SUPPLEMENTARY MATERIAL

Supplemental Methods

eMethod1. Participants

We evaluated cognitively unimpaired (CU) individuals from the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort. Participants included in this study had baseline [^{18}F]florbetapir amyloid (Aβ) Positron Emission Tomography (PET) and plasma measurement of p-tau181 and NfL in at least two visits (up to 24 months). All participants presented Mini-Mental State Examination (MMSE) scores ≥ 24 and Clinical Dementia Rating (CDR) of 0. The participants included in this study did not receive any disease-modifying therapies.

eMethod2. Imaging analysis

The standardized uptake value ratio (SUVR) images were acquired using the whole cerebellum grey matter as the reference region. A cortical composite of [^{18}F]florbetapir was calculated by averaging SUVRs in the cortical gray matter of frontal, anterior and posterior cingulate, lateral parietal, and temporal regions. Aβ PET positivity (+) was determined whether the composite value was greater than 1.11, based on previous validation studies[2,3]. We used SUVR value and equations previously established by the ADNI PET core to transform the Aβ PET SUVR to the Centiloid scale[4]. Based on ongoing clinical trials, the individuals with Centiloid values between 20-40 were classified as having intermediate Aβ levels[5,6].

eMethod3. Plasma biomarkers

Plasma phosphorylated tau 181 (p-tau181) and neurofilament light chain (NfL) were measured on single molecule array (Simoa) HD-X instruments (Quanterix, Billerica, MA, USA) at the Clinical Neurochemistry Laboratory, University of Gothenburg, Mölndal, Sweden. Baseline p-tau181 and NfL levels were considered outliers and excluded if their value was higher than three standard deviations of the population as proposed in the previous studies[7]. Based on this criterion, 10 individuals were excluded from the analyses.

eMethod4. Statistical analysis

Associations between biomarkers were tested using Pearson correlation. The slope of change of plasma p-tau181 and plasma NfL levels were calculated using linear mixed-effects (LME) models with subject-specific random slopes and intercepts, as follows:

$$Slope\ Plasma\ Biomarker = \beta_0 + \beta_1 Time_{ij} + (1 + \beta_1 Time_{ij} | v_{0i})$$

i. β_0 represents the intercept;

ii. $Time_{ij}$ represents the effect of time, fitted as a continuous measure in years from baseline;

iii. υ_{0i} is the subject-specific variation from the average intercept effect.

Time-to-event analysis was carried out to evaluate the risk of clinical progression to mild cognitive impairment (MCI). Adjusted hazard ratios (HRs) were calculated using Cox-proportional hazards models that were fitted with the following predictors: group, age, and years of education. The percentage of change in biomarkers was calculated between follow-up and baseline as follows: $\left(\frac{\text{Follow up}-\text{Baseline}}{\text{Baseline}}\right) * \left(\frac{100}{\Delta\text{time}}\right)$. The effect size was calculated as the mean of change in biomarker in the group divided by the standard deviation[11]. We estimated the sample size required for a clinical trial testing a hypothesized 25% drug effect on longitudinal reduction in biomarker[11-13] with 80% of power at a 5% level using a well-validated formula[11,14] . We used 12-month changes in tau PET (^{18}F-flortaucipir SUVR in the temporal lobe) and structural MRI (whole cortex atrophy assessed with tensor-based morphology)[11] in Aβ PET positive individuals previously reported in the literature to estimate the sample size for clinical trials using neuroimaging biomarkers as surrogate outcomes.

eMethod5. CSF measures

CSF $A\beta_{1-42}$ levels were measured using fully automated Elecsys immunoassays (Roche Diagnostics).[8] Measurements outside the analytical range (< 200 pg/mL or > 1700 pg/mL for $A\beta_{1-42}$) were set to their respective technical limit. Aβ positivity was defined as CSF $A\beta_{1-42}$ < 977 pg/mL[9,10].

Supplemental Tables and Figures

eTable 1. CU participant's demographics and key characteristics.

	Aβ PET negative	**Aβ PET positive**	**Aβ PET Centiloid 20-40**
Number	174	83	25
Age at baseline, years (SD)	71.8 (6.02)	74.8 (5.90)	76.6 (6.41)
Male, No. (%)	88 (51.2%)	27 (32.9%)	20 (38.5%)
MMSE at baseline, score (SD)	29.1 (1.3)	29.0 (0.9)	29.0 (1.2)
Education, years (SD)	16.9 (2.46)	16.2 (2.74)	16.0 (3.18)
Race/ethnicity (%)			
American Indian/Alaska Native	1 (0.57%)	-	-
Asian	10 (5.75%)	1 (1.20%)	1 (3.58%)
Black	4 (2.30%)	6 (7.23%)	4 (14.29%)
White	155 (89.08%)	75 (90.37%)	23 (82.14)
More than one	4 (2.30%)	1 (1.20%)	-
***APOE*ε4, number (%)**	38 (22.1%)	35 (42.7%)	16 (30.8%)
Global Aβ PET at baseline, SUVR	1.02 (0.05)	1.32 (0.178)	1.15 (0.03)
Plasma p-tau181 at baseline, pg/ml	14.1 (10.4)	17.1 (7.8)	15.3 (13.2)
Plasma NfL at baseline, pg/ml	32.4 (10.8)	37.1 (14.0)	36.1 (15.8)

Continuous variables are presented as mean (standard deviation). *APOE* ε4 = Apolipoprotein E ε4; p-tau181 = tau phosphorylated at threonine 181; Neurofilament light chain (NfL).

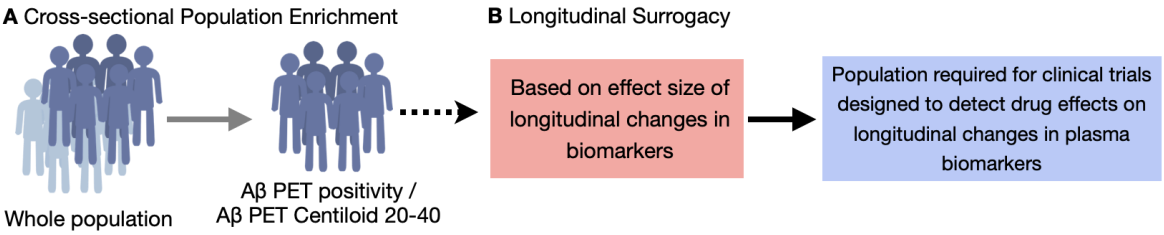
eTable 2. Repeated measures estimate of the slopes used in the supplementary eFigure 3, eFigure 4 and eFigure 5.

Effect	β (SE)	t (DF)	p-value	95% CI for β
Plasma p-tau181				
Intercept	16.79 (1.27)	13.25 (340.6)	<0.0001	(14.3 to 19.3)
Years from baseline	0.10 (0.45)	1.18 (238.2)	0.817	(-0.78 to 0.98)
Plasma NfL				
Intercept	37.41 (1.03)	36.21 (362)	<0.0001	(35.5 to 39.4)
Years from baseline	2.04 (0.45)	4.6 (192)	<0.0001	(1.15 to 2.92)

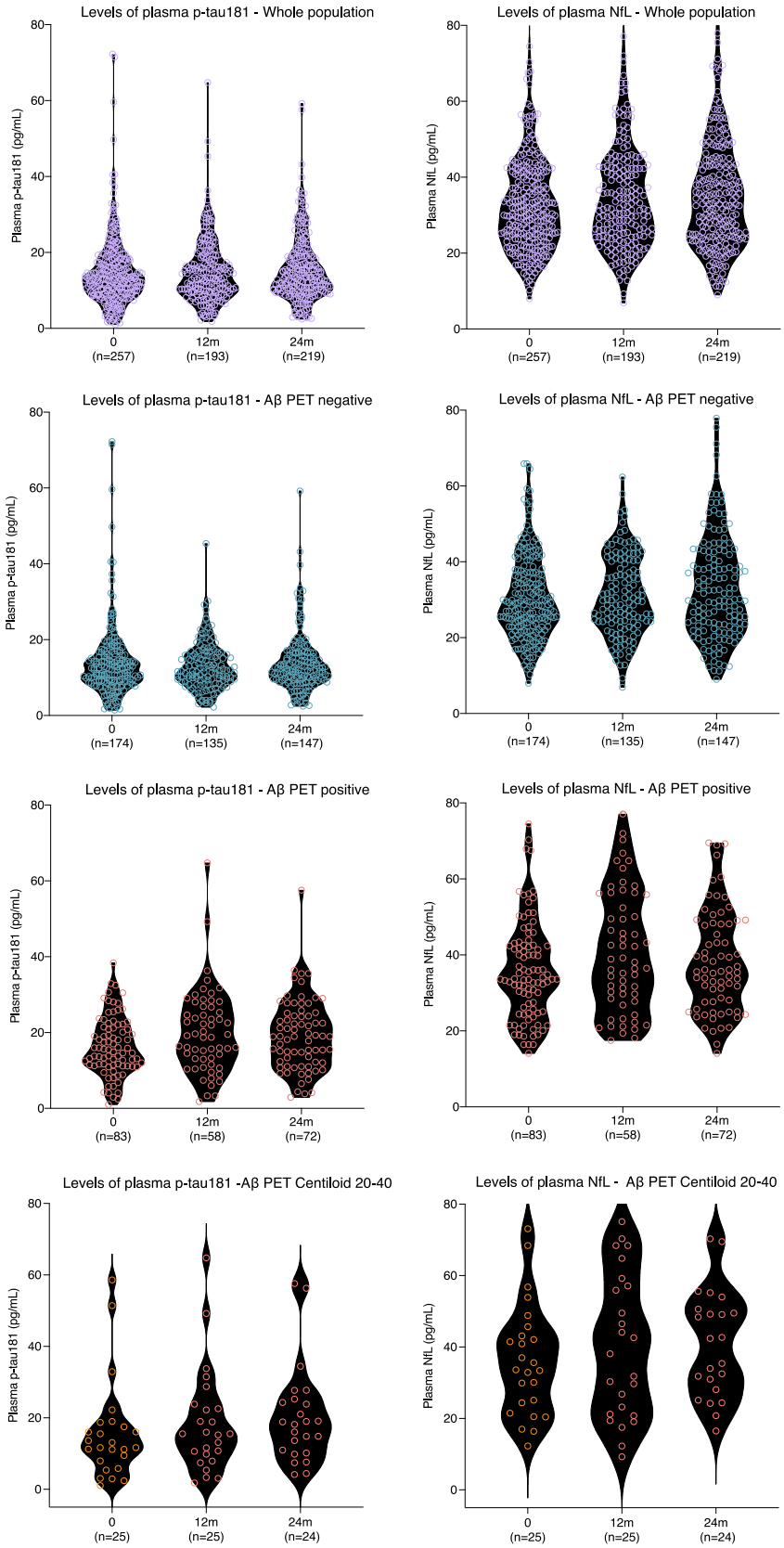
eTable 3. Sample size estimates per study arm required for clinical trials in CU individuals enriched using different strategies.

	Whole population	Aβ PET positivity	Aβ PET Centiloid 20-40	Aβ CSF positivity	*APOE* ε4 carriership
Plasma p-tau181					
12 months	28,090	11,796	3,112	6,786	5,974
24 months	4,442	2,520	1,216	5,535	14,572
Plasma NfL					
12 months	4,556	3,886	1,608	4,470	4,230
24 months	1,724	1,434	698	2,168	3,294

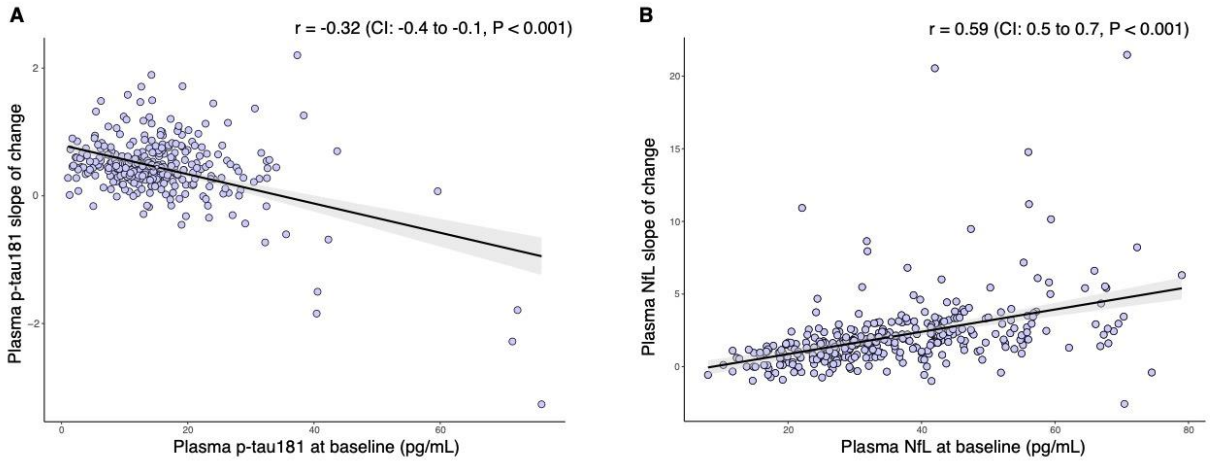
Sample size estimates per study arm for a clinical trial targeting a 25% reduction in change in plasma biomarker with 80% power and 0.05 alpha. *APOE*ε4 = Apolipoprotein E ε4.



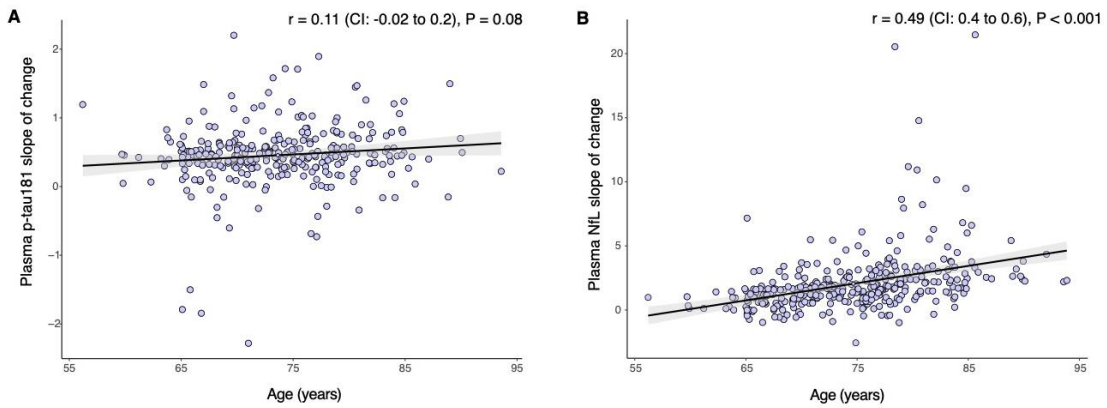
eFigure 1. Assessment of the utility of plasma biomarkers for clinical trials. (**A**) Previous studies assessed the utility of plasma markers for selecting individuals most likely to progress over time (cross-sectionally – population enrichment). (**B**) In the present study, we evaluated the utility of plasma biomarkers to monitor drug effects (longitudinally – surrogacy).


Levels of plasma p-tau181 - Whole population
Plasma p-tau181 (pg/mL)
0
20
40
60
80
0 (n=257)
12m (n=193)
24m (n=219)
Levels of plasma NfL - Whole population
Plasma NfL (pg/mL)
0
20
40
60
80
0 (n=257)
12m (n=193)
24m (n=219)
Levels of plasma p-tau181 - Aβ PET negative
Plasma p-tau181 (pg/mL)
0
20
40
60
80
0 (n=174)
12m (n=135)
24m (n=147)
Levels of plasma NfL - Aβ PET negative
Plasma NfL (pg/mL)
0
20
40
60
80
0 (n=174)
12m (n=135)
24m (n=147)
Levels of plasma p-tau181 - Aβ PET positive
Plasma p-tau181 (pg/mL)
0
20
40
60
80
0 (n=83)
12m (n=58)
24m (n=72)
Levels of plasma NfL - Aβ PET positive
Plasma NfL (pg/mL)
0
20
40
60
80
0 (n=83)
12m (n=58)
24m (n=72)
Levels of plasma p-tau181 -Aβ PET Centiloid 20-40
Plasma p-tau181 (pg/mL)
0
20
40
60
80
0 (n=25)
12m (n=25)
24m (n=24)
Levels of plasma NfL - Aβ PET Centiloid 20-40
Plasma NfL (pg/mL)
0
20
40
60
80
0 (n=25)
12m (n=25)
24m (n=24)

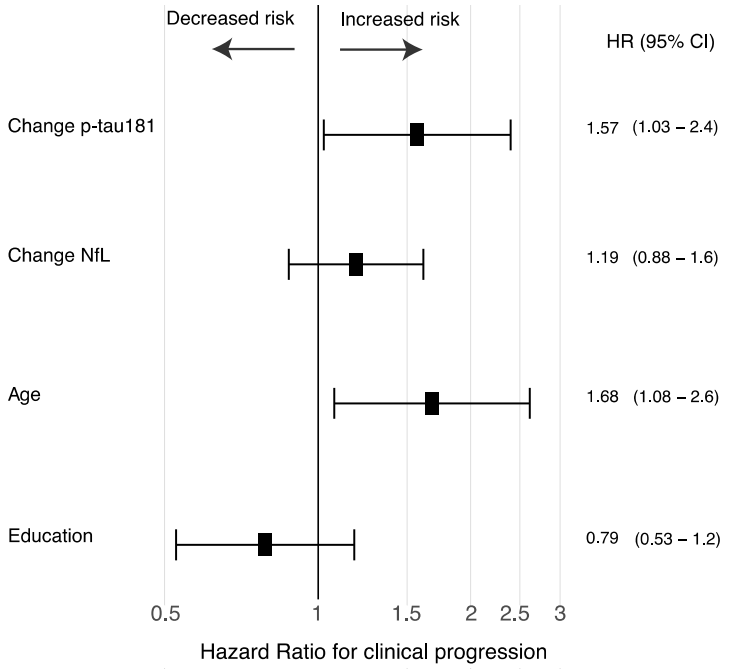
eFigure 2. Plasma p-tau181 and NfL estimates across time frames. The violin plots show the overall levels of plasma p-tau181and NfL concentrations across CU older individuals available in each visit and according to Aβ PET status. Note that violin plots at follow-up visits (12-24 months) are only partially composed by the same individuals. Some individuals had baseline and 12-month follow-up only, and others had baseline and 24-month follow-up only. This limits the comparison of the rates of changes between 12 and 24 months.



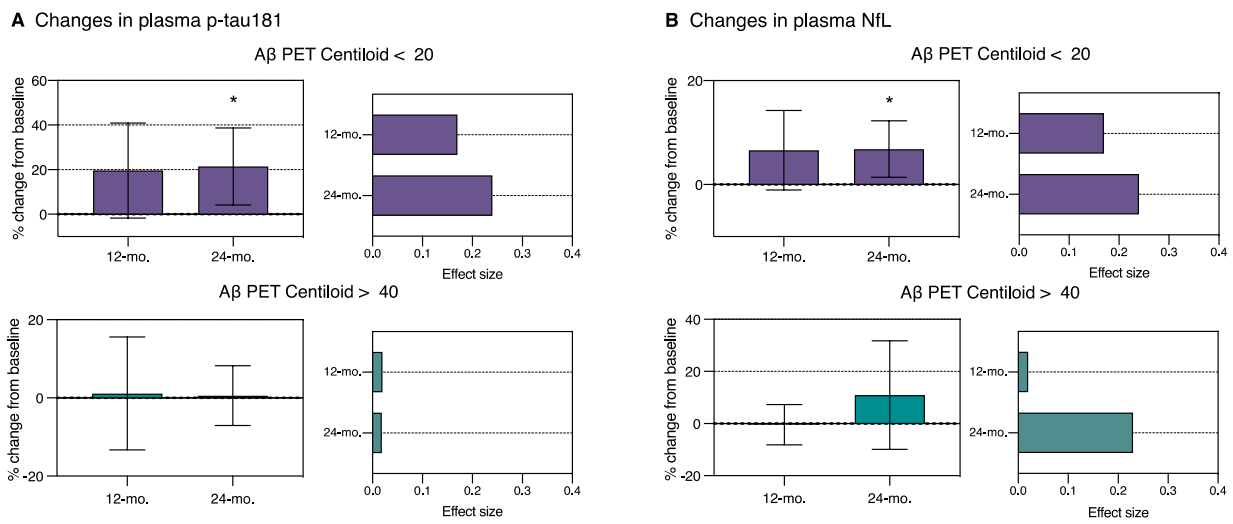
eFigure 3. Correlation between baseline biomarkers levels and their corresponding longitudinal changes in CU individuals. (**A**) The plot shows a negative Pearson correlation between the baseline levels and longitudinal changes in p-tau181. (**B**) The plot shows a positive Pearson correlation between the baseline levels and longitudinal changes in NfL.



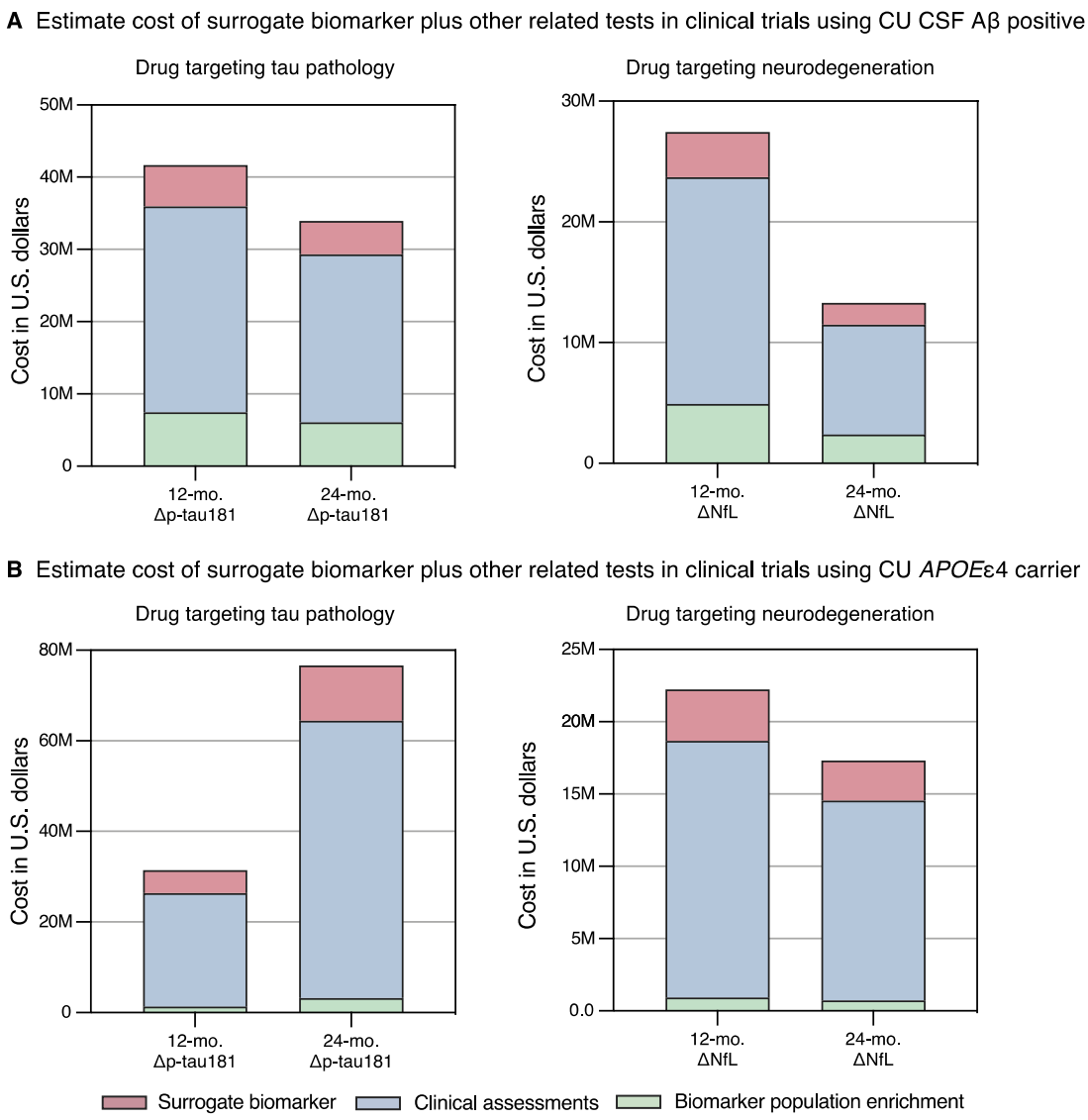
eFigure4 – Longitudinal change in plasma NfL, but not in p-tau181, positively associated with participants' age at baseline. The plot (**A)** shows no significant Pearson correlation between longitudinal changes in p-tau181 levels and age at baseline. The plot (**B)** shows a significant Pearson correlation between longitudinal changes in NfL levels and age at baseline.



eFigure 5. Longitudinal change in plasma p-tau181, but not in NfL, is associated with an increased risk of progression to MCI. The squares represent the hazard ratio values with a 95% confidence interval of the variables for a clinical progression from CU to MCI over 24 months. The model accounted for years of age and education.



eFigure 6. Percentage of change and effect size of plasma biomarkers over 12 and 24 months in individuals with the lowest (Centiloid < 20) and highest (Centiloid > 40) baseline Aβ. The bar plots show the percentage of changes with their respective 95% confidence intervals for plasma (**A**) p-tau181 and (**B**) NfL concentrations in CU older individuals over 12 months and over 24 months in relation to the biomarker value at the baseline visit. The effect size was calculated as the ratio between the mean and standard deviation of the percentage of change overtime points. (*) indicates that the 95% confidence interval did not cross the zero line, and therefore, the longitudinal progression was significantly different from zero.



eFigure 7. Cost-effectiveness of plasma biomarkers as surrogates for clinical trials using different population enrichment strategies. The figure **A** shows the estimated cost of clinical trials using plasma p-tau181 and NfL as a surrogate using CSF Aβ42 to define Aβ positivity as a population enrichment strategy (Aβ positivity was defined as CSF Aβ42 < 977 pg/mL, **eMethod5**). The figure **B** shows the estimated cost of clinical trials using APOE ε4 allele carriers as a population enrichment strategy. For the calculations, we used the following hypothesized values: plasma markers = $200; CSF markers = $200; lumbar puncture procedure = $300; APOE ε4 allele genotype = $100; Recruitment/consenting/clinical assessment = $1,000. Assessments (excepted with the population enrichment) were calculated to 2 time-points (baseline and follow-up). We estimated an attrition rate of 10% in the calculations. Δ = longitudinal change.

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