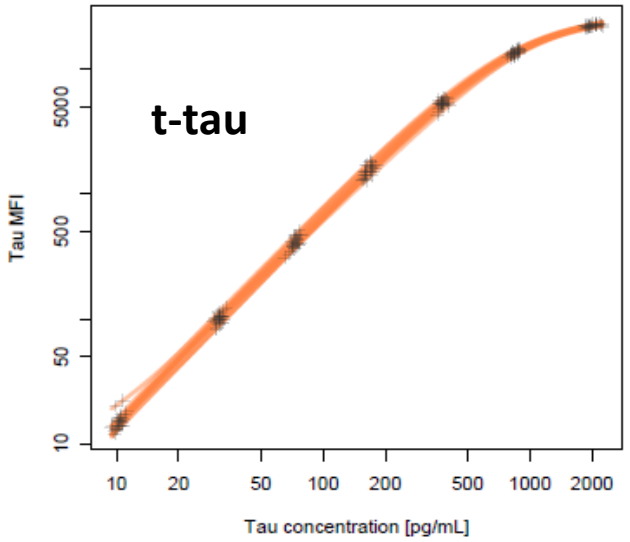


Abeta '07 are ADNI 1 BASELINE CSF results on 12 selected subjects, using Innogenetics AlzBio3 xMAP.
Abeta '12 are never before analyzed replicate CSF aliquots (stored at -80 ^{0}C)from the 12 selected subjects.
The analyses done in 2007 were done as one batch that included all ADNI 1 BASELINE CSF samples.
The analyses done in 2012 were done as one batch (different lots of reagents and calibrators than used in the 2007 analyses), using Fujirebio/Innogenetics AlzBio3 xMAP immunoassay.

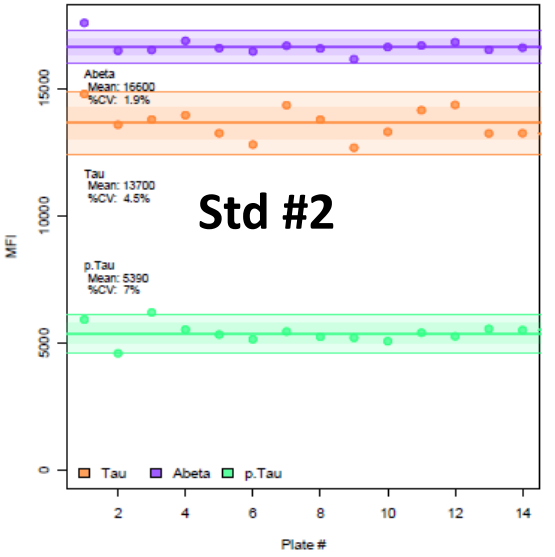
Calibrator reproducibility

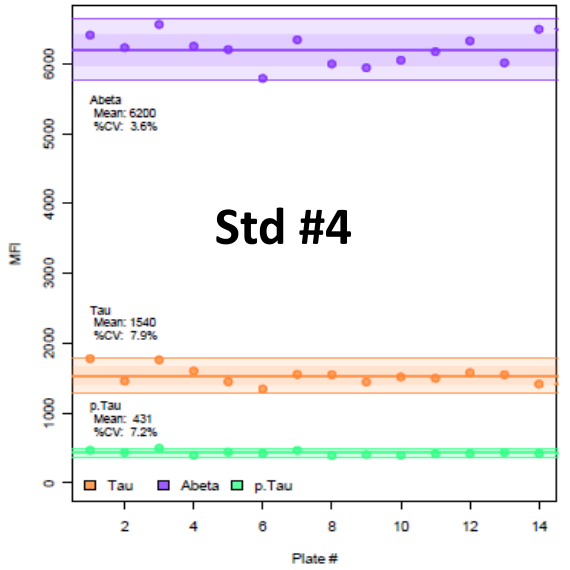
Median MFI vs nominal concentration





MFI values for each run

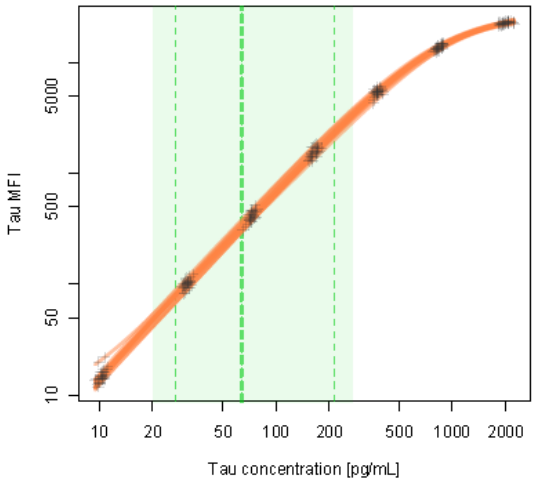


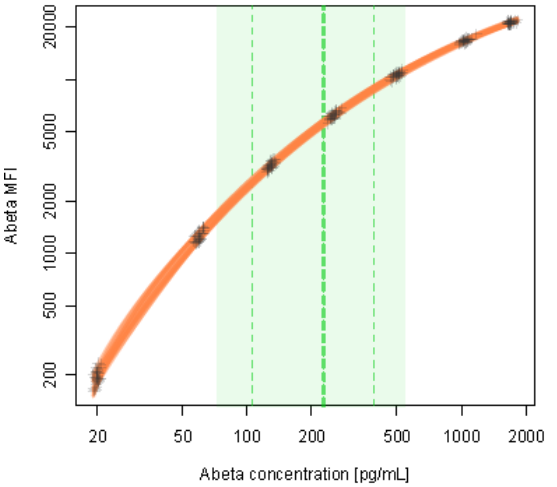


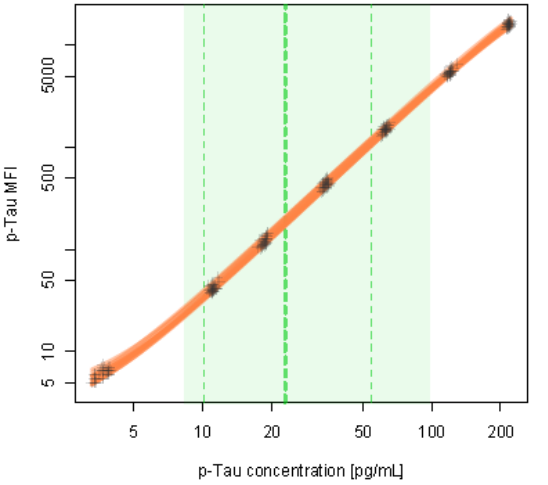
Precision of back-calculated stds

Analyte	Sample	N	Mean	SD	CV	5% CI	95% CI
Tau	Standard1	27	2011	120	5.97	1847	2235
	Standard2	28	848.2	30.44	3.59	800.2	890.9
	Standard3	28	377.6	12.98	3.44	358.8	404.5
	Standard4	27	165.8	7.134	4.3	154.9	178.8
	Standard5	28	73.1	2.886	3.95	67.25	77.68
	Standard6	28	31.51	1.084	3.44	29.79	33.6
	Standard7	28	10.31	0.4109	3.99	9.53	11.06
Abeta	Standard1	27	1699	46.88	2.76	1644	1806
	Standard2	27	1046	43.62	4.17	982.6	1138
	Standard3	28	500.9	18.14	3.62	467.4	530.7
	Standard4	28	254.1	9.289	3.66	239	273.8
	Standard5	28	128.7	3.464	2.69	124	135.4
	Standard6	28	59.93	1.937	3.23	56.92	62.92
	Standard7	28	20	0.4089	2.04	19.27	20.71
p-Tau	Standard1	28	217.3	2.81	1.29	212.4	222.1
	Standard2	27	120.6	3.01	2.5	115.2	126.2
	Standard3	28	63	1.362	2.16	60.75	65.66
	Standard4	28	34.49	0.7841	2.27	33.07	35.7
	Standard5	28	18.62	0.465	2.5	17.68	19.23
	Standard6	28	11.13	0.2863	2.57	10.71	11.71
	Standard7	27	3.648	0.2192	6.01	3.334	3.944

ADNI subject CSF biomarker concentration ranges in relationship to calibrators' ranges







t-tau

$A\beta_{1\text{-}42}$

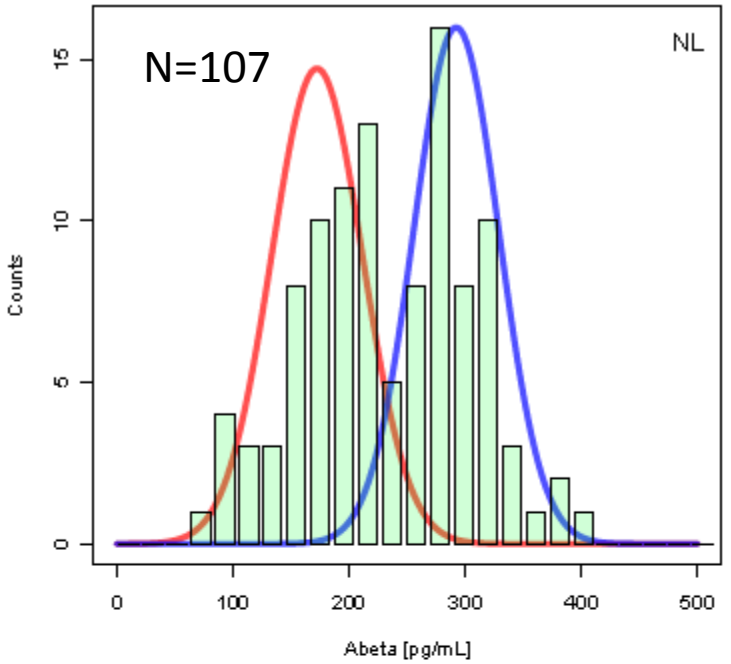
$p\text{-}tau_{181}$

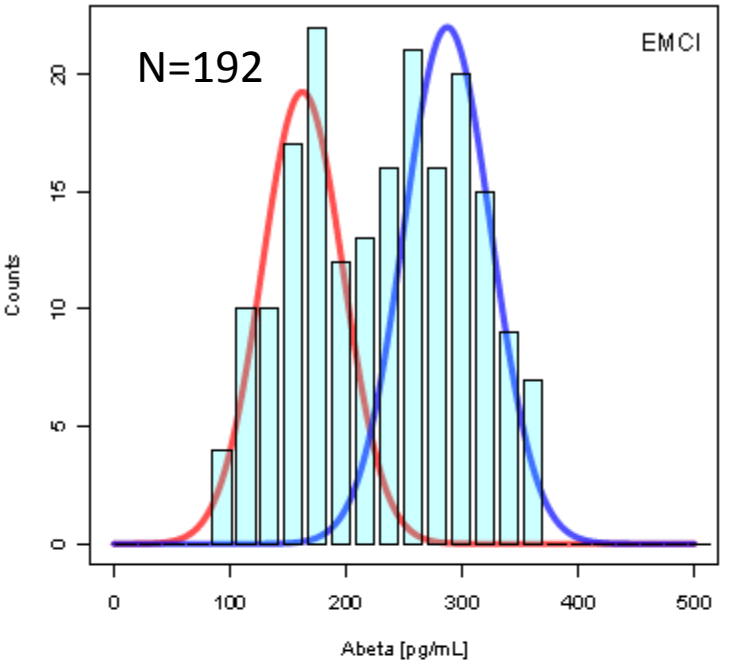
*The range of biomarker concentrations obtained in ADNI GO & ADNI 2 CSF samples is indicated by the green shaded ranges

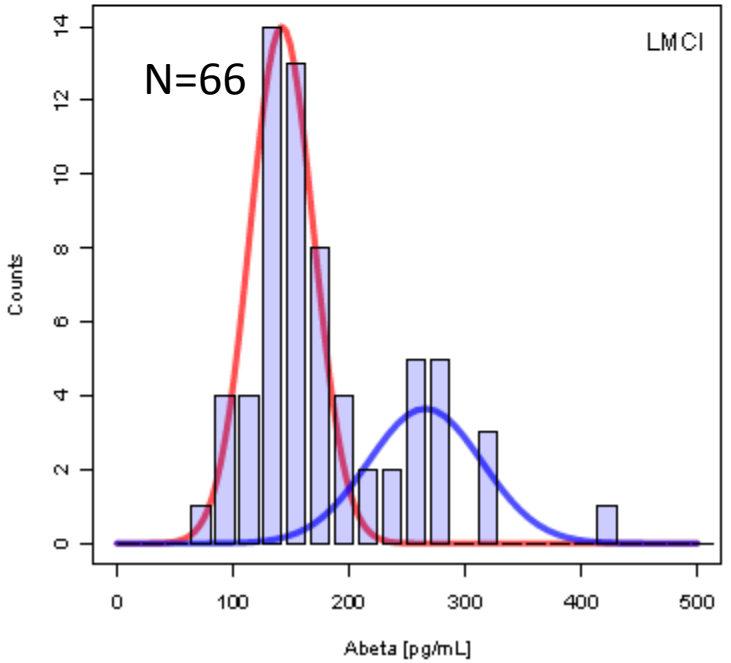
ADNI GO & ADNI 2 CSF biomarkers

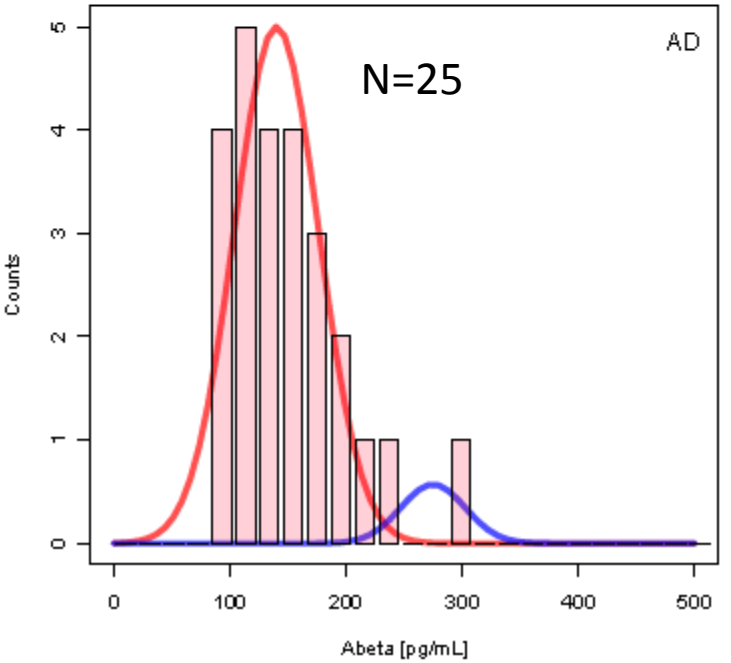
	APO e4 (%)	$A\beta_{1-42}$ (pg/mL)	t-tau (pg/mL)	p-tau$_{181}$ (pg/mL)	t-tau/$A\beta_{1-42}$	p-tau/$A\beta_{1-42}$
Normal (107)	22%	233±71	73±34	21.3±8.0	0.37±0.27	0.10±0.6
EMCI (192)	39%	231±72*	81±53**	22.6±11***	0.45±0.49****	0.11±0.09*****
LMCI (66)	53%	181±68	103±55	30±16	0.68±0.45	0.18±0.12
AD (25)	58%	151±52	134±59	33±13	0.97±0.49	0.22±0.12

* $A\beta_{1-42}$: p<0.000001 vs AD; p<0.00001 vs LMCI, p=0.83 vs NL. ** t-tau: p<0.000005 vs AD, p<0.005 vs LMCI, p=0.86 vs NL.
p-tau$_{181}$:p<0.0005 vs AD, p<0.00005 vs LMCI; p=0.91 vs NL. * t-tau/ $A\beta_{1-42}$: p<0.0000001 vs AD, p<0.00005 vs LMCI, p=0.99 vs NL
*****p-tau$_{181}$/ $A\beta_{1-42}$: p< 0.00005 vs AD, p<0.000001 vs LMCI; p=0.99 vs NL

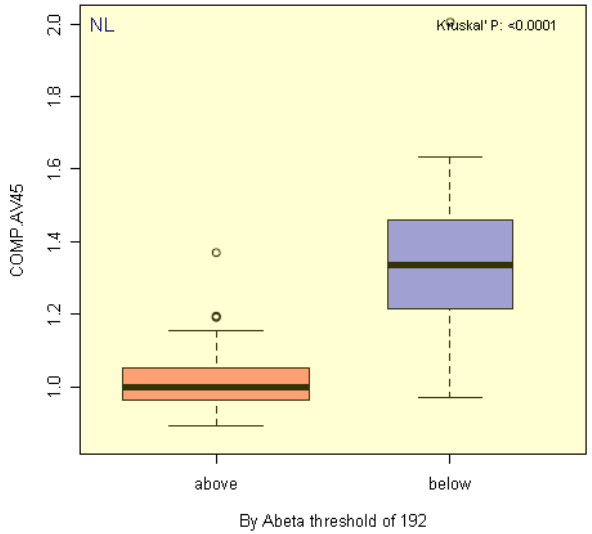

N=107
NL
Counts
Abeta [pg/mL]

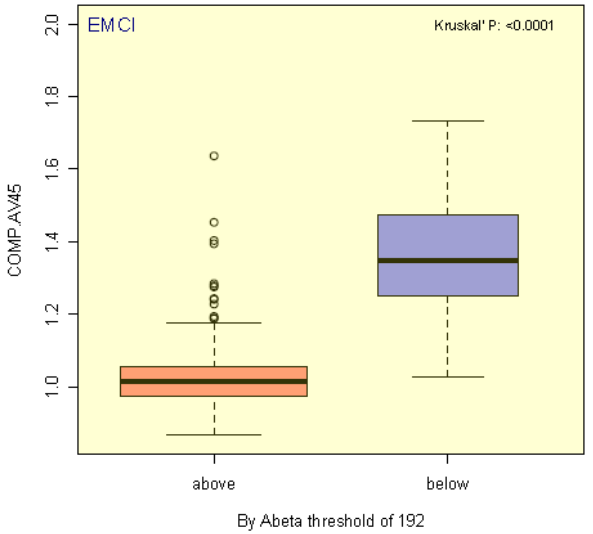

N=192
EMCI
Counts
Abeta [pg/mL]

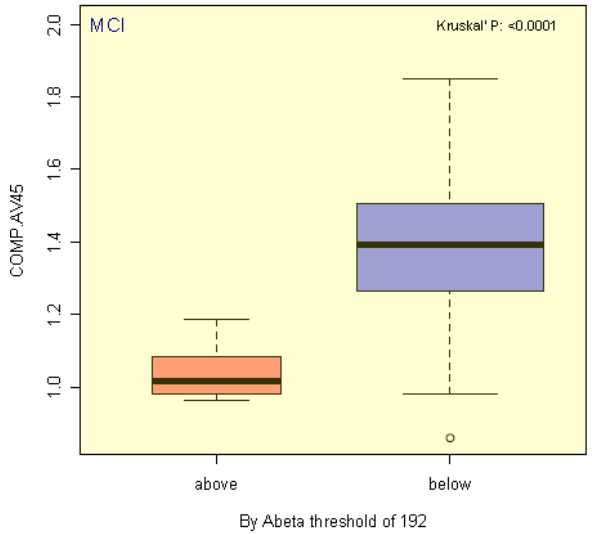

N=66
LMCI
Counts
Abeta [pg/mL]

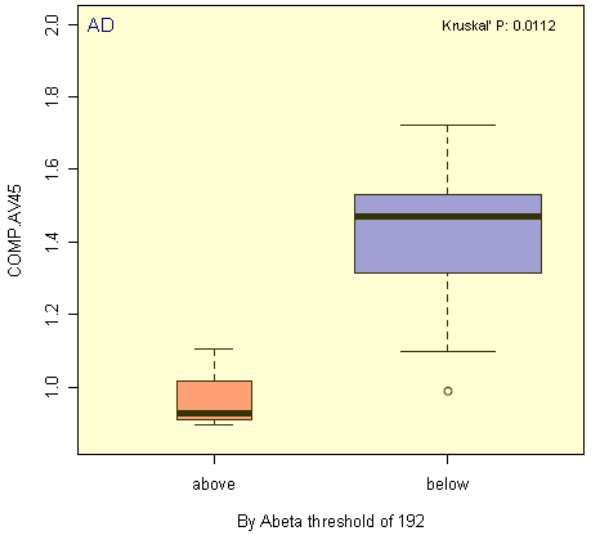

N=25
AD
Counts
Abeta [pg/mL]

Baseline AV45 SUVR results in ADNI subjects with CSF $A\beta_{1-42}$ >192 pg/mL or <192 pg/mL





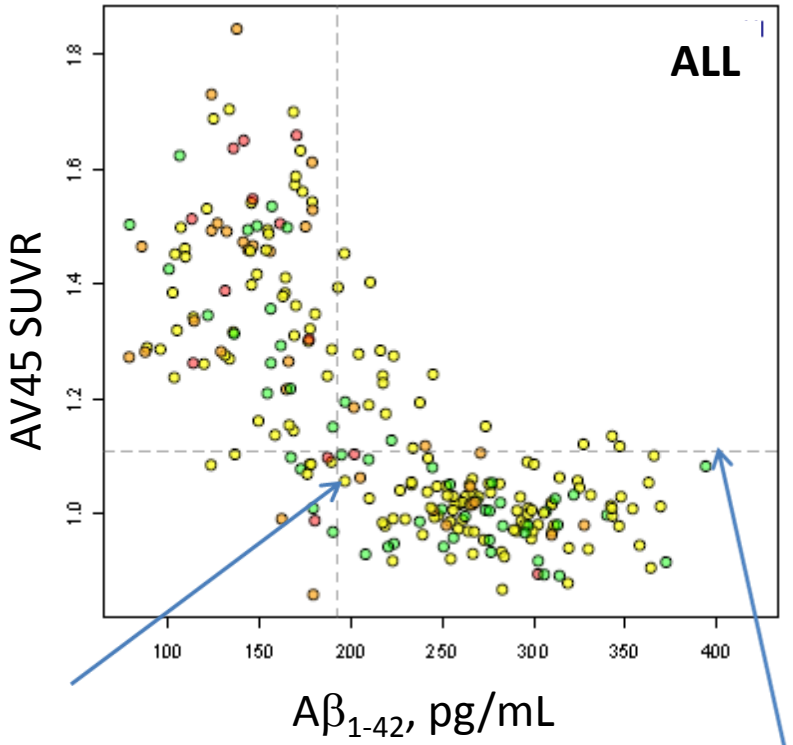


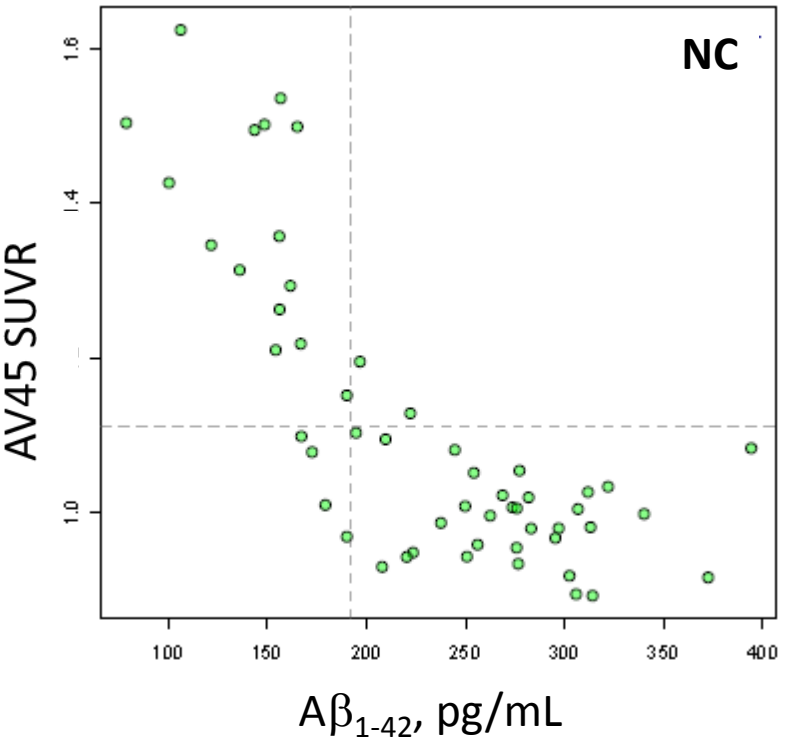


Baseline AV45 SUVR results for ADNI subjects (mean±SD) with $A\beta_{1-42}$ <192 pg/mL or >192

	$A\beta_{1-42}$ <192pg/mL	$A\beta_{1-42}$ >192pg/mL	*p*
ALL n=374	1.37±0.20	1.03±0.10	<0.0001
NC n=103	1.34±0.21	1.02±0.08	<0.0001
EMCI n=187	1.35±0.18	1.04±0.12	<0.0001
LMCI n=62	1.38±0.22	1.03±0.06	<0.0001
AD n=22	1.42±0.19	0.98±0.11	<0.05

AV45 SUVR vs CSF $A\beta_{1-42}$ in ADNI GO and ADNI 2 subjects

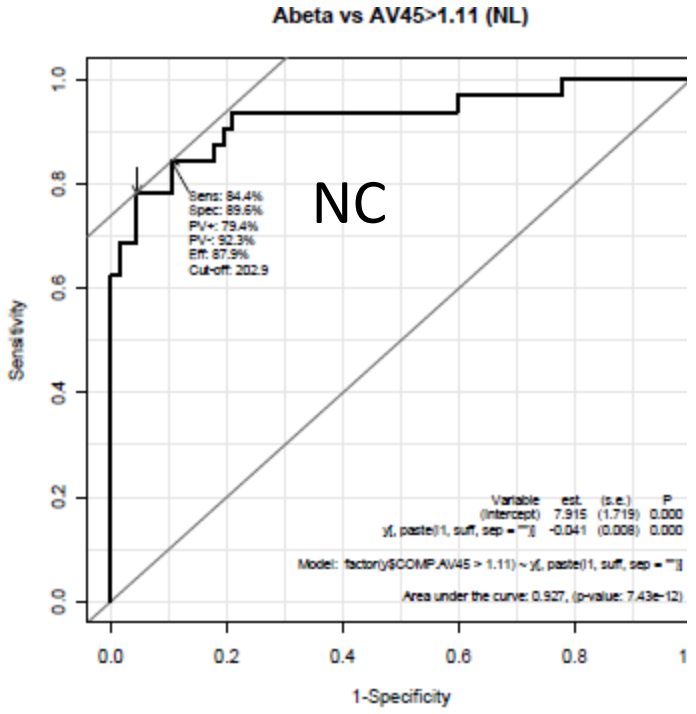


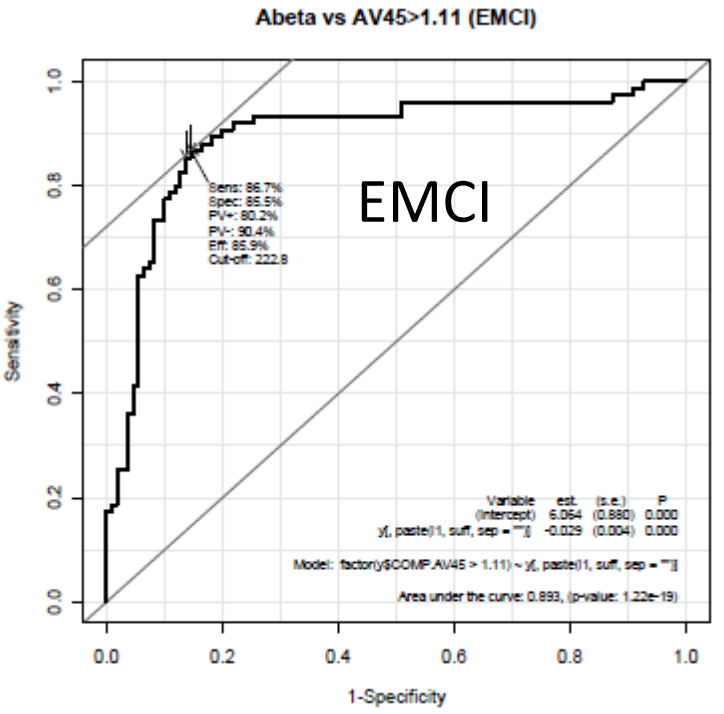


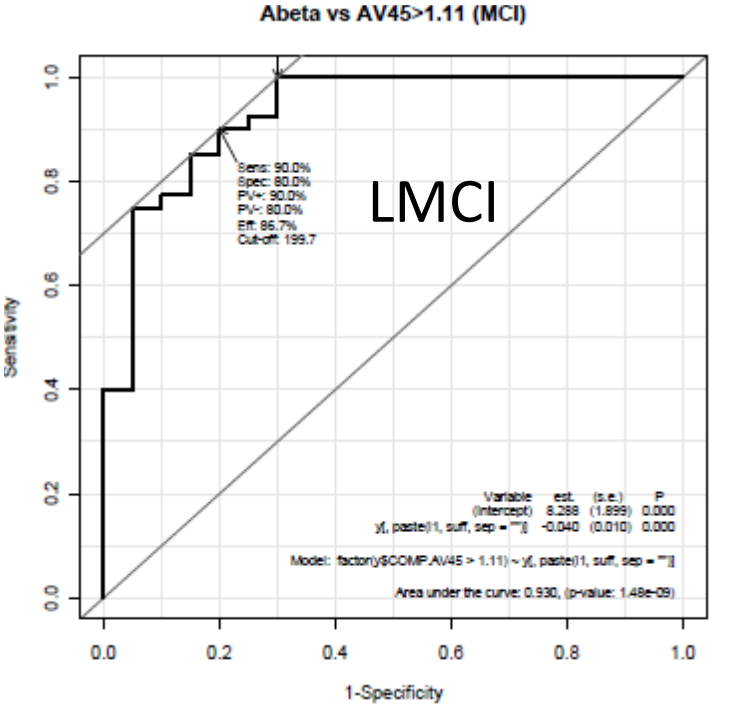
192 pg/mL pathologically based Cutpoint (Shaw, et al, 2009)

1.11 SUVR cutpoint as described by Landau and Jagust (ADNI web site)

ROC curve analyses: CSF $A\beta_{1\text{-}42}$ vs AV45 (1.11 cutpoint)





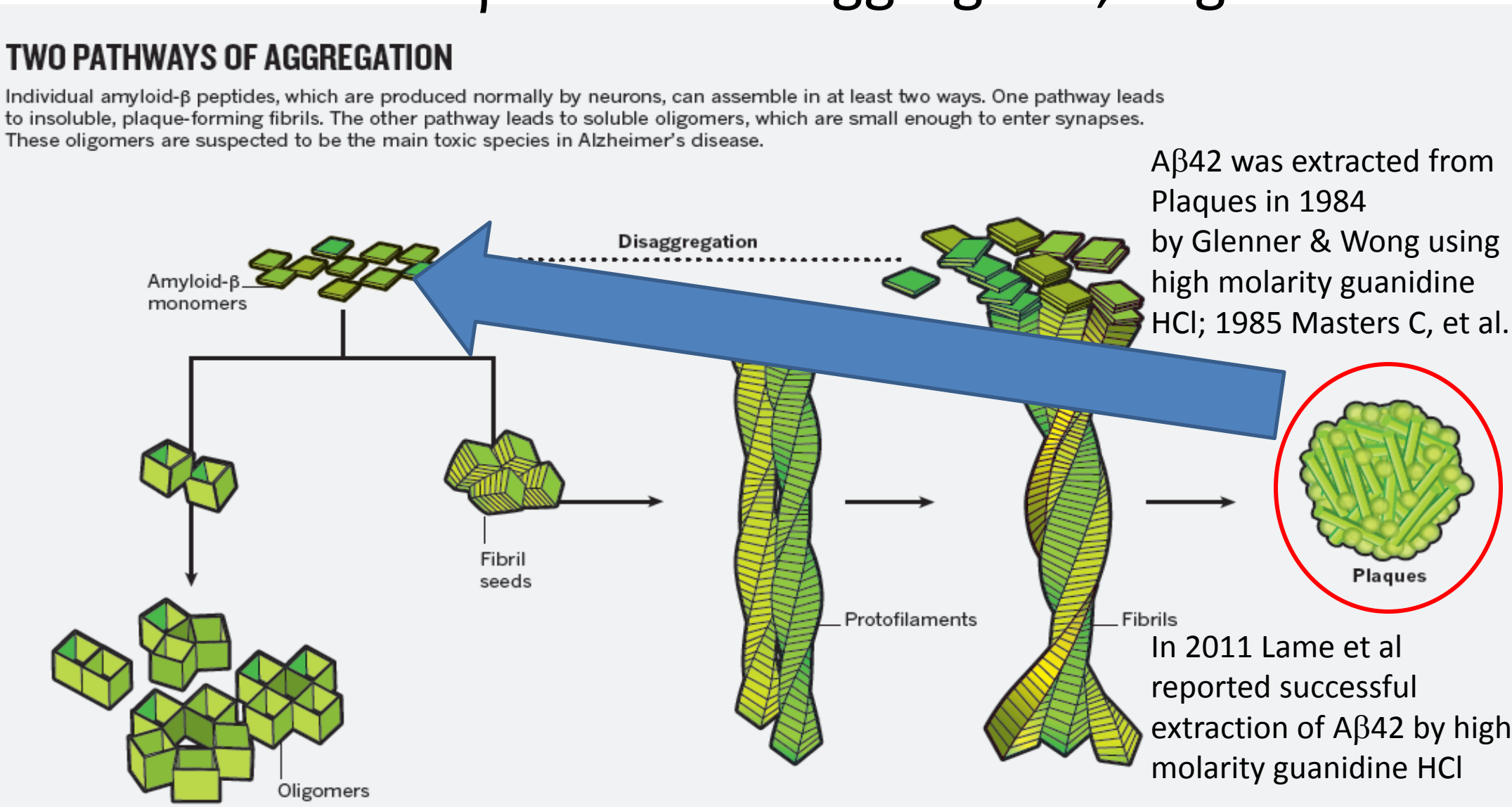


AUC	0.93	0.89	0.93
Sens	84.4%	86.7%	90.0%
Spec	89.6%	85.5%	80.0%
PPV	79.4%	80.2%	90.0%
NPV	92.3%	90.4%	80.0%
Accuracy	87.9%	85.9%	86.7%
Cutpoint	203 pg/mL	223 pg/mL	200 pg/mL

Candidate reference method for $A\beta_{1\text{-}42}$

- Development of an mrmHPLC/tandem mass spectrometry direct sample preparation methodology for quantification of $A\beta_{1\text{-}42}$.
- The method uses high concentration of guanidine HCl to denature all species of $A\beta_{1\text{-}42}$ (monomer, dimer, higher oligomers, complexes with other proteins, aggregates)
- A group of investigators is pursuing this collaboratively [Erin Chambers(Waters Co), Rand Jenkins(PPD), Les Shaw(UPenn) and Kaj Blennow(Goteborg)] as an ***AA/GBSC-sponsored effort to develop a candidate reference method. This will have several spin-off benefits including use for assigning accuracy-based values to CSF pools and harmonization of CSF Aβ1-42 across centers.***
- ***Each of the individual laboratories is in the process of validating their methodology and an interlab round robin study is in planning stage***
- At least 4"lab years" of work has been put into this project collectively and the group is optimistic about having viable assays ready for testing in the near future.
- The clinical performance of this type of methodology has to be determined.

Treatment of CSF with guanidine HCl to release "denatured" Aβ1-42 from aggregates, oligomers



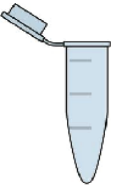
Aβ42 was extracted from Plaques in 1984 by Glenner & Wong using high molarity guanidine HCl; 1985 Masters C, et al.

In 2011 Lame et al reported successful extraction of Aβ42 by high molarity guanidine HCl

ADNI 2: Biomarker Core

- SRM LC/MSMS method for Aβ peptides in CSF

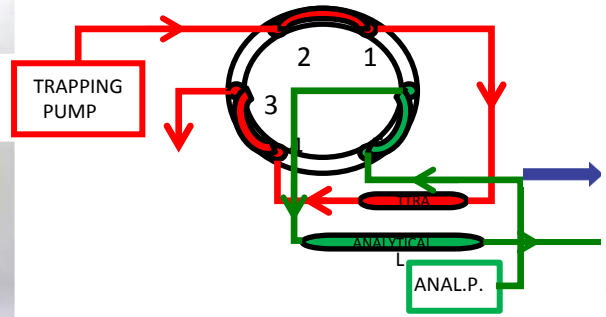
- N^{15}-Aβ peptide ISTDs added to CSF samples
- Guanidine·HCl
- SPE extraction – 96 well Format
- 2D HPLC/SRMtandem mass spectrometry



Protein LoBind Tubes

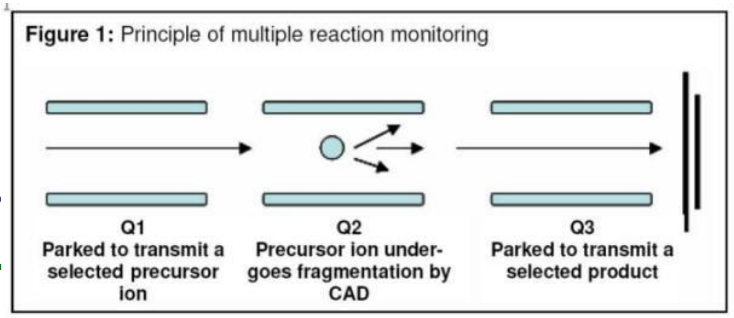
Oasis MCX μElution Plate

ACQUITY UPLC



Injection position of switching valve - 2D chromatography

Korecka M et al, AAIC Poster #P1-317



m/z 1129.0 → *m/z* 1078.8

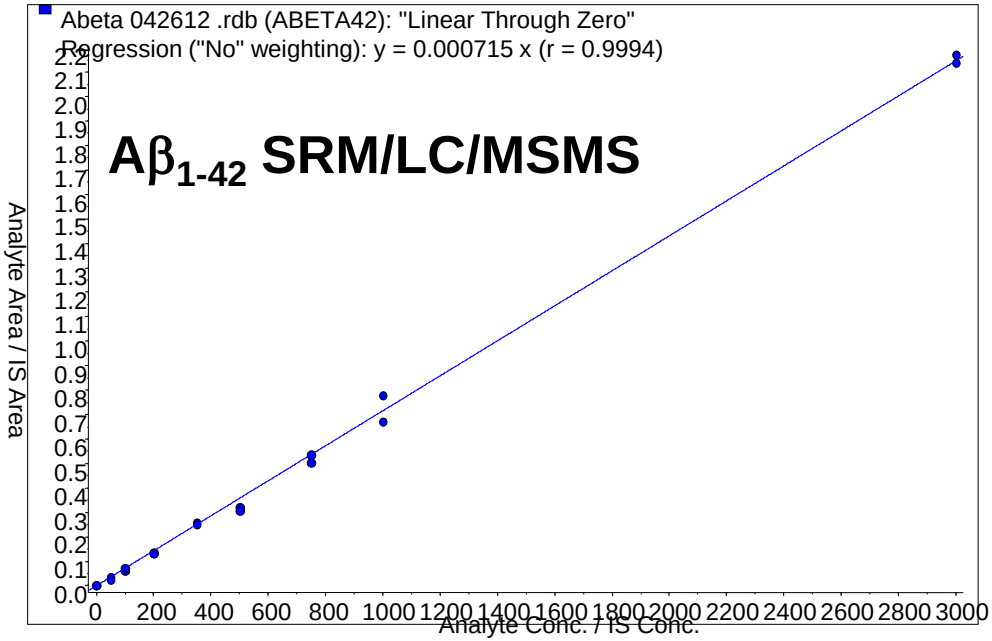
($A\beta_{1-42}$ precursor [4+] ion) (product [4+] ion)

Tandem mass spectrometer API 5000 (ABSciex)

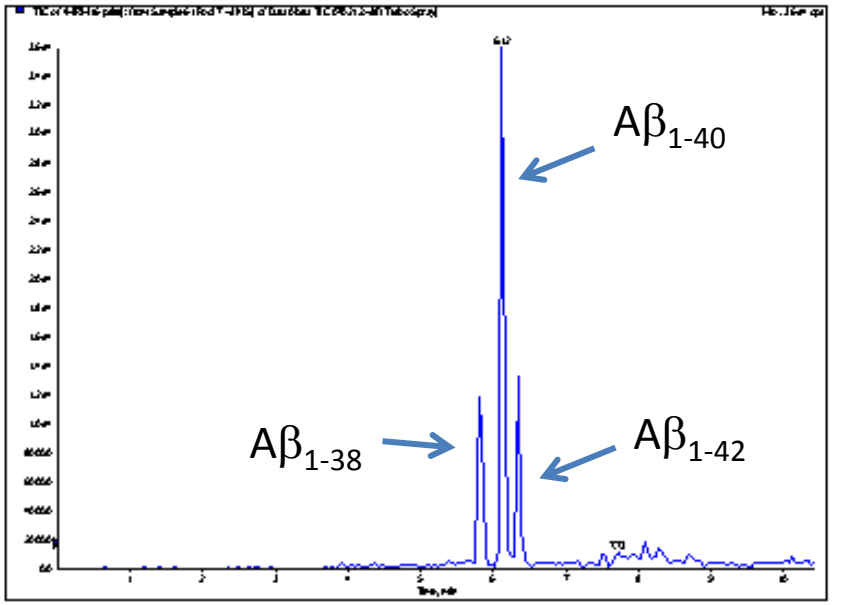
ADNI 2: Biomarker Core

- Established
  - Surrogate matrix
  - LLOQ/UPOQ
  - Linearity
  - Precision performance
  - Recovery from hCSF
  - Equivalence between surrogate matrix and hCSF

More details at the Korecka et al AAIC poster #P1-317



Typical calibration curve for the quantitation of amyloid beta 1-42 (single analyte assay). An artificial CSF with addition of BSA (4mg/mL) was a matrix for calibrators preparation.



hCSF pool sample

Efforts underway to improve standardization and achieve harmonization

- ADNI(standardization of imaging and biofluid biomarkers in AD)
- Alz Assn: International qc program; Global Biomarker Standardization Consortium
- CAMD[Coalition Against Major Diseases Biomarker Working Group]
- PPMI (standardization of biofluid and imaging biomarkers in PD)
- BIOMARKAPD(standardization of established and new biomarkers for AD & PD)
- UPenn/Wash U collaboration on ELISA/xMAP/PiB relationships(Anne Fagan, David Holtzman, John Morris, John Trojanowski)
- UPenn ADRC studies in neurodegenerative diseases with autopsy diagnosis:

  xMAP/ELISA data integration(Jon Toledo, David Irwin, Li-San wang, John Trojanowski)
- Collaborative studies between labs for xMAP immunoassay:
  - With Mayo Clinic (Ron Petersen & Roy Dyer)
  - With Japan ADNI (Takeshi Iwatsubo, Ryosun Kuwano & Hiroyuki Arai)
- ABSI: Innogenetics-sponsored workgroup on standardization guidelines

  [Alzheimer's Biomarkers Standardization Initiative]

It takes a great team effort!

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