# ADNI 2 Biomarker Core review
# Aims & Challenges as we plan for ADNI 3

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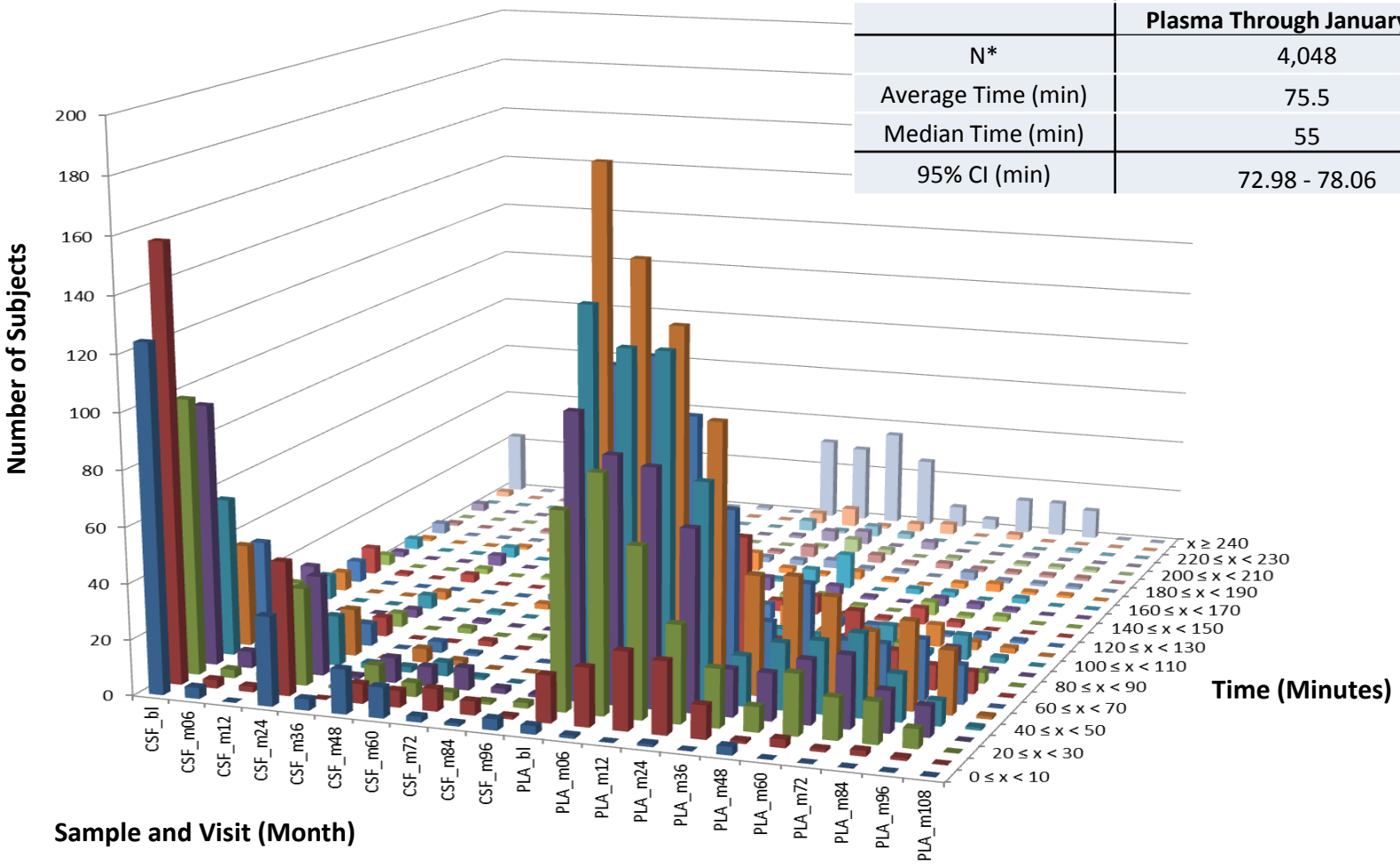
# Biomarker Core

- Biofluid report update
- 2015 ADNI 2 4th batch analyses of CSF completed and uploaded 4/14/15
- mrm/tandem mass spectrometry reference method for $A\beta_{1\text{-}42}$, developed, published & awaiting final mass assignment by the IRMM to the reference $A\beta_{1\text{-}42}$ we are using. This will permit final calculation of ADNI 1 BASELINE CSF **$A\beta_{1\text{-}42}$** data, and upload results (will include **$A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}38}$** ) on LONI. Work over past ~3yrs in collaboration with Alz Assn GBSG
- Highlights of CSF biomarker longitudinal studies-implications of the trajectories for CSF $A\beta_{1\text{-}42}$ , t-tau & p-$tau_{181}$
- Studies reported on the ADNI/LONI website & studies newly approved by RARC/NIA/ADNI using ADNI biofluids
- Initiated planning process: move from the AlzBio3 immunoassay to an automated platform for CSF $A\beta_{1\text{-}42}$, t-tau, p-$tau_{181}$
- Aims for ADNI 3

# ADNI Biofluid processing tracking: Time from Sample Collection to Freezing for ADNI GO and ADNI 2 CSF and PLA

| | Plasma Through January 2015 | CSF Through January 2015 |
|---|---|---|
| N* | 4,048 | 1,174 |
| Average Time (min) | 75.5 | 52.4 |
| Median Time (min) | 55 | 28 |
| 95% CI (min) | 72.98 - 78.06 | 45.68 - 59.03 |




| ADNI GO and 2 | Total (CSF) | Total (PLA + SER) | Grand Total as of 1/26/2015 | Grand Total as of 4/1/2014 |
|---|---|---|---|---|
| Number of Biofluids Collected as of January 26th, 2015 | 1,279 | 8,182 | **9,461** | **8,600** |
| Number of Aliquots in Bank | 39,561 | 124,559 | **164,120** | **145,442** |

| All ADNI | Total (CSF) | Total (PLA + SER) | Grand Total as of 1/26/2015 | Grand Total as of 4/1/2014 |
|---|---|---|---|---|
| Number of Biofluids Collected as of January 26th, 2015 | 2,240 | 16,931 | **19,171** | **17,201** |
| Number of Aliquots in Bank | 66,130 | 229,079 | **295,209** | **287,420** |

# ADNI GO+2 CSF $A\beta_{1\text{-}42}$, t-tau & p-$tau_{181}$ analyses

| Subjects | BASELINE | 24 months* |
|---|---|---|
| **NC** | 158 | 88 |
| **SMC** | 96 | --- |
| **EMCI** | 275 | 120 |
| **MCI** | 155 | 77 |
| **AD** | 131 | 16 |
| **TOTAL** | **815** | **301** |

*Includes batch analysis uploaded on LONI/ADNI April 14, 2015: 112(BL + 24 mos) longitudinal sets of CSF samples.

# ADNI GO+2 BASELINE results

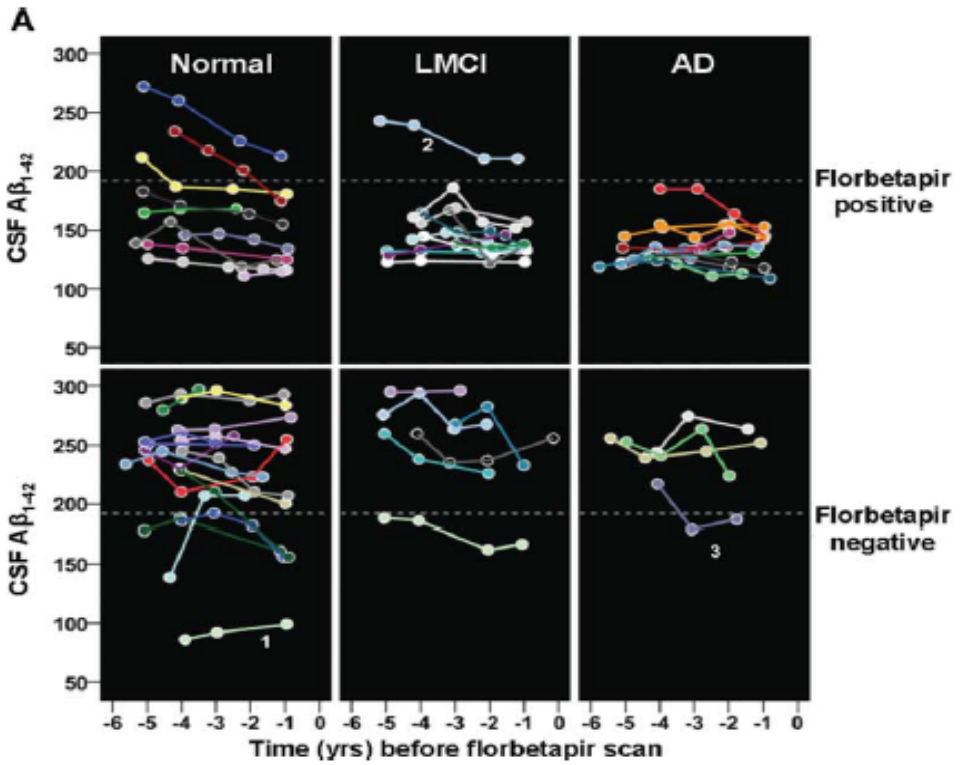
| | N | % APOE ε4 | t-tau (pg/mL) | $A\beta_{1-42}$ (pg/mL) | p-$tau_{181}$ (pg/mL) | t-tau/$A\beta_{1-42}$ | $ptau_{181}/A\beta_{1-42}$ | LRTAA2i |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| AD | 131 | 66.4 | 133±62 | 137±38 | 59±36 | 1.04±0.6 | 0.47±0.3 | 0.79±0.3 |
| | | | | | | | | |
| LMCI | 155 | 57.4 | 101±56 | 159±50 | 48±27 | 0.72±0.5 | 0.35±0.2 | 0.63±0.4 |
| | | | | | | | | |
| EMCI | 275 | 42.1 | 76±48 | 184±51 | 37±21 | 0.49±0.4 | 0.24±0.2 | 0.40±0.4 |
| | | | | | | | | |
| NC | 158 | 26.7 | 69±34 | 195±51 | 35±19 | 0.40±0.3 | 0.20±0.2 | 0.32±0.3 |
| | | | | | | | | |
| SMC | 96 | 33.3 | 65±31 | 201±49 | 38±21 | 0.36±0.2 | 0.21±0.2 | 0.31±0.3 |

# Longitudinal CSFs for ADNI I & “carryover” subjects

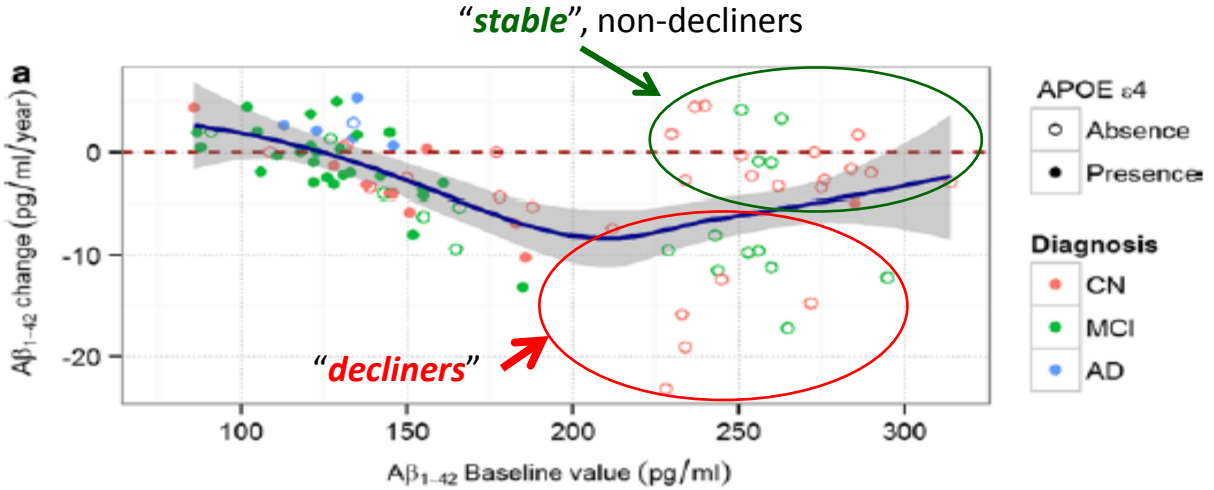
| Subjects | BL | Mo 12 | 24 | 36 | 48 | 60 | 72 | 84 |
|---|---|---|---|---|---|---|---|---|
| ADNI 1 | 419 | 328 | 104 | 82 | 66 | 37 | 21 | 4 |
| | | | | | | | | |
| ADNI 2 | 815 | -- | 301* | -- | // | // | // | // |
| | | | | | | | | |

*-- additional collections over next 8-9 months; //-- collections in ADNI 3

# ADNI subjects with BASELINE ***normal*** CSF $A\beta_{1\text{-}42}$: In some CSF $A\beta_{1\text{-}42}$ remains ***stable/normal*** & in some ***declines to abnormal***



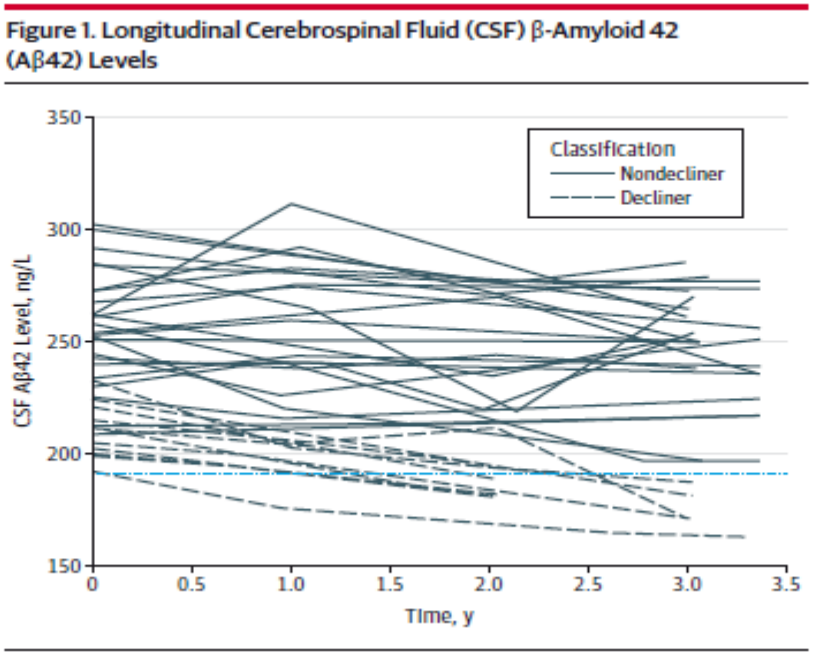

Landau et al, 2013




Toledo, et al, 2013

**20 *stable*, -0.5pg/mL/yr**

**14 *decliners*, -9.2 pg/mL/yr**



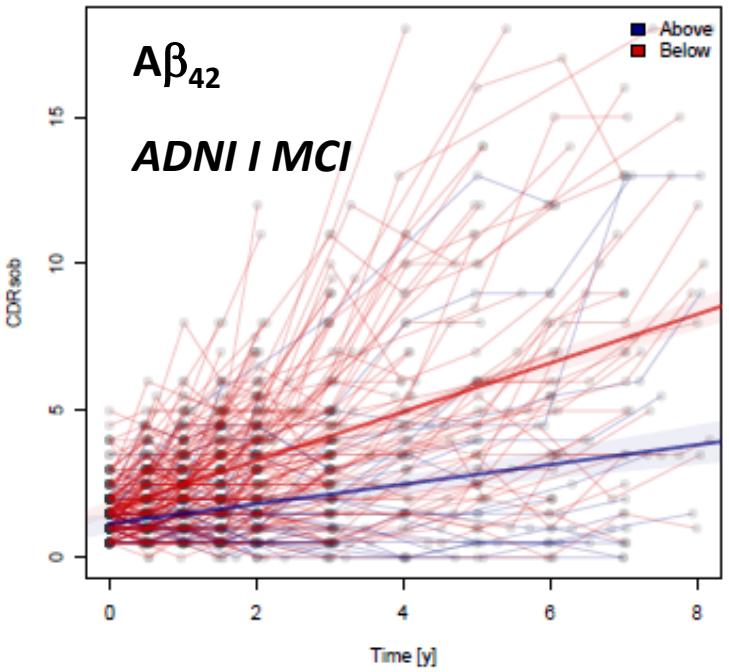

Mattsson, et al, 2015

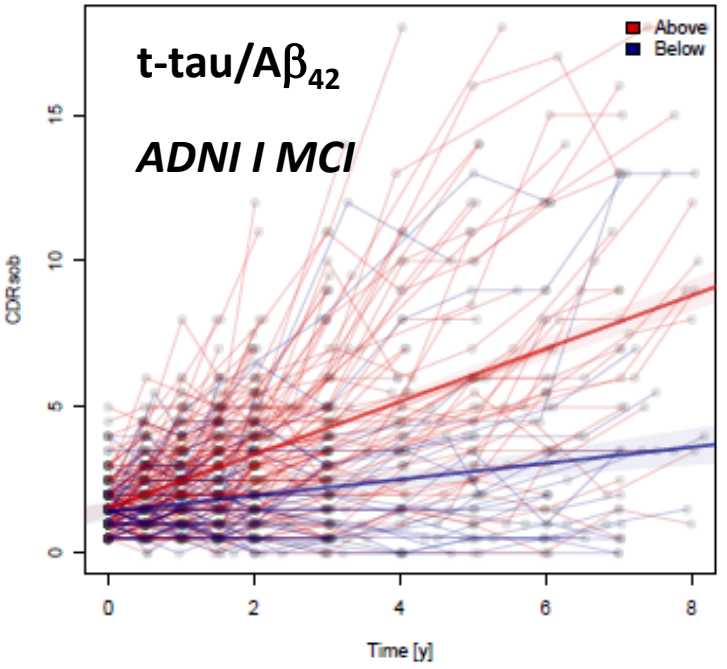
# ADNI 1 cognitively normal subjects with "non-pathological" $A\beta_{1-42}$ at BASELINE*

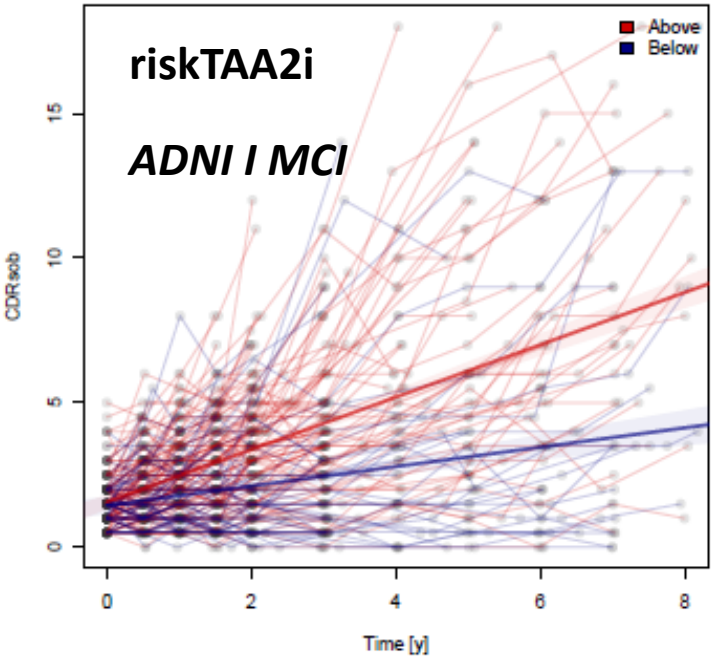
- Longitudinal CSFs out to 3-4 years, n=35, all above-cutpoint $A\beta_{1-42}$ at BASELINE
- $A\beta_{1-42}$: In ~2/3rds remained stable and non-pathological, but in ~1/3rd declined toward pathologic at a mean rate of 9.2 pg/mL/yr
- Majority of "decliners" and "stables" were APOE $\varepsilon$4 negative
- "stables" and "decliners" were not statistically different at BASELINE for mean cognitive & memory tests; were different for mean $A\beta_{1-42}$ : 257 vs 211pg/mL, $p<0.001$; comparable HV values.
- Important questions for ADNI 2 and ADNI 3:
  - which biomarker, imaging, genetic factors predict which MCI & cog normals will decline….
  - Which candidate biomarkers will add to predictive performance of $A\beta_{1-42}$, tau and ptau:
    - Neurogranin, Vilip 1, SNAP-25, YKL-40, total $\alpha$-SYN and PS-129, others?
    - Blood biomarkers?
  - Longitudinal changes in these candidate biomarkers

* Subjects in the ADNI 1 add-on study, sponsored by an anonymous donor, included a total of 141 subjects with 3 or more CSFs collected longitudinally between 2005 - 2014

# Rates of decline for CDRsob: Pathologic vs non-Pathologic biomarker CSF biomarkers (ADNI I dataset)




riskTAA2i: logistic regression model that includes Ab42, t-tau and APOE ε4 allele count

| **Cutpoint** | **192 pg/mL** | **0.39** | **0.5** |
|---|---|---|---|
| Biomarker | **$A\beta_{42}$** | **t-tau/$A\beta_{42}$** | **riskTAA2i*** |
| **CSF pathologic** | **+1.10/yr** | **+1.14/yr** | **+1.14/yr** |
| **CSF non-pathologic** | **+0.26/yr** | **+0.31/yr** | **+0.45/yr** |
| ***p*** | <0.0001 | <0.0001 | <0.0001 |

# New biomarkers in NIA/ADNI/RARC-approved studies

| Biomarker | Fluid | # | ADNI study | Investigator |
|---|---|---|---|---|
| Proteome/RBM | plasma | 1,065 | BL & yr1; multiple publications | HSoares;Pfizer/PPSB/FNIH |
| Proteome/RBM | CSF | 317 | BL ADNI 1; multiple publications | WPotter,etal/PPSB/FNIH |
| BACE & sAPP | CSF | 402 | BL ADNI 1; recent publication | MSavage;merck/PPSB/FNIH |
| α-Synuclein;xMAP | CSF | 390 | BL ADNI 1; several publications | JZhang; University of Wash |
| Proteome/ MRM/tandem MSMS | CSF | 331 | BL ADNI 1; 567 tryptic peptides associated with 221 proteins; recent publication, another planned | ADNI PPSB/FNIH; LHonigsberg |
| Neurogranin; IA | CSF | 416 | BL ADNI 1; manuscript submitted | KBlennow; Sahlgrenska UHosp |
| DDE; LC/msms | plasma | 211 | AD vs controls, ADNI 1 | ALevey; Emory University |
| Vilip 1; HS IA | CSF | 612 | ADNI 1, GO, 2 in longitudinal samples 2015 AAIC abstract, pub. planned | AFagan; Wash University |
| Phospho-α-SYN;xMAP | CSF | 567 | Study in BL & longitudinal samples | JZhang; University of Wash |
| YKL40, SNAP25 & neurogranin | CSF | 612 | Studies in longitudinal samples | AFagan; Wash University |
| Metabolic networks & pathways in AD | serum | 833 | Studies in BL samples | RKaddoura-Daouk; Duke Univ, Phenomenone |

# BIOMARKER CORE AIMS FOR THE ADNI-3 RENEWAL

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- It is important to take into account the heterogeneity of AD in the ADNI-3 Biomarker Core in ADNI3. Many studies emphasize this including ADNI data showing that that a very small minority (4/22) of ADNI subjects with clinical AD/MCI only had AD plaque and tangle pathology at autopsy, while 82% had plaques and tangles in addition to TDP-43 and/or alpha-synuclein ($\alpha$-syn) inclusions as well as hippocampal sclerosis in some cases (Toledo et al, ANP Commun, 1:65, 2013). These findings are echoed in a larger study of non-ADNI Penn subjects (Toledo et al, ANP, 124:23-35, 2012).

- Further, we have used a CSF total $\alpha$-syn assay in ADNI CSF samples that may enable detection of co-morbid LBs in MCI/AD subjects in ADNI 3 (Toledo et al, ANP, 126:683-697, 2013) and we also have access to a phospho-$\alpha$-syn immunoassay that could be incorporated into ADNI-3 (Wang et al, Sci Trans Med, 4:121-20, 2/15/2012).

- TDP-43 biomarkers are not yet available so we work with Hugo Vanderstichele at ADx and Andreas Jeromin at Quanterex on TDP-43 ELISA based assays, but it is not yet certain if a TDP-43 immuno-assay will be ready for use in ADNI-3. We also need to address the issue of co-morbid cerebro-vascular disease (CVD), but information on CVD may come from imaging rather than chemical biomarker studies.

With this as background, the Aims of the Biomarker Core in ADNI 3 are as follows:

# Aims for ADNI 3

1. Continue to collect, aliquot, store, curate and track all biofluid samples collected from subjects in ADNI-1, ADNI-GO, ADNI-2 and ADNI-3 and <u>continue</u> regular reconciliation & reviews with the clinical core at UCSD.
2. Implement new immunoassay platform for CSF $A\beta_{1\text{-}42}$, t-tau and p-$tau_{181}$, following review of all possible systems in consultation with PPSB BBWG.
3. Implement our newly validated mrmMass Spec assay to measure $A\beta_{1\text{-}42}$, $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}38}$ in all ADNI CSFs.
4. Continue longitudinal studies of ADNI biomarkers that are most informative including CSF t-tau, p-$tau_{181}$ and $A\beta_{1\text{-}42}$. Neurogranin and Vilip 1 data recently available and total $\alpha$-syn and PS129-$\alpha$-syn, YKL40, SNAP25, a 2nd neurogranin study (with emphasis on longitudinal CSFs) data will be available later this year. We propose to add the most promising of these as opportunities to improve on our CSF AD panel in order to chart the onset and progression of AD and determine earliest predictors of cognitive decline.
5. Continue ongoing assessments of best combinations of biomarkers for prediction of cognitive and functional decline, both within-biomarker core as well as collaborative studies with other cores and investigators.
6. Implement other validated and promising new biomarker immunoassays such as those that measure other species of tau and Aβ in CSF, including fragments of tau and Aβ as well as oligomers thereof in addition to other biomarkers such as VILIP1, YKL40, neurogranin, total $\alpha$-syn and phospho-$\alpha$-syn. We are following closely any promising lead for plasma biomarkers with predictive value and exosomes as a potentially valuable carrier of CNS biomarkers in the circulation. The results from these ongoing studies will determine the likelihood of incorporating these new analytes into ADNI-3.
7. Continue international collaboration (AA GBSC & IFCC CSF WG) to establish a certified reference material for $A\beta_{1\text{-}42}$ that supports improved analytical platform to platform, lot-to-lot performance, and to promote harmonization across analytical methods for these widely used CSF biomarkers.
8. Establish validated Mass Spectrometry-based assay to identify and measure t-tau and tau isoforms in CSF.
9. Partner with the PPSB and other investigators who pursue additional approved Add-On studies of AD biomarkers in plasma and/or CSF in order to bring these assays on-line for use in ADNI 3

# Selection of a new automated immunoassay platform for ADNI 3

- Move from a manual RUO immunoassay to a fully automated immunoassay platform for ADNI 3: due diligence started Q4, 2014.
- Need for preliminary validation data for ADNI 3 grant submission
- Sought after features:
  - precision & accuracy based; dilutional linearity, parallelism
  - full automation;
  - commitment to the ADNI study;
  - on an IVD trajectory;
  - commitment to pre-competitive activities,

  to contribute to CSF biomarker QC/standardization
  - Commitment to data sharing consistent with the ADNI study
- Selection will be done in consultation with the ADNI PPSB/BBWG, chaired by Johan Luthman.

# Support of standardization efforts

- ADNI-longterm commitment to standardization of all methods
  - Open access to data generated following quality control
  - Has been in operation for 10 years
  - Benefits from lots of interaction, peer review, with the scientific community in academia, industry, governmental sectors
- Alz Assn Global Biomarker Standardization Consortium
  - Analytical methods standardization--strong support for improved performance of existing and new immunoassays for CSF biomarkers, and automation
  - The Alz Assn-supported international CSF QC program provides continuing feedback on quality both short and long term
  - Support for mrm/tandem mass spectrometry for direct measurement of absolute $A\beta_{1\text{-}42}$ concentration
  - IFCC/IRMM project to develop reference $A\beta_{1\text{-}42}$ peptide material and using mrm/msms and large pools of CSF with accurately measured $A\beta_{1\text{-}42}$
  - Need same for t-tau
- CAMD(Coalition Against Major Diseases) has made a substantial commitment to support FDA approval for the use of HV and CSF AD biomarkers in treatment trials
  - Hippocampal volume
  - CSF AD biomarkers