ADNI Biomarker Core

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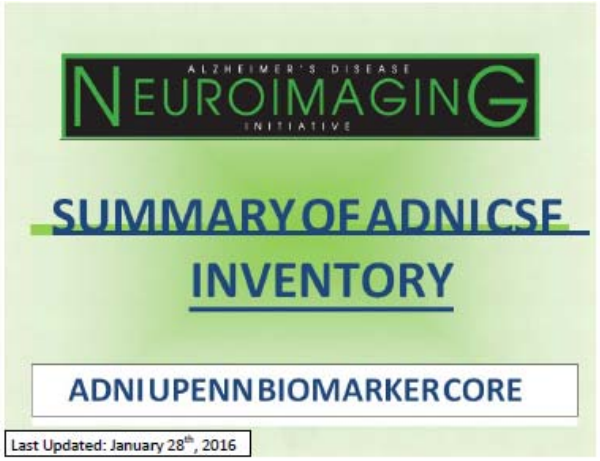
ADNI3 Aims for Biomarker Core

Aim 1: Receive, aliquot, store and curate biofluid samples following established ADNI SOP's and transfer samples to investigators approved by the RARC as described in the Administrative Core.

- Continue to collect, store, curate and track all biofluid samples collected from subjects in ADNI-1, ADNI-GO, ADNI-2 and ADNI-3
- Regular reconciliation and reviews with the clinical core at USC.
- Provide blinded sample aliquots for studies approved by the NIA/ADNI/RARC.
- Produce regular inventory reports
  - for ADNI biofluid collections
  - for biofluid aliquots per ADNI subject



ADNI3 Aims for Biomarker Core

Aim 2: Provide highly standardized $A\beta_{1-42}$, t-tau and p-tau_{181} measurements on all ADNI subject CSF samples using the Roche automated immunoassay platform(Cobas e601) and immunoassay reagents. In addition provide immunoassay-independent measurements of Aβ species ($A\beta_{1-42}$, $A\beta_{1-40}$ and $A\beta_{1-38}$) using a validated reference 2D-UPLC/tandem mass spectrometry method in baseline and longitudinal CSF samples. Continue collaboration with other investigators to achieve harmonization of these measurements across centers and different platforms in support of their use in clinical trials.

- ***Change:*** from manual RUO immunoassay to fully automated immunoassay platform for ADNI 3:
- ***Due diligence:*** started Q4, 2014, in consultation with ADNI Exec Comm & NIA & PPSB/BBWG/DDWG.
- ***Selection:*** in consultation with ADNI PPSB/BBWG/DDWG, chaired by Johan Luthman.
- ***Roche Elecsys:*** validation for $A\beta_{1-42}$ in CSF completed.
- ***External QC:*** Participation in the AlzAssn CSF QC program for $A\beta_{1-42}$
- ***Validation of t-tau and p-tau_{181}:*** scheduled for completion in summer, 2016
- ***Start analyses of all ADNI CSFs:*** FALL, 2016
- ***Continued collaboration:*** with Kaj Blennow & IFCC CSF WG to produce certified reference CSF pools with assigned reference $A\beta_{1-42}$ concentration values, measured with reference 2D-UPLC/tandem mass spectrometry, to provide certified reference materials for manufacturers of $A\beta_{1-42}$ calibrators--promoting harmonization across assay platforms.
- ***Review:*** pre-analytical factors for CSF collection.
- ***Tau measurement by mass spectrometry:*** work initiated

Why automation of CSF biomarkers?

- Eliminate as many manual steps as possible
- Promote best possible precision
  - Within-lab
  - *Between-labs*
- Improved lot-to-lot performance
- Enable IVD test approval → clinical laboratory test
- Can provide both accurate and precise data
- Use in treatment trials, especially international where local laboratory is essential(eg, China).

Method validation studies at UPenn for the Roche Elecsys immunoassay

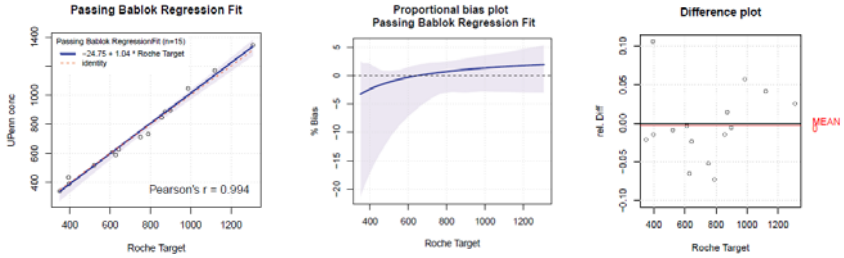
CSF Aβ1-42:

- Analytical studies
  - Short and long-term precision studies
  - Linearity
  - Comparison of Elecsys between UPenn and Roche
  - Comparison with a reference mrm/mass spectrometry method
  - Comparison with the RUO AlzBio3 immunoassay
  - Two sets of non-ADNI CSF samples utilized(250 residual CSF from routine clinic patients; 129 CSFs from the UPenn ADRC)
- ROC analyses for AD vs HC in 129 CSFs from the UPenn ADRC(62 AD, 67 HC)

SUMMARY

UPENN/Roche comparison (both use Roche Elecsys,15 CSF pools): PB regression—Y = 1.04X - 24.8; Pearson's r = 0.994

- Bias at cut-off <10%
- Slope is within 1.0 ± 0.1



Elecsys, AlzBio3 and LC-MS Abeta(1-42) measurements were performed for 250 samples from data set A and 129 samples from data set B

Data set A and B were not pooled as AlzBio3 measurements differed between the two sample sets

Correlation between

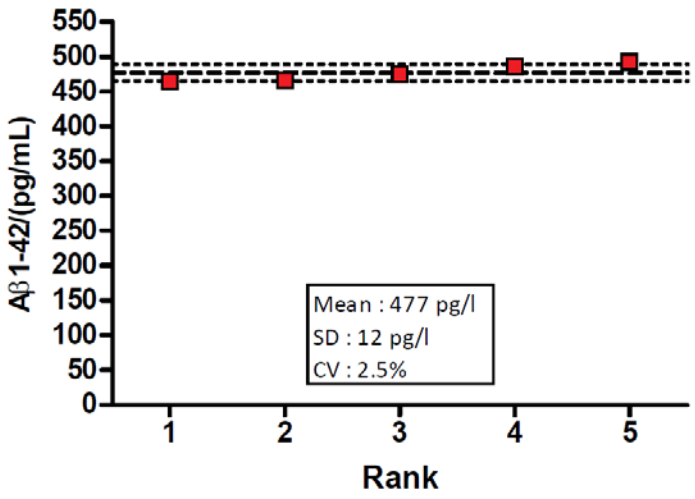
Elecsys and AlzBio3: Spearman's rho 0.86(A)/0.82(B)
Elecsys and LC-MS: Spearman's rho 0.95(A)/0.96(B)
LC-MS and AlzBio3: Spearman's rho 0.87(A)/0.77(B)

ROC-AUC analysis within the data set B(AD vs HC): equivalent performance of all 3 methods

**This study will be presented in a poster at the Toronto AAIC meeting & included in a symposium talk.*

Lab to lab performance

Alz Assn CSF QC program:



5 laboratory interlab reproducility

	PC1	PC2	CSF.01	CSF.02	CSF.03	CSF.04	CSF.05
Mean Concentration [pg/mL]	485.39	869.49	342.40	662.12	754.77	935.20	1472.09
Total* % CV (Reproducibility)	**1.99**	**2.16**	**3.25**	**2.61**	**5.07**	**2.24**	**3.22**
- 95% CI	(1.65; 2.49)	(1.67; 3.05)	(2.22; 6.04)	(1.96; 3.88)	(3.64; 8.33)	(1.84; 2.86)	(2.52; 4.46)
Site-to-Site %CV	0.66	0.76	0.00	0.46	3.49	0.75	1.75
Lot-to-Lot %CV	0.59	1.03	2.25	1.46	0.61	0.89	0.78
Intermediate precision** %CV	1.78	1.74	2.35	2.11	3.62	1.91	2.59
- 95% CI	(1.54; 2.09)	(1.50; 2.07)	(2.05; 2.74)	(1.86; 2.45)	(3.27; 4.06)	(1.71; 2.16)	(2.30; 2.95)
Day-to-Day %CV	1.35	1.41	1.75	1.44	0.00	0.95	1.58
Run-to-Run %CV	0.54	0.47	0.17	0.75	2.46	0.71	0.23
Within-Run (Repeatability)	1.02	0.90	1.56	1.35	2.66	1.50	2.04
- 95% CI	(0.89; 1.18)	(0.79; 1.05)	(1.37; 1.81)	(1.19; 1.57)	(2.34; 3.09)	(1.31; 1.74)	(1.79; 2.37)

Bittner, et al, Alz Dem, 2015.

Longitudinal CSFs

Rationale

Track longitudinal changes in the pathology underlying disease progression

Resources

ADNI1/GO/2 subjects who provided 1 or more CSF that have been analyzed:

CSF N	1	2	3	4	5	6	7	8	TOTAL
Subjects	573	481	113	46	21	17	7	1	1259
TOTAL CSF	573	962	339	184	105	102	49	8	***2322***

- ***60 longitudinal sets to be analyzed include 48 months CSFs for ADNIGO/2 subjects.***
- ***Challenge: continue as high a rate of longitudinal lp's as possible***

Study progress

Biomarkers studied: Beckett etal 2010; Vemuri etal 2010;Lo etal 2011

- $A\beta_{1\text{-}42}$, t-tau, p-tau_{181}: Biomarker core Toledo etal 2013; Landau etal 2013;Mattsson etal 2015
- YKL-40, Vilip-1, SNAP-25, Neurogranin: Anne Fagan et al
- α-SYN; Ser129-α-SYN: Jing Zhang, soon

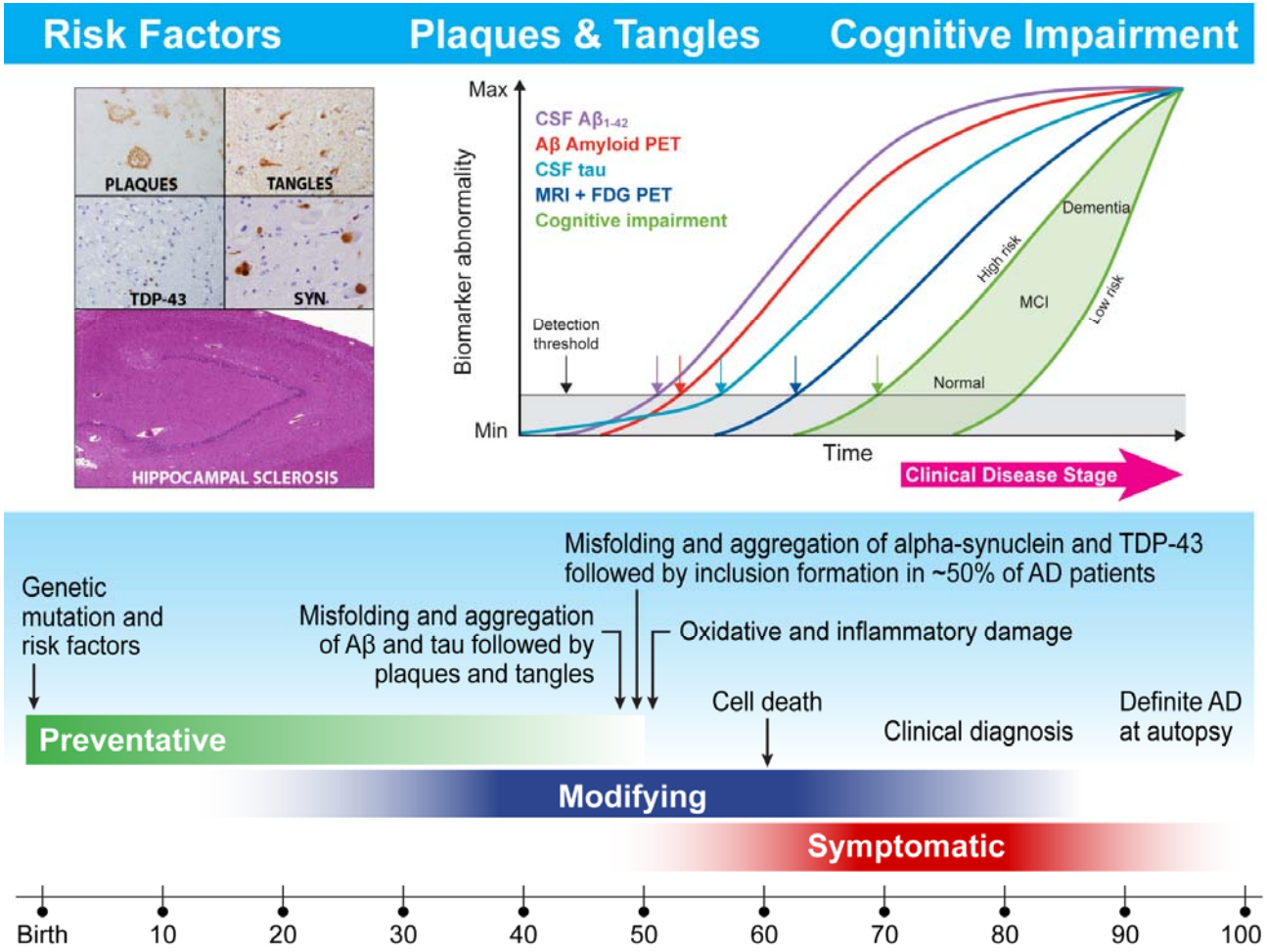
ADNI rollover subjects are very important contributors to these studies

ADNI1 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 ε4 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.
- Questions for 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 α-SYN and PS-129, others?
    - Blood biomarkers?
  - Longitudinal changes in these candidate biomarkers

* Subjects in the ADNI1 add-on study, sponsored by an anonymous donor, included a total of 142 subjects with 3 or more CSFs collected longitudinally between 2005 - 2014

Alzheimer's Disease: heterogeneity and biomarker timeline dynamics



Kang JH, et al, AlzDement, 2015

ADNI3 Aims for Biomarker Core

Aim 3: Collaboration with other investigators in the use of new tests for CSF (total and phospho-α-SYN; neurogranin; NFL; Vilip1 and TDP-43) and possibly blood biomarkers (metabolomic and lipidomic assays; $A\beta_{1-42}$ and tau proteins in neurally derived exosomes).

LOAD-*disease heterogeneity a key characteristic*.

- It is important to take into account the heterogeneity of AD in ADNI3. Many studies emphasize this including ADNI data showing that that less than half (42%) of ADNI subjects with clinical AD/MCI had only AD plaque and tangle pathology at autopsy, while 58% had plaques and tangles in addition to TDP-43 and/or alpha-synuclein (α-syn) inclusions as well as hippocampal sclerosis in some cases (Toledo et al, ANP Commun, 1:65, 2013; Cairns etal, Neuropath 2015). These findings are echoed in a larger study of non-ADNI Penn subjects (Toledo et al, ANP, 124:23-35, 2012).
- We have worked together with Jing Zhang and found that a CSF total α-SYN assay in ADNI CSF samples may enable detection of co-morbid LBs in MCI/AD subjects in ADNI1 subjects(Toledo et al, ANP, 126:683-697, 2013) and we now are continuing this by using a new total α-SYN and a phospho-α-syn immunoassay in non-ADNI patients and this 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 immunoassay 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.

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 ADNI1; multiple publications	WPotter,etal/PPSB/FNIH
BACE & sAPP	CSF	402	BL ADNI1; recent publication	MSavage;merck/PPSB/FNIH
α-Synuclein;xMAP	CSF	390	BL ADNI1; several publications	JZhang; University of Wash
Proteome/ MRM/tandem MSMS	CSF	306	BL ADNI1; 567 tryptic peptides/221 proteins; publication, another planned	ADNI PPSB/FNIH; LHonigsberg
AD Autoantibodies	serum	118	36 each NC, MCI, AD BL ADNI1;publication	RMcIntyre; St Francis Hospital
AD Autoantibodies	serum	100	50 BL MCI; 50 BL HC; publication	R Nagele; UMDNJ
Neurogranin; NFL; IA	CSF	416	BL ADNI1; multiple publications	KBlennow; Sahlgrenska UHosp
DDE; LC/msms	plasma	211	AD vs controls, ADNI 1	ALevey; Emory University
Tau; IA	plasma	595	BL ADNI1; publication	Kblennow; Sahlgrenska UHosp
Vilip 1; YKL-40; IA	CSF	612	Longitudinal samples, publication planned	AFagan; Wash University
T-& Phos-α-SYN; IA	CSF	567	Longitudinal samples, to be uploaded	JZhang; University of Wash
SNAP25 & neurogranin	CSF	612	Longitudinal samples	AFagan; Wash University
Metabolic networks	serum	833	Studies in ADNI1 BL samples; data uploaded	RKaddoura-Daouk; Duke Univ,

ADNI3 Aims for Biomarker Core

Aim 4: Collaborate in studies (a) of individual and combinations of CSF biomarkers for prediction of memory, cognitive and functional decline, (b) the effect of using individual and combinations of CSF biomarkers and associated cutpoints for reducing sample size thus improving efficiency of treatment trials, (c) study rates of change of CSF biomarkers over time to determine relationships between rates of change and future cognitive decline, (d) determine the prediction of uptake of tau ligand by CSF $A\beta_{1\text{-}42}$ below cutpoint, and (e) determine the concordance between $A\beta_{1\text{-}42}$ and amyloid-β plaque ligand uptake with the Biostatistics Core and other ADNI3 Cores as well as with other outside investigators.