Implementation of fully automated immunoassays for CSF A $\beta_{1-42}$ , t-tau and p-tau<sub>181</sub>in the Alzheimer's Disease Neuroimaging Initiative

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## Why automation of CSF biomarkers?

- Eliminate as many manual steps as possible
- Promote best possible precision & accuracy
  - Within-lab

### -Between-labs

- using common samples, eg AlzAssn QC program
- Same study population and pre-analytical protocol, eg, treatment trials
- Different study populations and pre-analytical protocols, eg, ADNI, BioFINDER
- Improved lot-to-lot performance
- Enable IVD test approval ——> clinical laboratory test
- CSF biomarker testing has IVD status in the EU for routine clinical use
- Use in treatment trials, especially international where local laboratory is essential(eg, China).

### ADNI3 Aims for Biomarker Core

**Aim 2:** Provide highly standardized  $A\beta_{1-42}$ , t-tau and p-tau<sub>181</sub> 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<sub>181</sub>: completed FALL, 2016
- Analyses of all ADNI CSFs: late FALL, 2016-early WINTER, 2017
- **Continued collaboration: with the GBSC/** AlzAssn and IFCC CSF WGs 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 validation of  $A\beta_{1-42}$  calibrators--promoting harmonization across assay platforms.
- **Review & participate in:** studies of pre-analytical factors for CSF collection.



### Between-labs performance: Alz Association QC program

Between laboratory CV (percent)						
	INNOTEST® β-AMYLOID (1-42) Fujirebio	Eurolmmune / ADx β-amyloid (1-42)	AlzBio3 β-amyloid (1-42) Fujirebio	Meso-Scale Human Aβ42	Elecsys® β-Amyloid(1−42) Roche Diagnostics	Lumipulse® β-Amyloid(1−42) Fujirebio
	ELISA	ELISA	Luminex	ECL V-PLEX	Fully automated	Fully automated
Round						
2014-14A	18		16	low n	2,9	
2014-14B	21		19	low n	4,4	
2014-15A	15		7,1	12	4,6	
2014-15B	17		14	12	3,4	
2104-16A	27	57	40	13	3	
2014-16B	17	19	30	11	2,5	
2015-17A	19	6,5	17	21	1,9	
2015-17B	14	8,2	15	20	3,2	
2015-18A	13	22	25	10	7,2	
2015-18B	13	16	13	9,4	4,7	
2015-19A	13	13	40	10	3	
2015-19B	13	13	15	13	1,5	
2016-20A	17,4	18	ND	10,5	2	
2016-20B	21,1	15,4	ND	14	Level out of range	
2016-21A	15	18	22	8,1	10	6,3
2016-21B	11	15	29	7,4	5,2	4
2016-22A	14	12	28	39	5,9	7,5
2016-22B	19	13	24	37	6,7	9,4
MEAN	16.5	17.6	22.1	15.5	4.2	6.8

KBlennow

### Analysis of 2401 ADNI1/GO/2 CSF samples

2401 ADNI pristine CSFs, collected from 9/7/2005 to 7/25/2016 were analyzed in 36 analytical runs at UPenn from 11-17-2016 to 1-20-2017:

• 402 ADNI1 BASELINE; 819 ADNIGO/2 BASELINE

• ADNI1: 112 HC, 192 MCI, 98 AD

• ADNIGO/2: 160 HC, 96 SMC, 277 EMCI, 154 LMCI, 132 AD

### Analysis of 2401 ADNI1/GO/2 CSF samples

2401 (1221 BASELINE + 1180 longitudinal) ADNI pristine CSFs, collected from 9/7/2005 to 7/25/2016 were analyzed in 36 analytical runs at UPenn from 11-17-2016 to 1-20-2017:

ADNI Phase	HC	SMC	EMCI	LMCI	AD
		1221 B/	ASELINE AD	NI CSFs	
ADNI 1	112			192	98
ADNIGO/2	160	96	277	154	132

## Analyses of ADNI1/GO/2 CSF A $\beta_{1-42}$ , t-tau, p-tau<sub>181</sub> using the Roche Elecsys fully automated immunoassay platform

At previous ADNI meetings, in AAIC abstracts and 2018 publication the following have been described:

- Rationale for moving from RUO to full automation
- Validation of  $A\beta_{1-42}$  for precision, accuracy, and clinical performance
- General statistics for  $A\beta_{1-42}$ , t-tau, p-tau<sub>181</sub>, t-tau/ $A\beta_{1-42}$ , p-tau<sub>181</sub>/ $A\beta_{1-42}$  in the ADNI1/GO/2 CSF samples
- Histogram distributions for  $A\beta_{1-42}$ , t-tau/ $A\beta_{1-42}$ , p-tau<sub>181</sub>
- Distributions based on FBP amyloid-β PET + or –
- Precision performance with an abnormal CSF pool; lot P09 comparison with lot P07
- Cut-point determinations
- Collaborative study with BioFINDER
- Concordance with FBP amyloid- $\beta$  PET
- Prediction of cognitive decline(CDRsob)
- Assessments of the contribution of CSF t-tau and p-tau<sub>181</sub> to the clinical utility of CSF A $\beta_{1-42}$
- Analyses of all DIAN samples and a re-analysis of a sub-set of ADNI CSFs as part of the joint study with DIAN-study includes  $A\beta 40$  statistical analyses underway

#### Frequency distribution plots: upper are mixture model plots, lower are FBP+ and FBP- for ADNI SMC/EMCI/LMCI/AD



Abeta [pg/mL]

log(Tau/Abeta)

log(PTau/Abeta)





Alzheimer's & Dementia 📕 (2018) 1-12

Featured Article

### CSF biomarkers of Alzheimer's disease concord with amyloid-β PET and predict clinical progression: A study of fully automated immunoassays in BioFINDER and ADNI cohorts

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## Precision performance; lot-to-lot performance







## Analyses of ADNI1/GO/2 CSF A $\beta_{1-42}$ , t-tau, p-tau<sub>181</sub> using the Roche Elecsys fully automated immunoassay platform

In order to further address the question of *what does CSF p-tau*<sub>181</sub> add to the clinical utilities of CSF  $A\beta_{1-42}$  we describe the following analyses for the ADNIGO/2 EMCI+MCI participants:

Stratify into 4 sub-groups using A<sub>β42</sub> and p-tau<sub>181</sub>

**A**myloid plaque burden +/- represented by  $A\beta_{42}$ + or - : [A $\beta$ + or A $\beta$ -]

**—T**au pathology +/- represented by p-tau<sub>181</sub>+ or - : [p-tau+ or p-tau-]

— As a pilot study, do the same thing except substitute  $A\beta 42/A\beta 40$  ratio + or - for  $A\beta$ + or  $A\beta$ -respectively using newly completed LC/MSMS analysis data

- Use the cut-points described in the earlier ppts to determine + or status for each biomarker.
- Test the hypothesis that having both Aβ+ & ptau+ [ie, Aβ+ | ptau+] is associated with much greater rates of cognitive, functional and memory decline as compared to having only one of the two pathologic states [ie, Aβ+ | ptau- or Aβ- | ptau+].
- Test the hypothesis that the least cognitive decline is associated with both classes of CSF biomarker being negative.
- Test this also for risk for progression from a diagnosis of MCI to a diagnosis of AD dementia.

#### ROC Curves for SMC+EMCI+LMCI+AD CSF biomarkers using FBP PET+/- as the clinical endpoint In the ADNI study-Roche Elecsys dataset\*



AUC values:		_ Sens	Spec I	Eff
p-tau/A $\beta_{1-42}$	0.944	91.3%	88.5%	90.2%
t-tau/A $\beta_{1-42}$	0.940	91.6%	87.4%	89.9%
$A\beta_{1-42}$	0.889	86.7%	81.7%	84.6%
p-tau <sub>181</sub>	0.845	79.9%	74.8%	77.8%
t-tau	0.803	74.0%	72.9%	73.5%

#### Cutpoint values:

au/A $eta_{ extsf{1-42}}$	0.021
u/A $β_{1-42}$	0.222
L-42	980 pg/mL
au <sub>181</sub>	21.8 pg/mL
iu	245 pg/mL

2017 ADNI dataset included in the collaboration with the Swedish BioFINDER study

\*SUVR of 1.1 used: Landau and Jagust

# Cut-point assessments for CSF A $\beta_{1-42}$ , t-tau & p-tau<sub>181</sub> in ADNI

t-tau/A $\beta_{1-42}$ , 0.22

- ROC with FBP PET as the endpoint:
  - Aβ<sub>1-42</sub>, 980 pg/mL
  - t-tau, 245 pg/mL p-tau<sub>181</sub>/A $\beta_{1-42}$ , 0.021
  - p-tau<sub>181</sub>, 21.8 pg/mL
- Disease-independent mixture modeling
  - $A\beta_{1-42}$ , 1016 pg/mL t-tau/ $A\beta_{1-42}$ , 0.19
  - t-tau, NA  $p-tau_{181}/A\beta_{1-42}$ , 0.018
  - p-tau<sub>181</sub>, NA

### • Prediction from BioFINDER study based on pre-analytic differences

- $A\beta_{1-42}$ , 880 pg/mL t-tau/ $A\beta_{1-42}$ , 0.33
- t-tau, 270 pg/mL p-tau<sub>181</sub>/A $\beta_{1-42}$ , 0.028
- p-tau<sub>181</sub>, 24 pg/mL

## Rates of clinical decline as a function of *Aβ42*|*p-tau*<sub>181</sub> status in ADNIGO/2 EMCI+LMCI-Roche Elecsys data



# Characteristics of ADNIGO/2 E+LMCI for the study of A $\beta$ 42|p-tau181 combinations

	Aβ <sub>42</sub> -   p-tau <sub>181</sub> -	$A\beta_{42}$ -   p-tau <sub>181</sub> +	<b>Α</b> β <sub>42</sub> + p-tau <sub>181</sub> -	Aβ <sub>42</sub> + p-tau <sub>181</sub> +
Ν	139	60	87	145
<i>APOE</i> ε4, N(%)	30(22%)	21(35%)	45(52%)	109(75%)
Education, yrs	16.3±2.6	16.2±2.4	16.1±2.7	16.2±2.7
A $\beta_{42}$ , pg/mL	1557±413	1612±618	689±176	672±159
p-tau <sub>181</sub> , pg/mL	16.4±3.8	33.1±13.3	16.4±4.4	39.1±13.8
t-tau, pg/mL	191±44	350±111	178±43	382±124
tau/A $\beta_{42}$	0.13±0.03	0.25±0.14	0.28±0.12	0.60±0.25
p-tau/A $\beta_{42}$	0.011±0.0028	0.024±0.016	0.026±0.012	0.061±0.028
# progressors	<b>5 (</b> 3.6% <b>)</b>	<b>5 (</b> 8.3% <b>)</b>	<b>11 (</b> 12.6% <b>)</b>	<b>56 (</b> 38.6% <b>)</b>
# non-progressors	114	48	59	68
Drop-outs	20	7	17	21

## Cox proportional-hazards analyses: comparisons across CSF p-tau<sub>181</sub> (+ or –) combined with A $\beta$ 42 (+ or -)



#### Hazard ratios:

Aβ42-/p-tau+	1.41(0.521 3.79)
Aβ42+/p-tau-	2.46(1.04 5.84)
A $\beta$ 42+/p-tau+	7.85(3 <i>.67 16.8</i> )

Cox proportional hazards models adjusted for gender, age, education years and APOE  $\epsilon$ 4 allele #.

## Summary

- Roche Elecsys immunoassays for Aβ42, t-tau and p-tau181 completed for 2401 ADNI1/GO/2 CSFs, and uploaded on the ADNI/LONI website, March 2017
- Precision and accuracy validations completed according to CLSI EP05; high level precision & good lot to lot performance
- General stats, Frequency distributions, mixture modeling & ROC with FBP PET as endpt described
- The t-tau/A $\beta_{1-42}$  and p-tau<sub>181</sub>/A $\beta_{1-42}$  ratios outperformed A $\beta_{1-42}$  alone for clinical utilities based on:
  - Comparisons to FBP PET in ROC analyses
  - Concordance with FBP PET
  - Disease-independent mixture modeling
  - This observation is consistent with: the BioFINDER study(Roche platform/flutemetamol PET; Hansson etal, 2018); with a WashU study in LOAD (Roche platform/PiB PET; Schindler etal, 2018) & with a study that used several new immunoassays including IT, MSD, EI/flutemetamol(Janelidze etal, 2018) as well as multiple other studies that used other immunoassay platforms and clinical endpoints:
    - Seeburger, 2015(OPTIMA study, N=227, autopsy-based diagnosis); Fagan, 2011(HASD, PIB PET based endpoint, N=103); Palmqvist, 2015(BioFINDER, Flutemetamol PET, N=366)
  - Mechanism possibilities: tau abnormality adds to predictive performance-supported by **A|T** analyses; normalization of variance.
- Cut-point assessments: ROC with FBP as endpoint; disease independent mixture modeling; extrapolation from BioFINDER study based on pre-analytical differences
- Prediction performance of BASELINE CSF AD biomarkers for cognitive decline documentation
- Continue ongoing work with ADNI and other studies toward goal of defining universal cut-points for Aβ<sub>1-42</sub>, t-tau and p-tau<sub>181</sub>.
- Continue to work with colleagues on pre-analytical and other factors to help minimize and control these sources
  of variability
- Collaboration on multimodal studies that include CSF, plasma, imaging, genetic, clinical parameters