BIOFLUID BASED BIOMARKERS
2022 YEAR IN REVIEW

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ISTAART
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Key themes of today

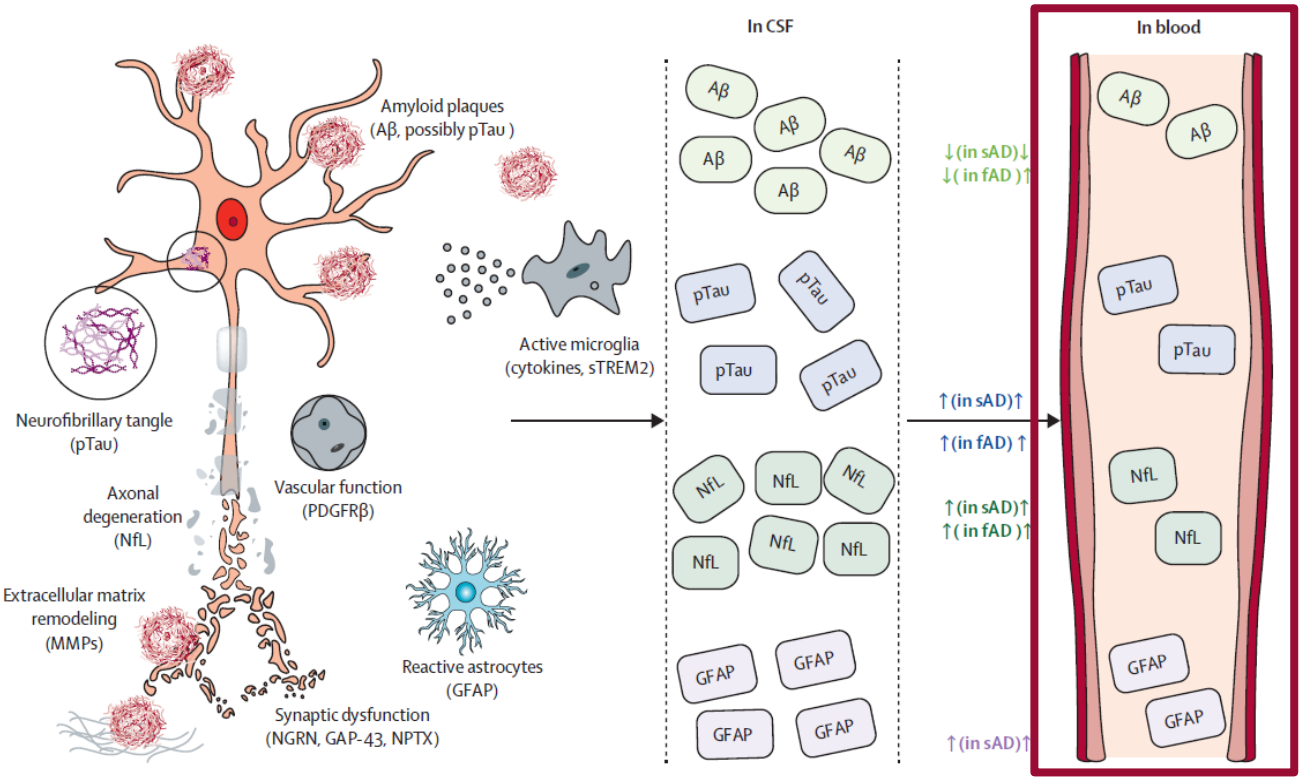
- Alzheimer's blood test towards implementation
  P-tau, Aβ42/40, GFAP, NfL

- Biomarkers for non-AD
  For FTD, DLB

- Novel biomarkers
  Discovery through proteomics
  MMP-10
  β-synuclein

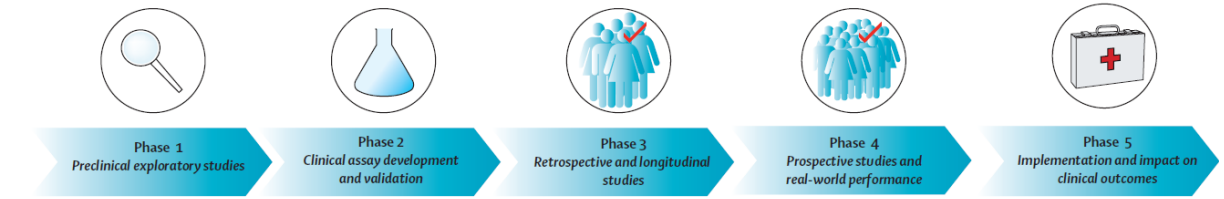
Alzheimer’s blood test

Key blood biomarkers for AD



Teunissen et al., Lancet Neurology
Complementary reading: Leuzy et al., EMBO Mol Med

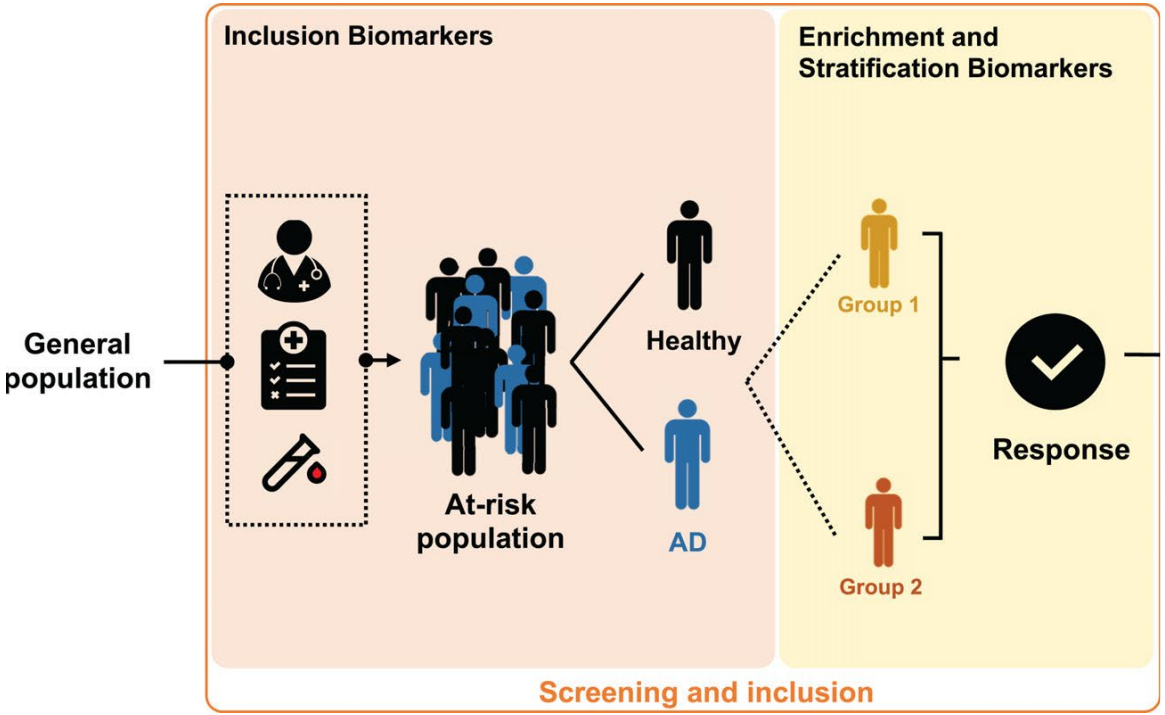
On the road towards implementation



	Phase 1 *Preclinical exploratory studies*	Phase 2 *Clinical assay development and validation*	Phase 3 *Retrospective and longitudinal studies*	Phase 4 *Prospective studies and real-world performance*	Phase 5 *Implementation and impact on clinical outcomes*
Primary aims	Biomarker identification and leads prioritisation: a) Hypothesis driven (Aβ, pTau, GFAP, NfL) b) Unbiased -omics data (proteomics, transcriptomics, etc)	*Technical:* a) LDTs and RUO assay development and validation (Aβ, pTau, GFAP, NfL) *Clinical:* a) Analysis of diagnostic accuracy in case-control studies (Aβ, pTau, GFAP, NfL)	*Technical:* a) Define criteria for a positive test—eg, cutoffs (Aβ, pTau, GFAP, NfL) *Clinical:* a) Diagnostic accuracy for early disease detection—eg, preclinical, prodromal (Aβ, pTau, GFAP, NfL)	*Technical:* a) IVD assay development and validation (Aβ, pTau, GFAP, NfL) *Clinical:* a) Establish diagnostic performance prospectively (Aβ, pTau, GFAP, NfL)	*Regulatory:* a) Integration in clinical guidelines (Aβ, pTau, GFAP, NfL) *Clinical:* a) Impact of biomarker testing on clinical decision making (Aβ, pTau, GFAP, NfL)
Secondary aims	*Not applicable*	*Technical:* a) Reproducibility of assays between laboratories (Aβ, pTau, GFAP, NfL) b) Preanalytical factor assessment (Aβ, pTau, GFAP, NfL) c) SOPs sample handling and storage (Aβ, pTau, GFAP, NfL) *Clinical:* a) Relation with gold standard measures—CSF, PET, autopsy (Aβ, pTau, GFAP NfL) b) Identification of covariates—age, sex, ethnicity, lifestyle—influencing biomarker concentrations in patients and healthy controls (Aβ, pTau, GFAP, NfL) c) Relation with disease characteristics —eg, cognitive performance (Aβ, pTau, GFAP, NfL) d) Disease specificity assessment for differential diagnosis (Aβ, pTau, GFAP, NfL)	*Technical:* a) Head-to-head comparisons of platforms and biomarker isoforms (Aβ, pTau, GFAP, NfL) *Clinical:* a) Impact of covariates on biomarker discriminatory performance (Aβ, pTau, GFAP, NfL) b) Develop algorithms for likelihood of positive results based on combinations of biomarkers (Aβ, pTau, GFAP, NfL) c) Analyse dynamics along Alzheimer's disease continuum and predictive capabilities in longitudinal studies (Aβ, pTau, GFAP, NfL) d) Establish context-of-use criteria for clinical settings and trials (Aβ, pTau, GFAP, NfL)	*Technical:* a) Reference methods and materials (Aβ, pTau, GFAP, NfL) b) Feasibility of biomarker implementation and adherence of patients to subsequent clinical work-up (Aβ, pTau, GFAP, NfL) *Clinical:* a) Validate context-of-use in clinical settings and trials (Aβ, pTau, GFAP, NfL) b) Establish predictive values (Aβ, pTau, GFAP, NfL) c) Define the potential cost-effectiveness of biomarker implementation (Aβ, pTau, GFAP, NfL) d) Monitor false negatives (Aβ, pTau, GFAP, NfL)	*Regulatory:* a) In-vitro diagnostic assay certification (Aβ, pTau, GFAP, NfL) b) Cost assessment and reimbursement (Aβ, pTau, GFAP, NfL) *Clinical:* a) Monitor diagnostic and prognostic performance across different settings (Aβ, pTau, GFAP, NfL) b) Guidelines for biomarker interpretation in primary care settings (Aβ, pTau, GFAP, NfL) c) Compare the cost-outcome effectiveness of different biomarker protocols and approaches (Aβ, pTau, GFAP, NfL)

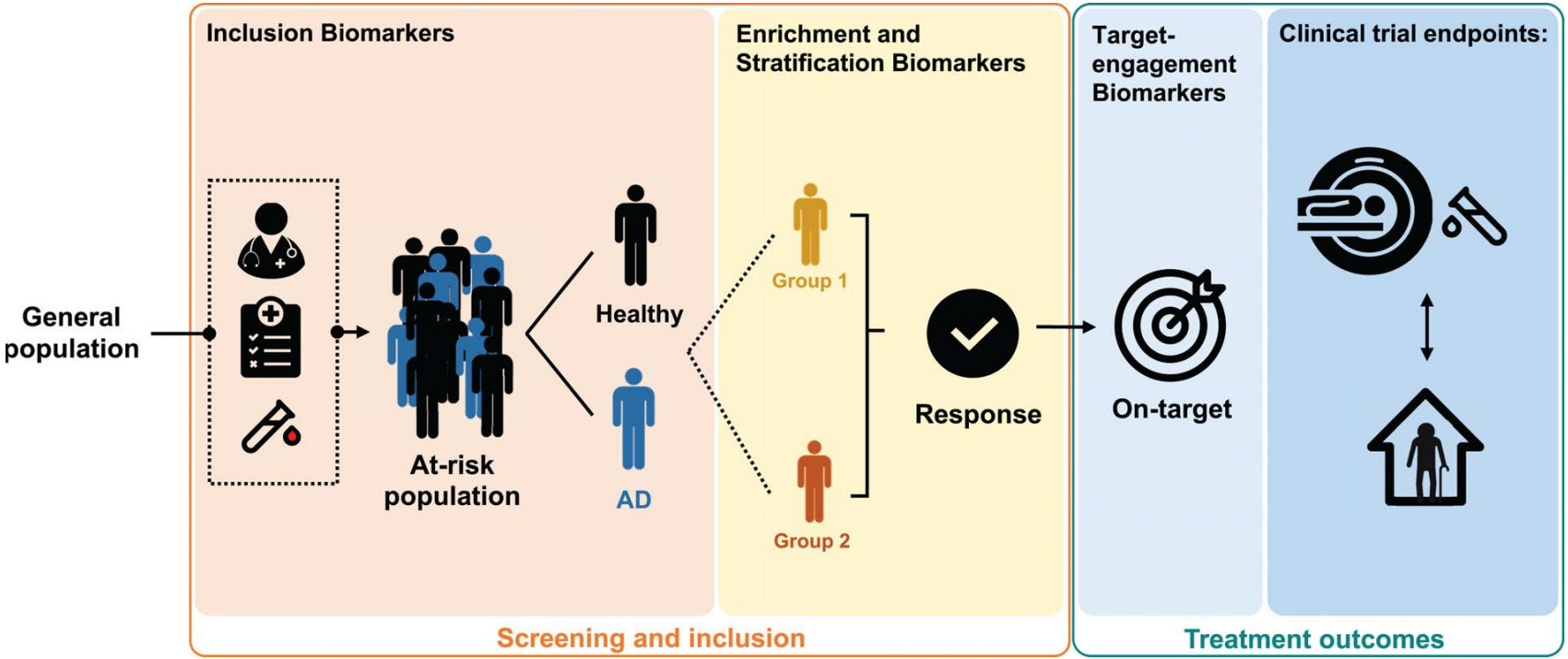
Teunissen et al., Lancet Neurology
Complementary reading: Leuzy et al., EMBO Mol Med

Appropriate use recommendations
Blood biomarkers in trials



Hansson et al., Alz & Dem

Appropriate use recommendations
Blood biomarkers in trials

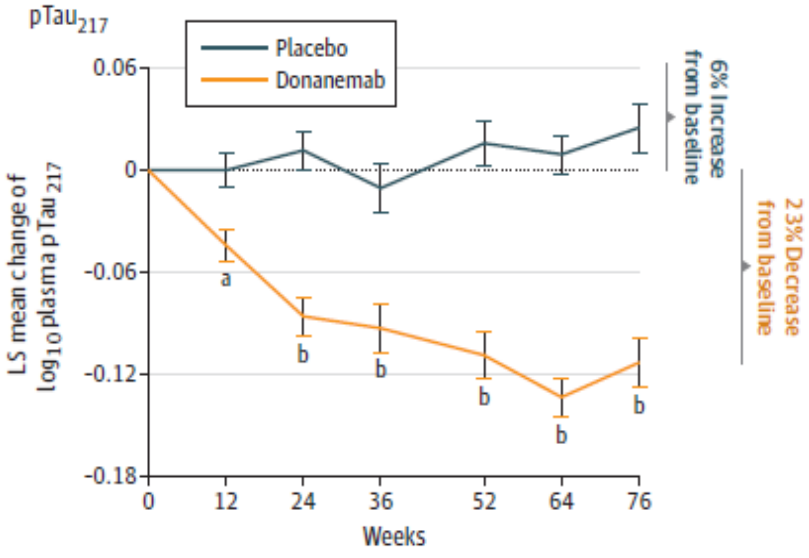


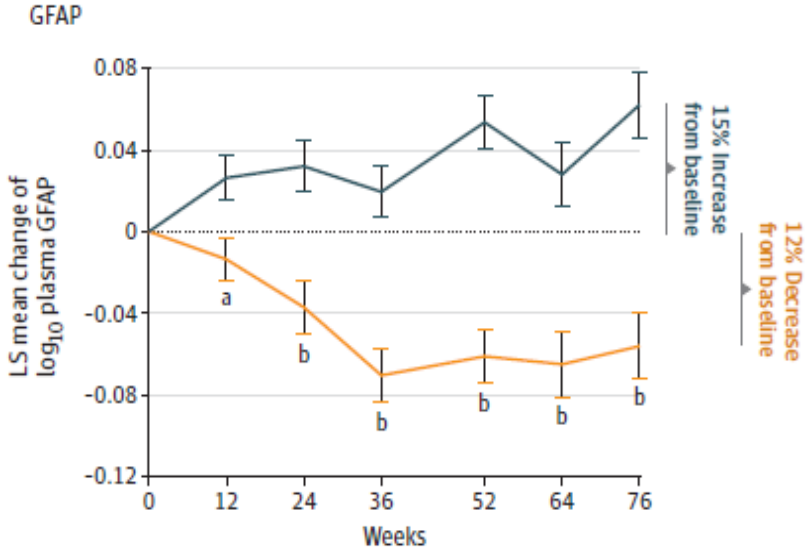
Hansson et al., Alz & Dem

Blood biomarkers with Donanemab

Results *TRAILBLAZER-ALZ*:

- Reduced cognitive decline in iADRS after 76 weeks
- Plasma pTau217 and GFAP change from baseline in the treatment arm



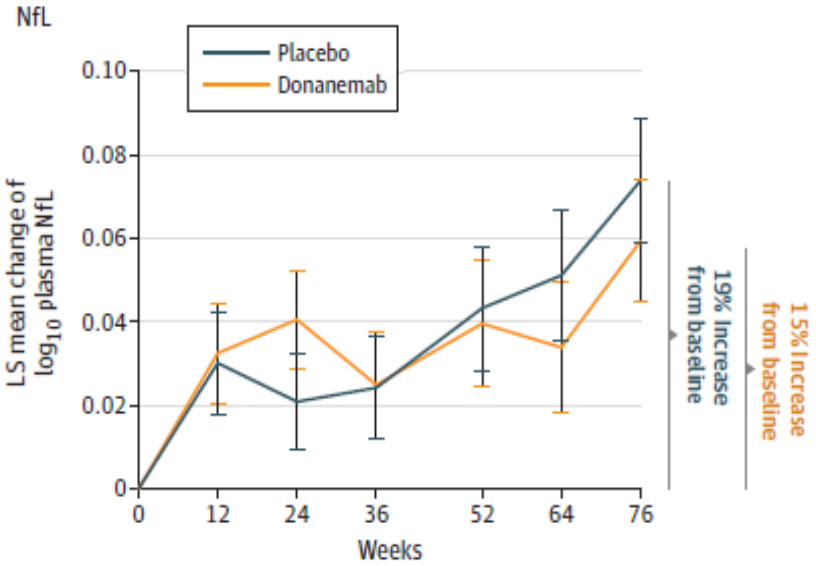


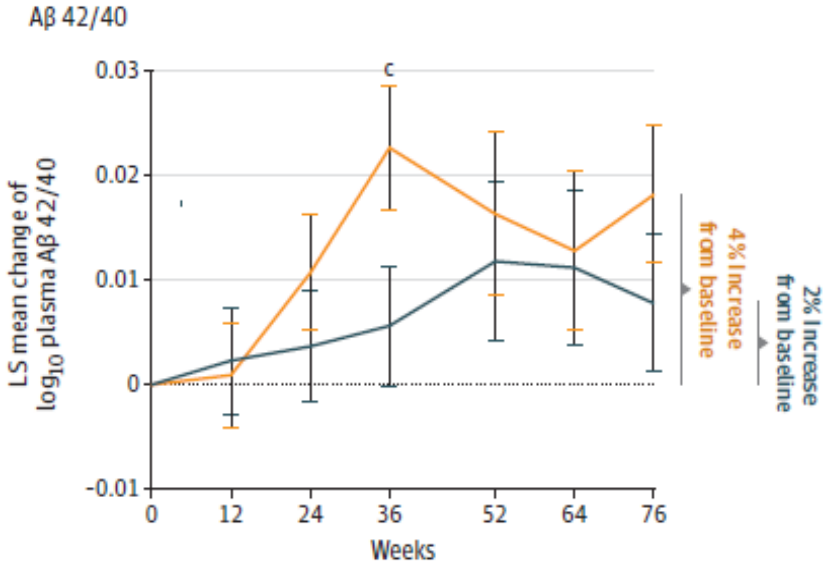
Pontecorvo et al., JAMA Neurology
Complementary reading: Mintum et al., 2021, N Engl J Med

Blood biomarkers with Donanemab

Results *TRAILBLAZER-ALZ* :

- Reduced cognitive decline in iADRS after 76 weeks
- Plasma pTau217 and GFAP change from baseline in the treatment arm, but not NfL and Aβ42/40



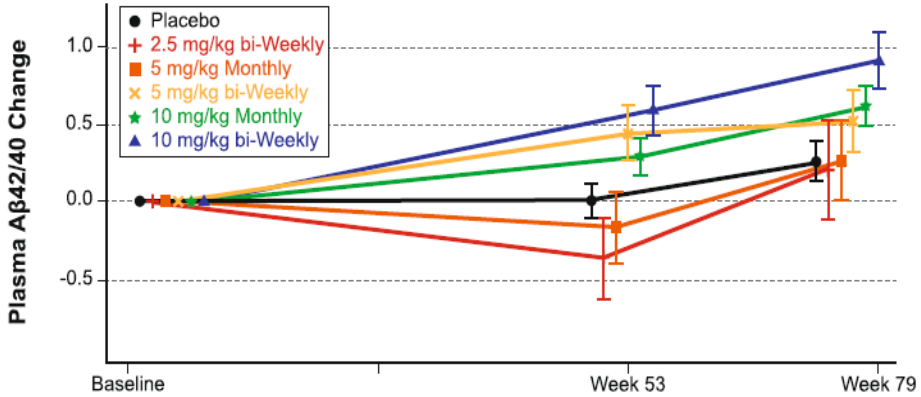


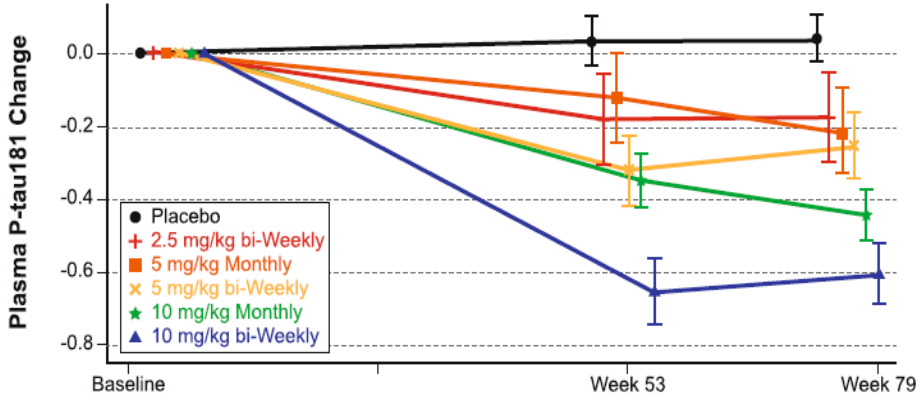
Pontecorvo et al., JAMA Neurology
Complementary reading: Mintum et al., 2021, N Engl J Med

Blood biomarkers with Lecanemab

Results from the Lecanemab proof-of-concept 201 core trial

- Plasma Aβ42/40 increased, P-tau181 decreased

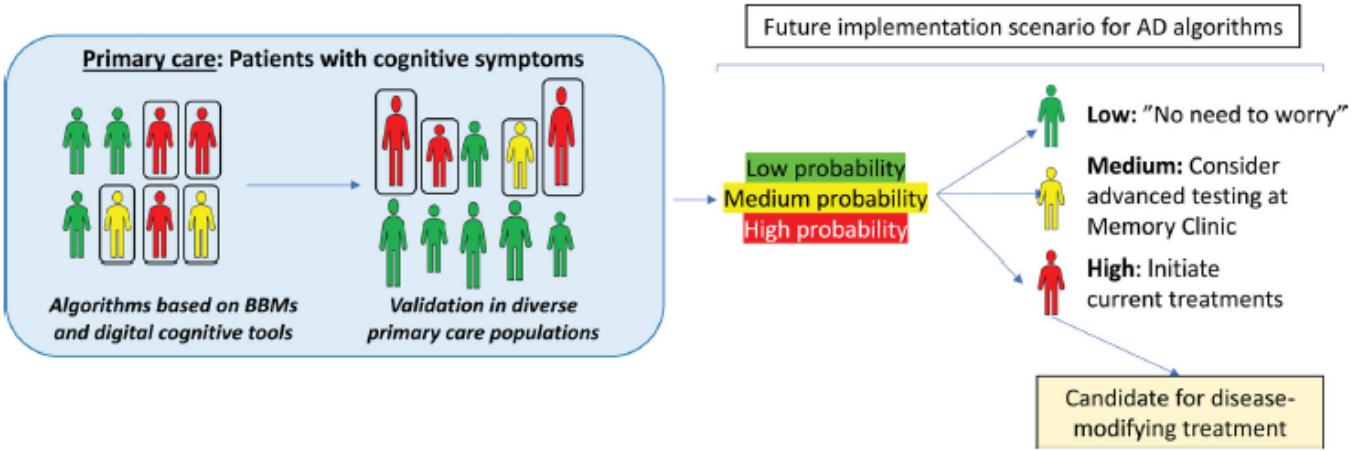




McDade et al., Alz Res Ther
Complementary reading: van Dyck et al., 2023, N Engl J Med

Appropriate use recommendations *Blood biomarkers in clinical setting*

- Specialized memory clinics: may be used in symptomatic patients, but should be confirmed by PET or CSF; more data needed in real-world settings
- Primary care: no data yet

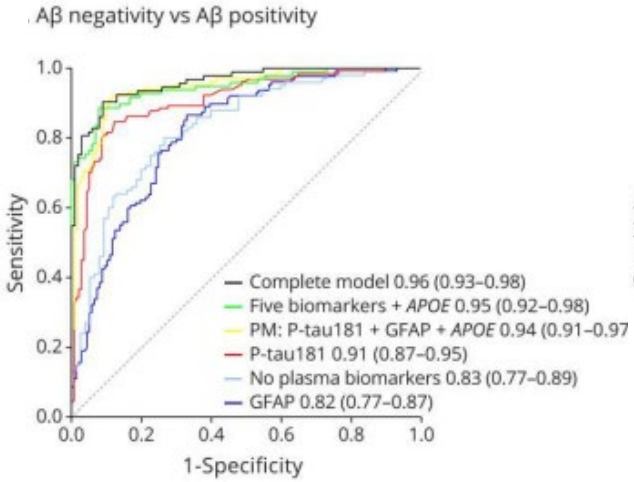


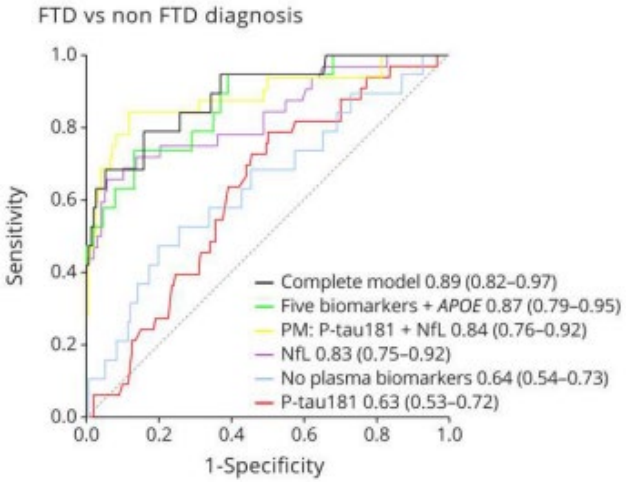
Hansson et al., Alz & Dem

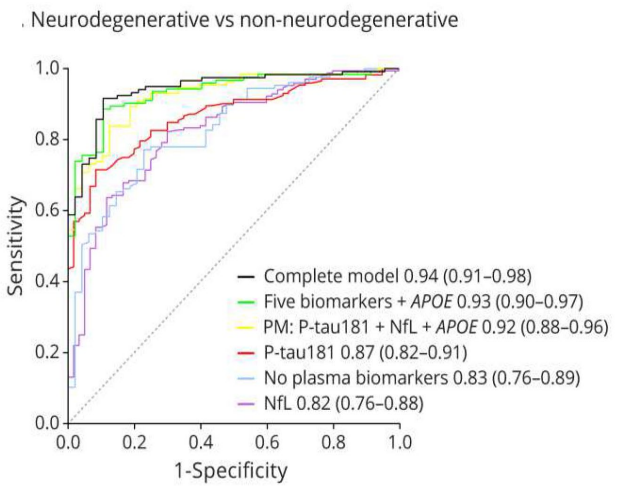
Blood biomarkers in clinical setting

Method: Plasma p-tau181, GFAP, NfL, t-tau and UCH-L1 measured in a prospective memory clinic cohort (n=385)

Results: P-tau181 and GFAP, or P-tau181 and NfL included in the parsimonious models (AUC 0.84 – 0.94)



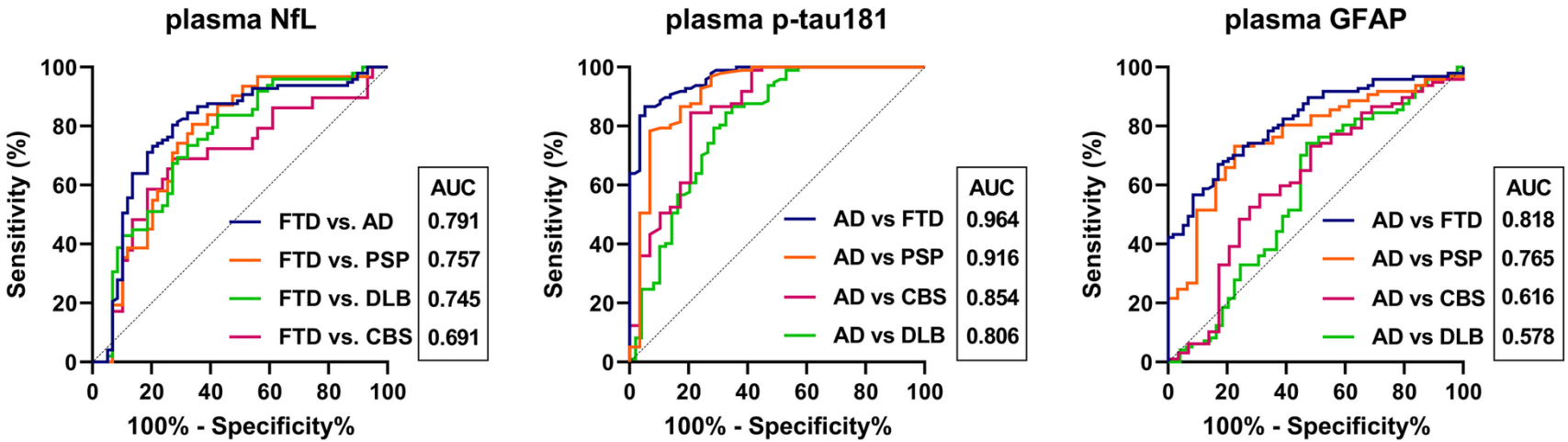




Sarto et al., Neurology

Blood biomarkers in clinical setting

Method: Assess plasma P-tau181, GFAP and NfL in 316 consecutive patients submitted to the neuropathology lab

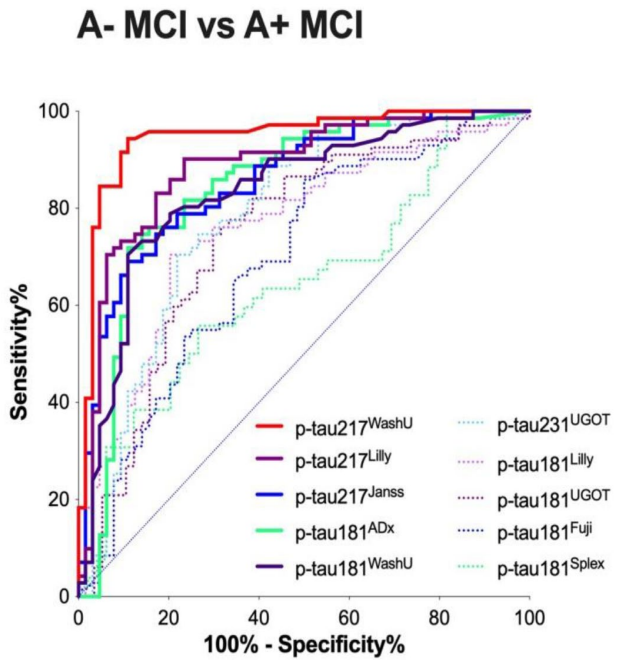


Baiardi et al., Alz Res Ther.

Complementary reading: Benussi et al., Alz Res Ther; Angioni et al., J Prev Alz Dis, Götze et al., Neurobiol of Disease

Key research priorities
Determine the best performing assays

Aim: Compare plasma P-tau assays and isoforms in 135 participants with MCI for A+
Result: P-tau217 AUCs: 0.95 – 0.86; P-tau181 AUCs: 0.84 – 0.64; P-tau231 AUC=0.78

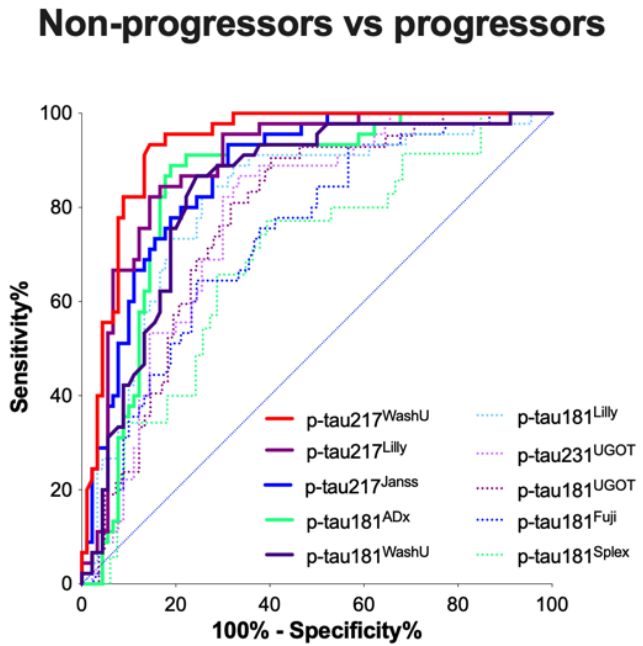


Janelidze et al., Brain
Complementary reading: Ashton, Alzheimer's & Dementia

Key research priorities

Determine the best performing assays

Aim: Compare plasma P-tau assays and isoforms in 135 participants with MCI for clinical progression
Result: P-tau217 AUCs: 0.93 – 0.87; P-tau181 AUCs: 0.85 – 0.69; P-tau231 AUC=0.78

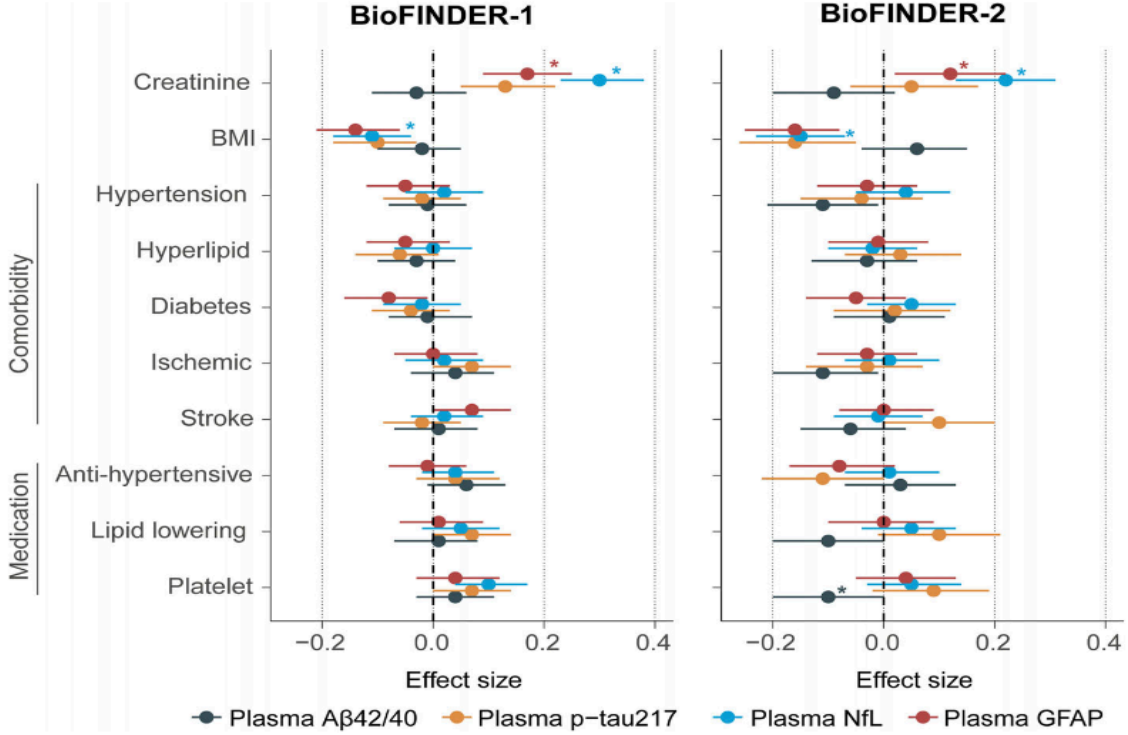


	Required volume, ml	intra-assay CV, %	Inter-assay CV, %	Samples below LLOD, %	LLOD, pg/mL
p-tau217^{WashU}	1[a]	3.3[b]	3.5[b]	0	NA[c]
p-tau217^{Lilly}	0.07	6.8	10.1	15.6	0.150
p-tau217^{Janss}	0.2	23.7	12.4	0	0.013
p-tau181^{ADx}	0.1	11.1	3.8	16.3	2.312
p-tau181^{WashU}	1[a]	3.7[b]	0.4[b]	0	NA[c]
p-tau231^{UGOT}	0.8	7.6	8.5	0	1
p-tau181^{Lilly}	0.07	6.0	11.2	0	0.864
p-tau181^{UGOT}	0.8	8.2	10.9	0	0.5
p-tau181^{Fuji}	0.13	NA[d]	NA[d]	0	0.052
p-tau181^{Splex}	0.06	4.8	13.5	0	0.190

Janelidze et al., Brain
Complementary reading: Ashton, Alzheimer's & Dementia

Key research priorities

Determine factors affecting the results

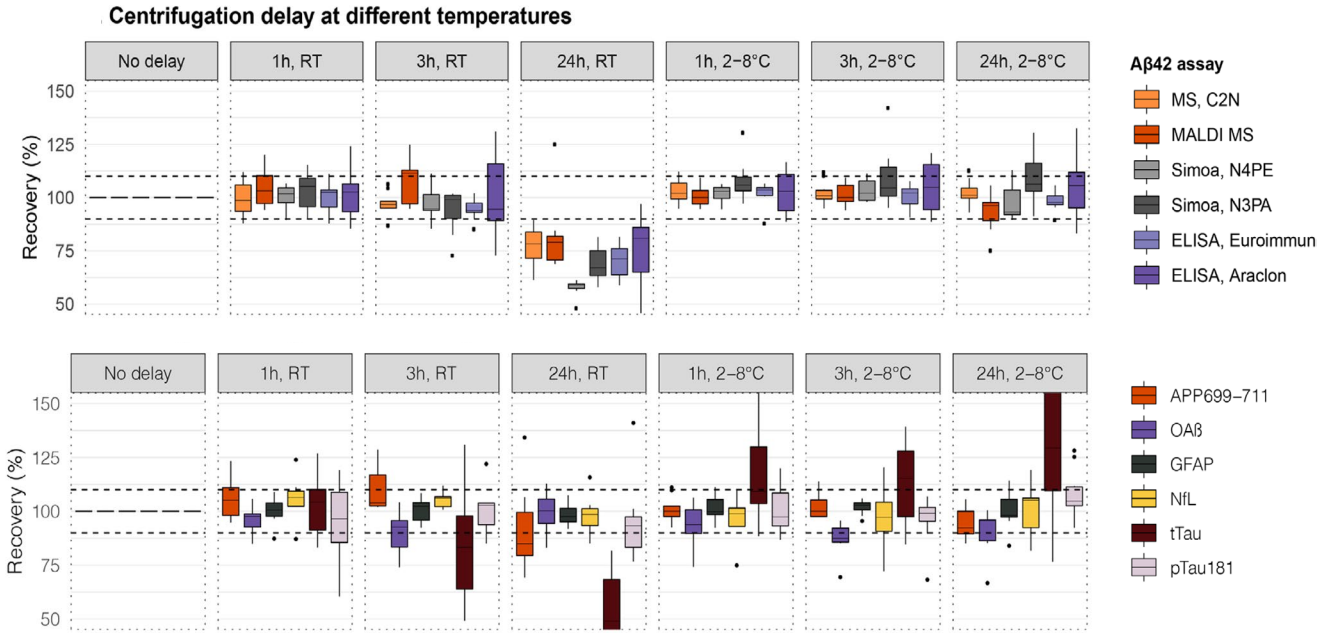


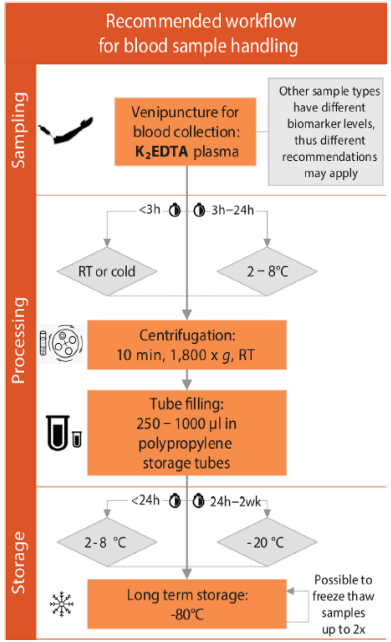
Pichet Binette et al., Alz&Dem

Complementary reading: Syrjanen et al., Alz&Dem; Tang et al., Alz Res Ther

Key research priorities
Determine factors affecting the results

Aim: Develop analyte- and platform independent standardized operating procedure for pre-analytical plasma sample handling

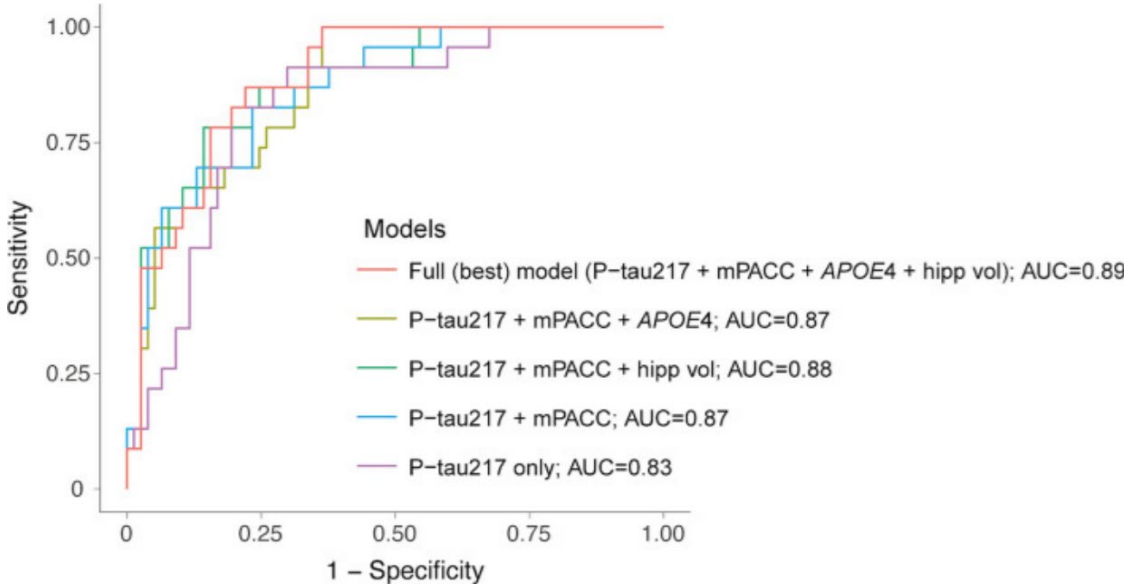




Verberk et al., Alz&Dem

Key research priorities
Do we need to make panels?

Aim: identify patients with MCI (n=110) at risk to progress to AD-dementia with combinations of AD plasma biomarkers and other easily accessible measures
Result: P-tau217 alone had the best performance; negligible contribution of Aβ42/40, GFAP and NfL

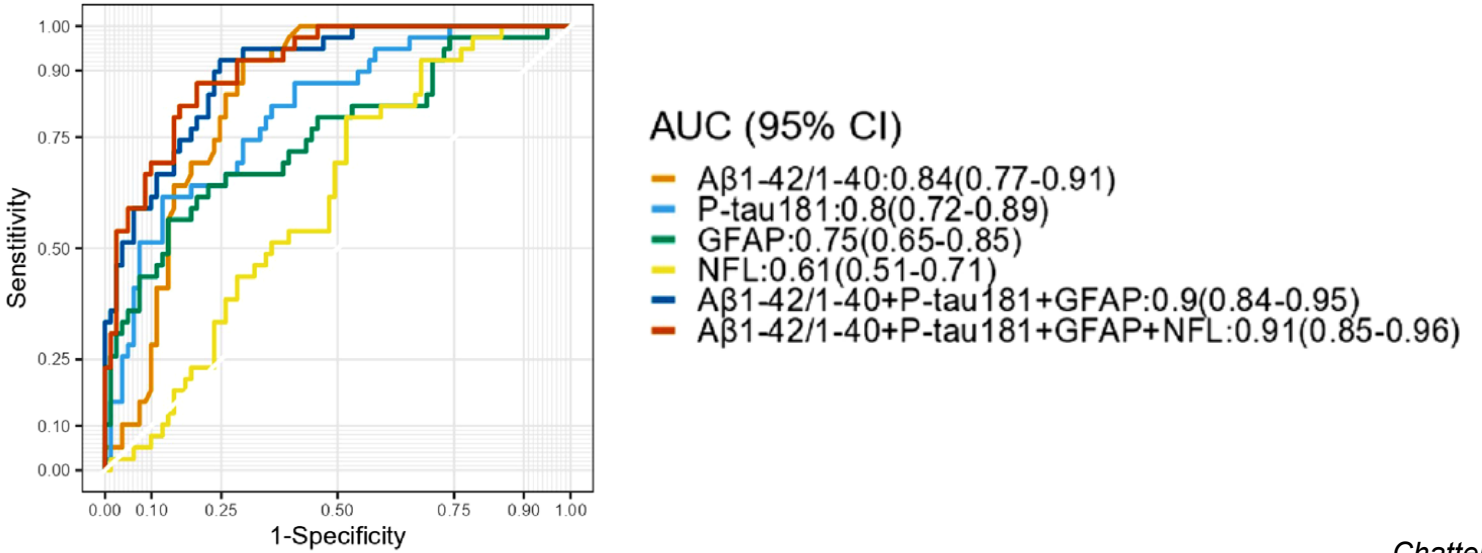


Pichet Binette et al., Alz Res Ther
Complementary reading: Planche et al., Neurology

Key research priorities
Do we need to make panels?

Aim: Compare the diagnostic performance of the plasma AD markers head-to-head (n=100)
Result: Single markers: AUC 0.61 – 0.84. Panel: AUC 0.90 – 0.91

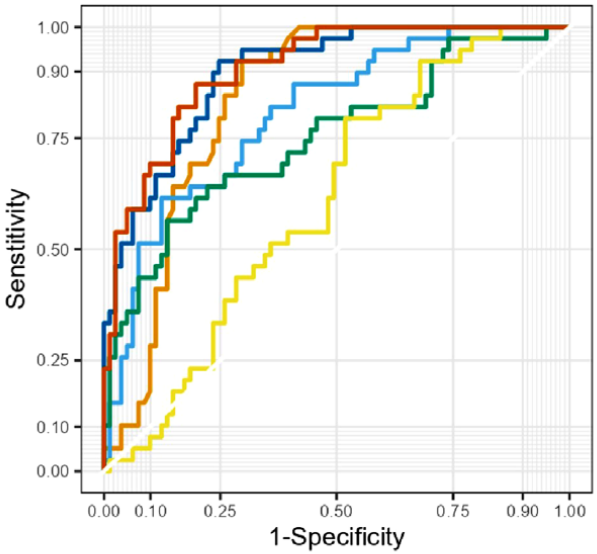
CU Aβ- versus AD Aβ+ (AIBL)



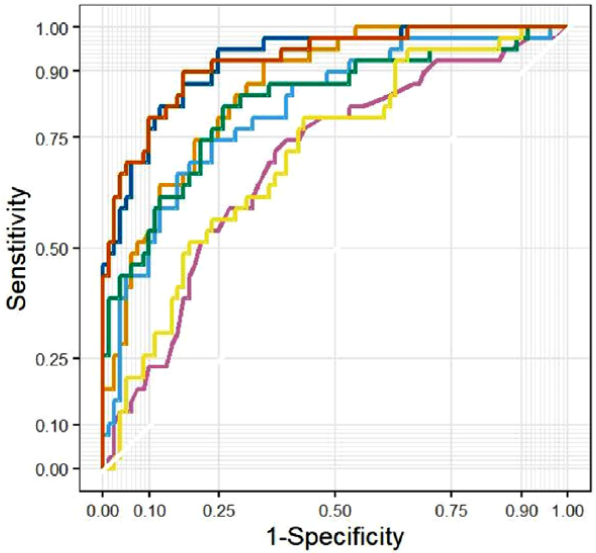
Chatterjee et al., Alz&Dem

Panel with or without risk factors?

CU Aβ- versus AD Aβ+ (AIBL)



AUC (95% CI)
- Aβ1-42/1-40:0.84(0.77-0.91)
- P-tau181:0.8(0.72-0.89)
- GFAP:0.75(0.65-0.85)
- NFL:0.61(0.51-0.71)
- Aβ1-42/1-40+P-tau181+GFAP:0.9(0.84-0.95)
- Aβ1-42/1-40+P-tau181+GFAP+NFL:0.91(0.85-0.96)



AUC (95% CI)
- Base Model(BM):0.69(0.59-0.79)
- BM+Aβ1-42/1-40:0.86(0.79-0.92)
- BM+P-tau181:0.81(0.73-0.9)
- BM+GFAP:0.83(0.74-0.91)
- BM+NFL:0.71(0.61-0.81)
- BM+Aβ1-42/1-40+P-tau181+GFAP:0.92(0.88-0.97)
- BM+Aβ1-42/1-40+P-tau181+GFAP+NFL:0.92(0.87-0.97)

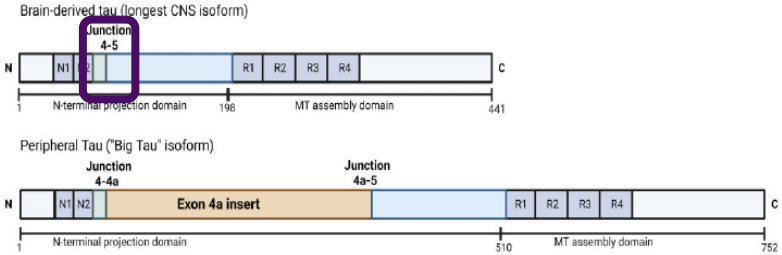
Results panels (red, blue):
- Without risk factors (left): AUC 0.90 and 0.91
- With risk factors (right): AUC 0.92

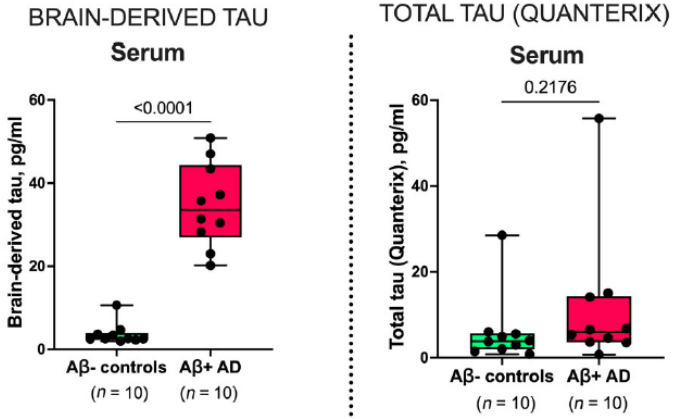
Risk factors: age, sex, APOE ε4

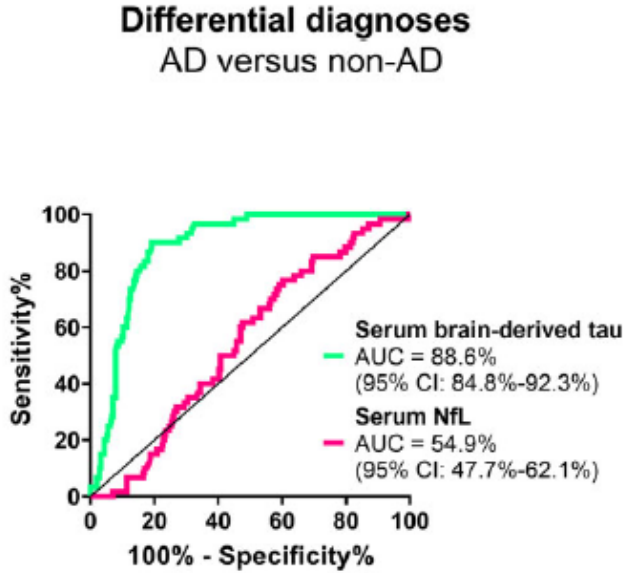
Chatterjee et al., Alz&Dem

Key research priorities
Make brain-specific assays

Method: Assay developed for brain-derived tau (capture for a continuous exon 4-5)





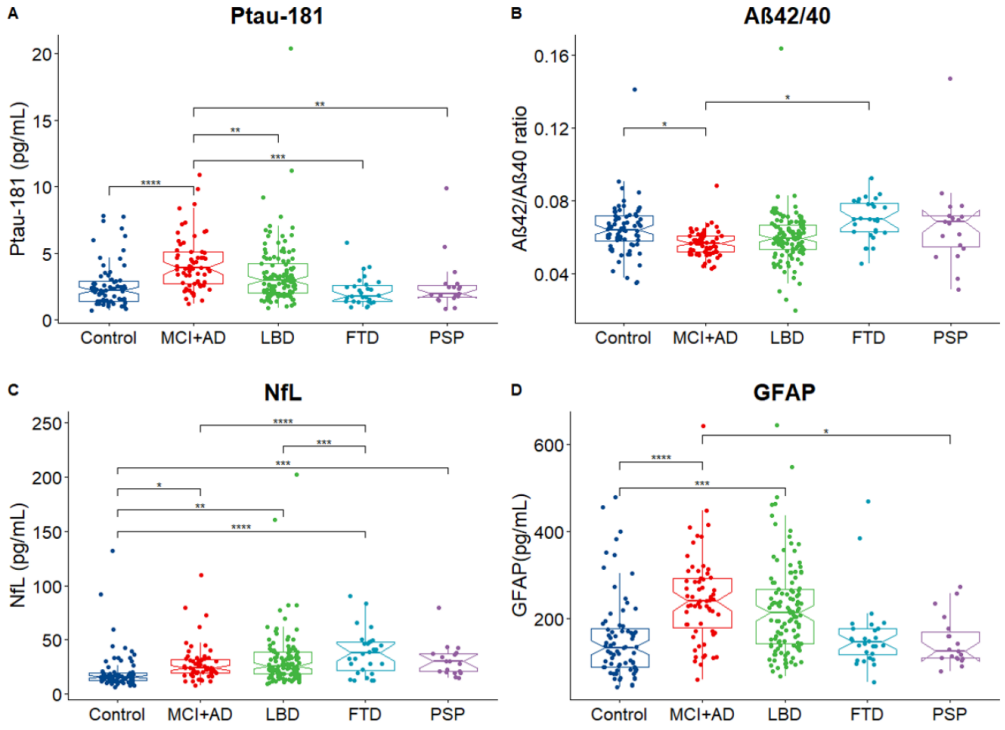


Gonzalez-Ortiz et al., Brain

Biomarkers for non-AD

Plasma biomarkers for AD exclusion

Aim: Differentiate LBD, FTD and PSP from AD using the AD plasma biomarkers



P-tau ↑ in MCI-AD vs. the other dementias

Aβ42/40 ↓ in MCI-AD vs. FTD

NfL ↓ in MCI-AD vs. FTD

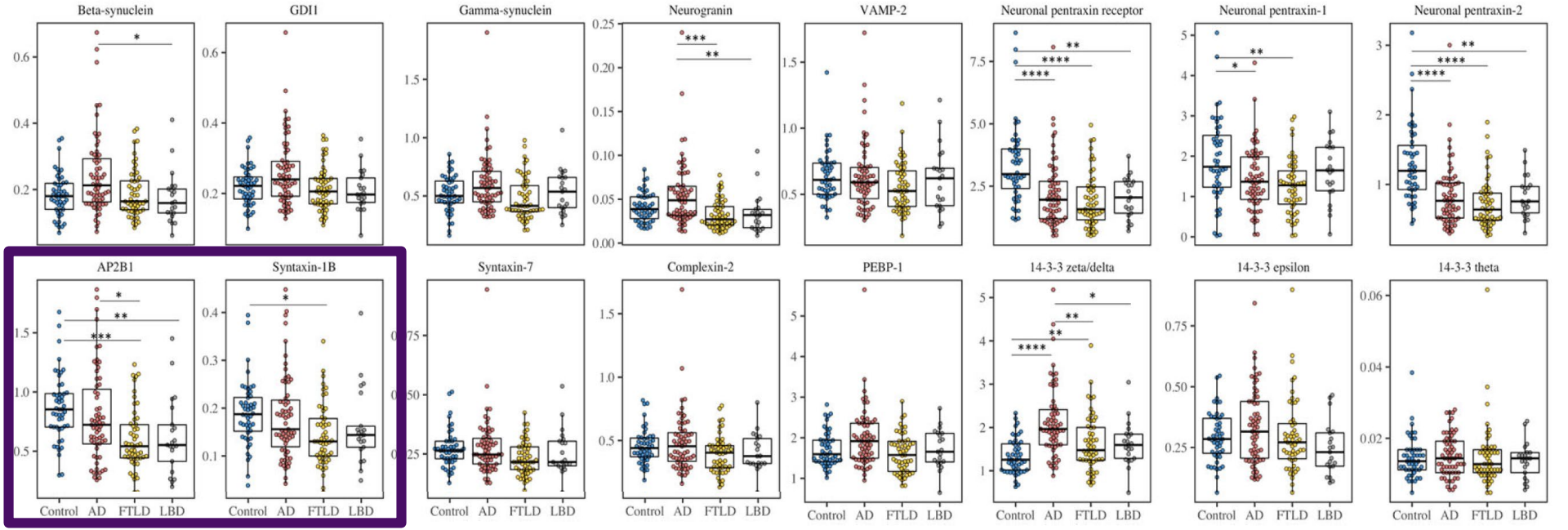
GFAP ↑ in MCI-AD vs. PSP

Chouliaras et al., JNNP

Complementary reading: Thijssen et al., DADM

Synaptic CSF biomarkers specific for FTD and DLB

Method: CSF synaptic dysfunction panel by LC-MS for differentiation of AD (n=63), FTLD (n=53) and LBD (n=21)



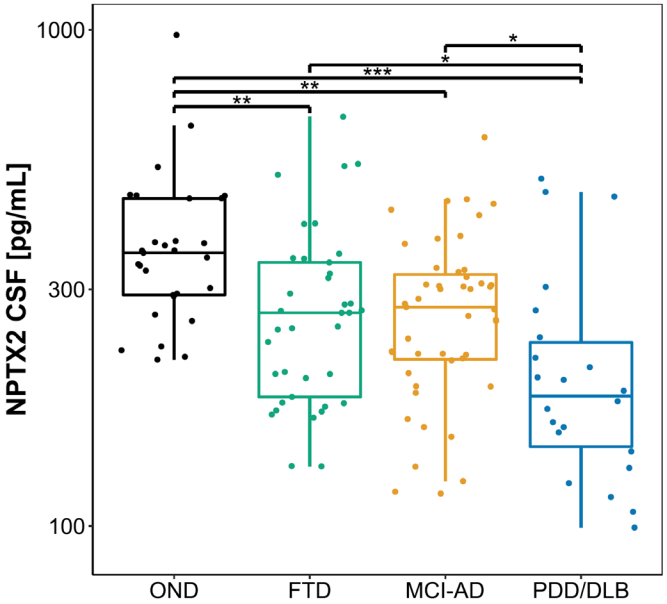
Nilsson et al., Alz&Dem

Complementary reading: Sogorb-Esteve et al., Alz Res Ther

CSF NPTX2 for differential diagnosis

Aim: Explore diagnostic potential of CSF NPTX2 for FTD vs. MCI-AD and PDD/DLB

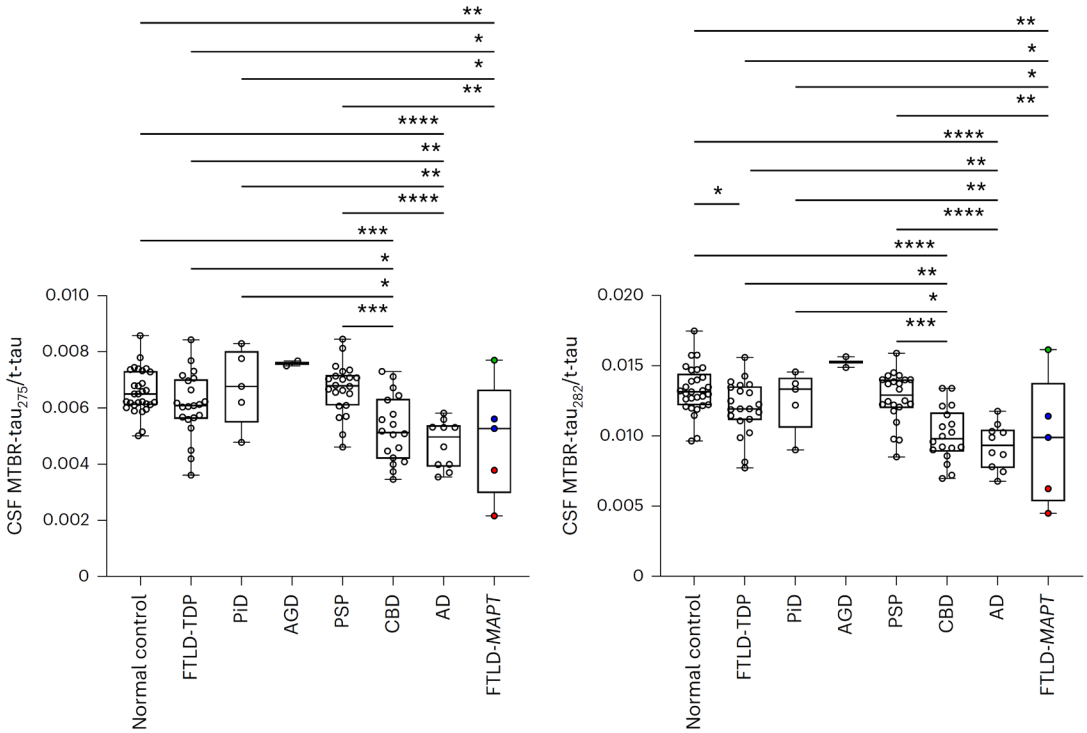
Results: AUCs NPTX2 versus FTD: 0.74 (OND), 0.67 (MCI-AD), 0.78 (PDD/DLB)



Bolsewig et al., JAD

4R isoform-specific tau for FTD subtypes

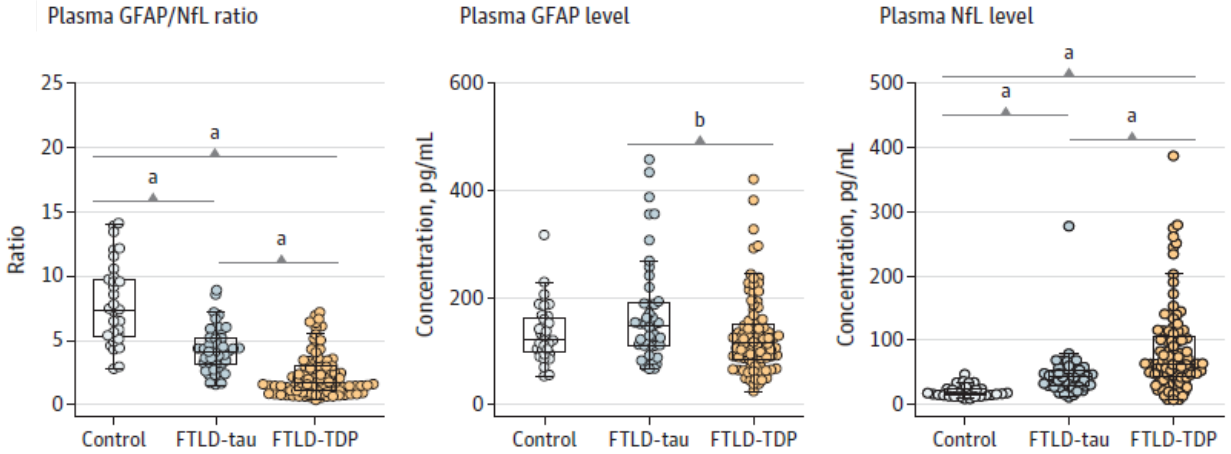
Method: Test CSF levels of 4R isoform-specific MTBR-tau species in different 4R tauopathies (CSF MTBR-tau$_{275}$ and MTBR-tau$_{282}$)

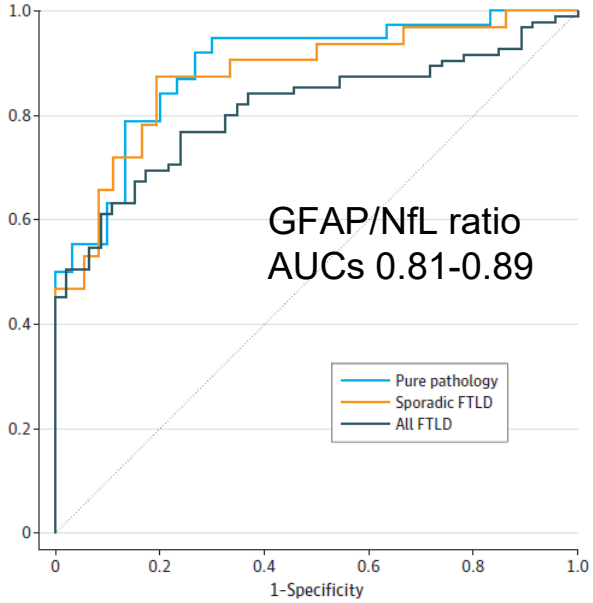


Horie et al., Nat Med

GFAP/NfL ratio for FTD subtypes

Aim: discriminate FTLD-tau (n=46) from FTLD-TDP (n=95) using GFAP/NfL ratio



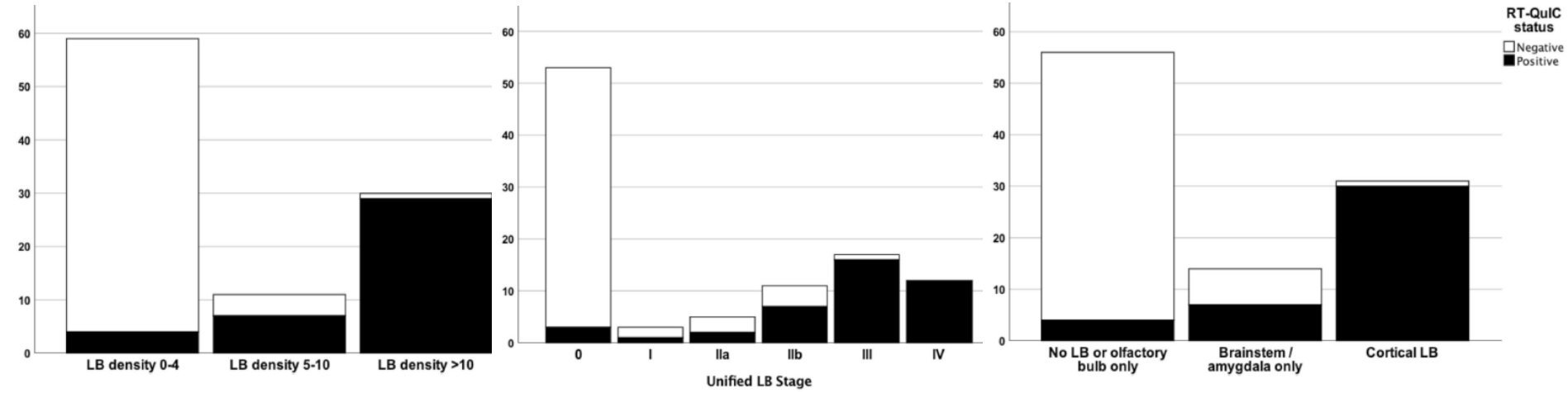


Cousins et al., JAMA neurol

Complementary reading: Katisko et al., Alz Res Ther (serum TDP-43 by FTLD-type)

CSF αSynuclein RT-QuIC for DLB

Aim: Assess performance of CSF αSynuclein RT-QuIC in relation to Lewy body disease



Hall et al., Acta Neuropathol Commun

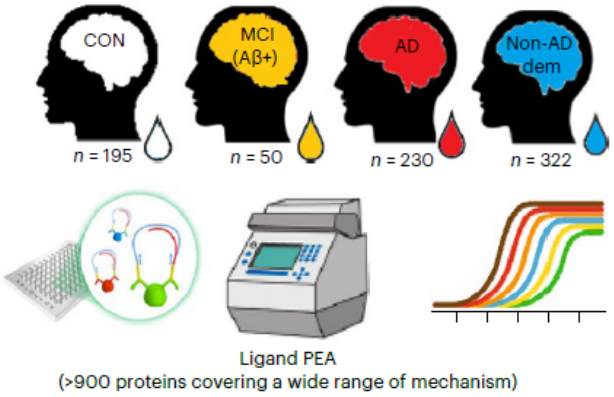
Complementary reading: review by Bellomo et al., Neurology

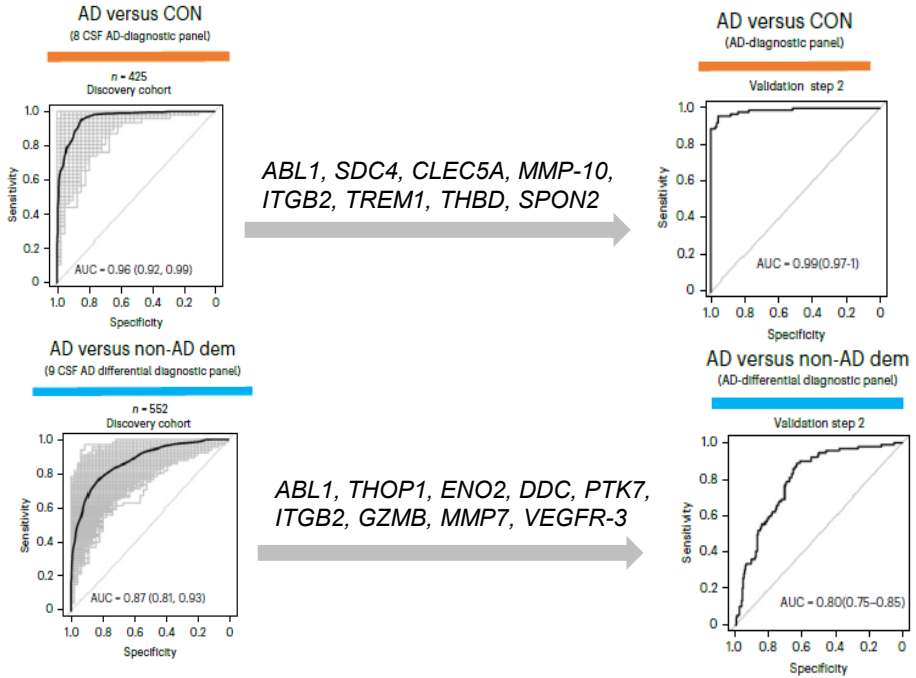
Biomarker discovery and novel biomarkers

Biomarker discovery in CSF

Method: >900 proteins with OLINK to identify novel biomarkers for AD and non-AD dementias

Panels selected and translated into customary panels

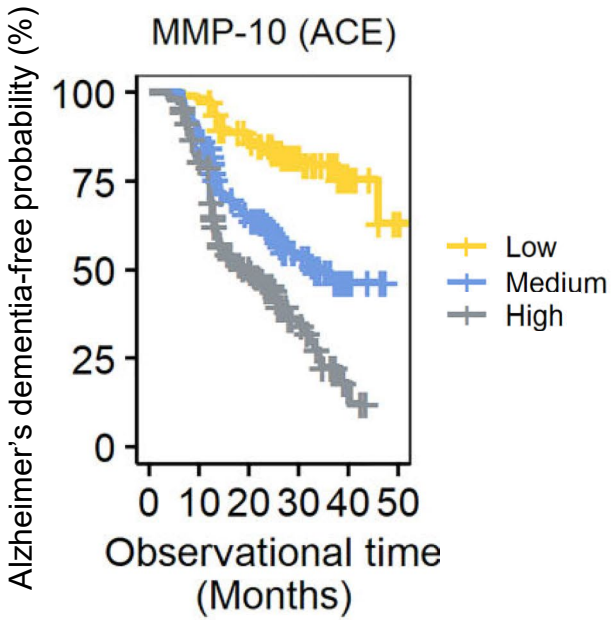


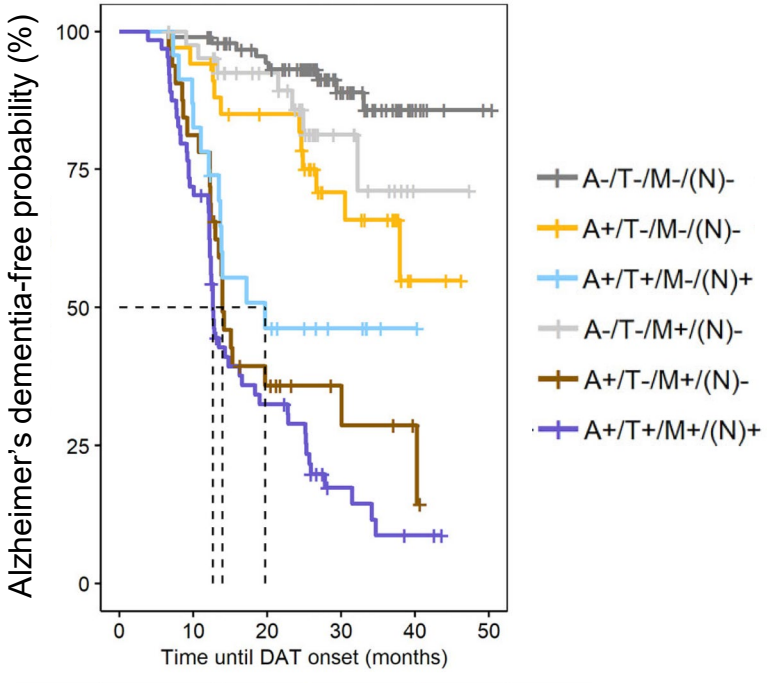


Del Campo et al., Nature aging

Novel biomarkers: CSF MMP-10

Result: CSF MMP-10 predicts conversion to Alzheimer's dementia, with added value to A/T/N

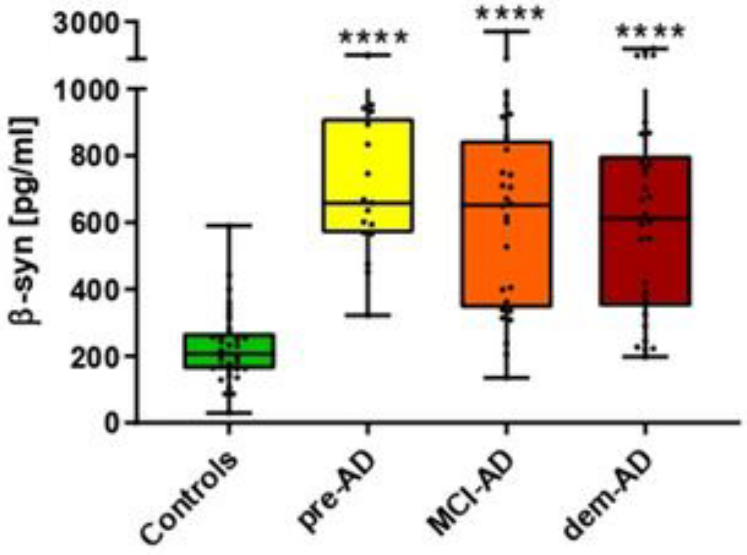


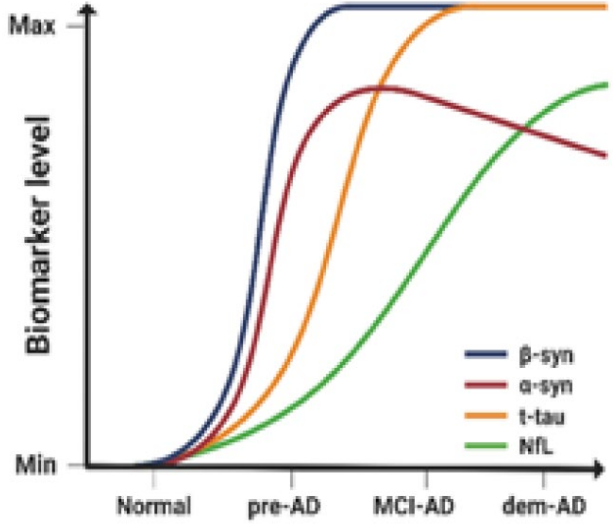


Martino Adami et al., Brain

Novel biomarkers: CSF β-synuclein

Results: AUCs: 0.91 – 0.99

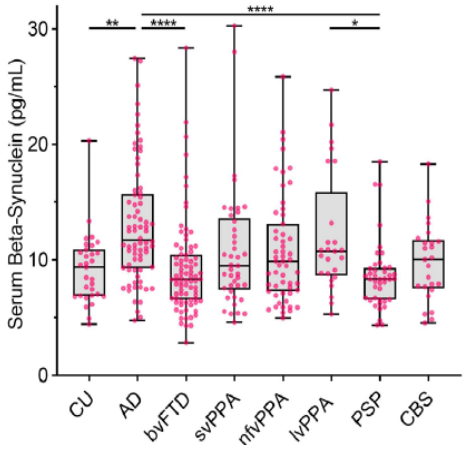


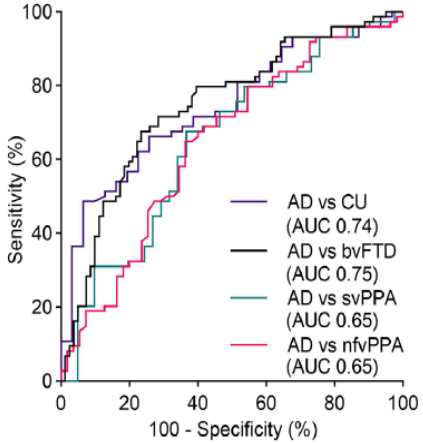


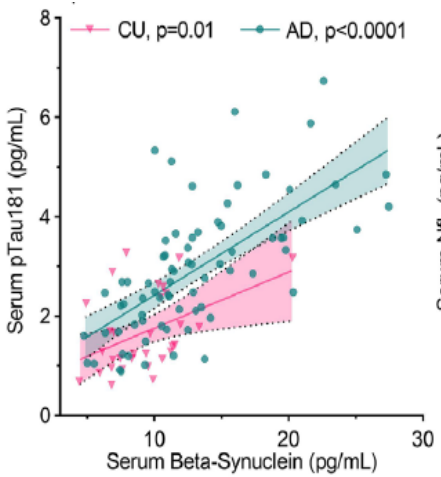
Barba et al., J Neurol Neurosurg Psychiatry

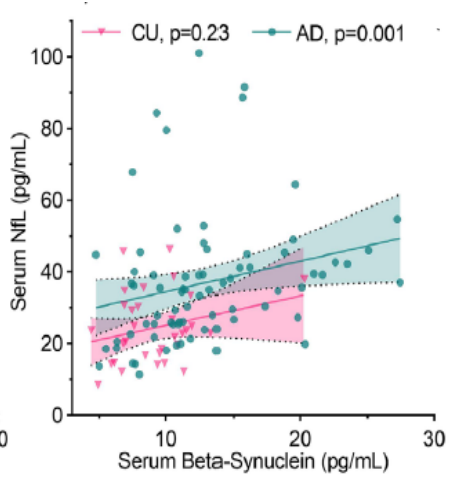
Novel biomarkers: serum β-synuclein

Results: Specific increase in AD and association with serum pTau181 and NfL









Oeckl et al., Alz & Dem

Complementary reading: Oeckl et al., Annal Neurol; Halbgebauer et al., Int J Mol Sci; Halbgebauer et al., Neurology

Take home points

- **Important steps are being taken to bring the Alzheimer's blood test to the market, but still a lot to do, e.g.:**
  - Define the most clinically and technically robust assays
  - Define how to use the markers: panels or standalone, with or without clinical measures
  - Define how to interpret the markers: e.g. binary outcomes with or without gray zones
  - Assess real-world settings, including peripheral hospitals and primary care
  - Obtain more longitudinal data (intra-individual stability)
  - Better address ethnical diversity
  - Further define the roles in clinical trials and further assess their value: Are they already good enough? Do we need more? Can they become primary endpoints over time?

- **There is an important role for CSF still:**
  - Biomarker discovery
  - Develop diagnostic biomarkers for differential diagnosis (e.g. specific for FTD, DLB)

Thank you!

PhD students of the
Amsterdam UMC neurochemistry lab

Survey responders

Marta del Campo and Ana Pereira

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