# Supplemental Online Content

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This supplemental material has been provided by the authors to give readers additional information about their work.

## eMethods

### Positron Emission Tomography (PET)

For amyloid PET imaging, a target dose of 10 millicurie (mCi) (370 megabecquerel (MBq)) of florbetapir was injected 50 minutes prior to acquisition of a 20-minute PET brain scan (4 frames x 5 minutes). The composite standardized uptake value ratio (SUVR) was calculated from 6 predetermined cortical regions with the whole cerebellum as a reference region [1]. The SUVR was then converted to Centiloid (CL) units [2].

For tau PET imaging, a target dose of 10 mCi (370 MBq) of flortaucipir was injected 75 minutes prior to acquisition of a 30-minute PET brain scan (6 frames x 5 minutes). For study eligibility, global neurofibrillary tangle density was estimated by the SUVR of tau PET signal in a weighted cortical target region (Multiblock barycentric discriminant analysis (MUBADA) derived region) relative to a parametrically derived white matter reference region (parametric estimate of reference signal intensity (PERSI)) [3,4]. For measuring tau neurofibrillary tangle change with treatment, regional tau SUVRs for the cerebral lobes were calculated using the Automated Anatomical Labelling atlas [5] while MUBADA region was used for global tau measurements. For tau PET longitudinal change assessments, a cerebellar gray matter region derived from the cerebellar crustaneous atlas-based region was used as a reference in our regional and global tau SUVR calculations [6].

### Plasma Assays

The P-tau217 assay is a digital immunoassay for the quantitative determination of tau phosphorylated at threonine 217 in human K2EDTA plasma. The Simoa HD-X analyzer uses P-tau217 immunoassay reagent to perform jobs (one job equals a single value, so if run in duplicate, that equals two jobs) using Single Molecule Array (Simoa) technology. This is a three-step P-tau217 digital immunoassay. In the first step, target antibody-coated paramagnetic capture beads are combined with sample. Target molecules present in the sample are captured by the anti-phospho-tau217 antibody coated capture beads. After washing, biotinylated detector anti-total tau antibodies are mixed with the capture beads. The detector antibodies bind to the captured target. Following a second wash, a conjugate of streptavidin-ß-galactosidase (SBG) is mixed with the capture beads. SBG binds to the biotinylated detector antibodies, resulting in enzyme labeling of captured target. Following a third wash, the capture beads are resuspended in a resorufin ß-D-galactopyranoside (RGP) substrate solution and transferred to the Simoa Disc. Individual capture beads are then sealed within microwells in the array. If target has been captured and labeled, the ß-galactosidase hydrolyzes the RGP substrate into a fluorescent product that provides the signal for measurement. A single-labeled target molecule results in a sufficient fluorescent signal to be detected and counted in 30 seconds by the Simoa optical system. At low target concentration, the percentage of bead-containing wells in the array that have a positive signal is proportional to the amount of target present in the sample. At a higher target concentration, when most of the bead-containing wells have one or more labeled target molecules, the total fluorescence signal is proportional to the amount of target present in the sample. The concentration of target in unknown samples is interpolated from the calibration curve using a log-log power regression without weighting.

Precision: Precision was determined by running validation samples with a minimum of two (2) replicates. A minimum of twenty (20) runs (no more than one [1] run per day for at least twenty (20) days was performed by multiple scientists (within-lab) on multiple analyzers. The cumulative data from the minimum of twenty (20) runs described above was analyzed for repeatability and within-lab (total) precision.

Validation samples were placed in a random sequence on the plate or within the run. *A priori* criteria for with-in lab and total precision was a ≤ 20% CV for 80% (16/20) of samples.

> *Precision criteria were met with 100% of samples (20/20) having a ≤ 20% CV (and 80% of samples (16/20) having a ≤ 15% CV). Furthermore, 10 of the 20 samples used for the precision study covered a range of 0.2 U/ml – 0.4 U/ml.*

Accuracy (analytical): Accuracy was assessed via percentage agreement studies, which utilized appropriate sample matrix spiked with known levels of P-tau217 calibrator material to span the range. Results were compared with expected results and expressed as recovery (%RE). Percent (%) agreement was calculated by dividing the number of

correct results by the total number of results and multiplying by 100. *A priori* acceptance criteria for accuracy were 80 – 120% RE with 80% agreement across samples.

*Accuracy (analytical) criteria were met with 83% of samples with valid points (15/18) were within 80-120% RE*

Analytical measurement range (AMR): The analytical measurement range (AMR) is the range of values that the P-tau217 method can directly measure on a specimen without additional dilution beyond the standard dilution, concentration, or other pretreatment that is not part of the standard assay process. Precision criteria established in the Validation Plan are met throughout the AMR.

*The AMR is 0.200 U/ml to 6.04 U/ml*

Sensitivity (analytical): Defined here as the Lower Limit of Quantitation, it was characterized as the lowest level of P-tau217 (after correction for standard dilution) that can be measured with acceptable precision as defined in the method specifications.

*The sensitivity is 0.200 U/ml*

Specificity (analytical): Specificity was tested using an irrelevant primary antibody- bead conjugate (Neuro 4-Plex E, Quanterix, 103653) and secondary detection antibody (N4PE, Quanterix, 103655). The testing will be completed on separate runs / same sample(s). The concentration of the samples assessed was ≥ 0.4 Us/mL.

*The method demonstrated specificity in that no signal was generated with the irrelevant antibody pairs.*

Cross-reactivity: Cross reactivity was tested using Quanterix non-phosphorylated Tau (Cat# 101552). This was spiked into two (2) separate K2EDTA plasma samples.

*No cross reactivity was seen with spiked Tau protein in the P-tau217 assay.*

Parallelism: Parallelism was measured using a sample that had a high endogenous concentration of P-tau217. One sample was run on instrument at the standard 1:2 dilution and further dilutions of 1:4 and 1:8. *A prior* acceptance criteria for parallelism was a mean of 80-120% RE of the standard 1:2 dilution.

*%RE failed for dilutions 1:4 and 1:8. No dilutions further than the standard 1:2 dilution are allowable for this assay*

Dilutional Linearity: Dilutional linearity was measured using a sample spiked with P-tau217 peptide (CPC Scientific). One sample was run on instrument at the standard 1:2 dilution and further onboard dilutions of 1:4 and 1:8. A prior acceptance criteria for dilutional linearity was a mean of 80-120% RE of the standard 1:2 dilution.

*%RE passed for dilutions 1:4 and 1:8. Together with the Parallelism experiments, no dilutions further than the standard 1:2 dilution is allowable for this assay.*

Interference:

Hemolysis: Acceptance criteria are defined as hemolytic sample results 80–120% RE of baseline established with the 0 hemolytic sample.

*Based on studies performed with this assay method, Hemolysis is not an interfering substance for this method.*

Lipemia: Acceptance criteria are defined as lipemic sample results 80–120% RE of baseline established with the 0 lipemia sample.

*Based on studies performed with this assay method, Lipemia is not an interfering substance for this method.*

Drug tolerance (donanemab): Acceptance criteria are defined as donanemab spiked sample results 80–120% RE of baseline established with sample diluent controls.

*At expected plasma concentrations based on pharmacokinetic analysis,* donanemab *is tolerated in this method. Note this is only a relevant issue for measuring P-tau217 in patients who have been dosed with* donanemab.

Reportable range: The Reportable Range was determined based upon the acceptability of the precision experiments in conjunction with the acceptability of the parallelism experiment

*Reportable range is 0.200 – 6.04 U/ml*

Sample stability:

Freeze/thaw: Samples demonstrated acceptable stability up to at least five freeze/thaw cycles, defined as mean of 80-120% RE of baseline.

Short term stability: Room temperature up to at least 6 hours is acceptable. 4°C up to at least 168 hours is acceptable. For both, acceptable is defined as mean of 80-120% RE of baseline.

Long-term stability: is being assessed to a planned four years.

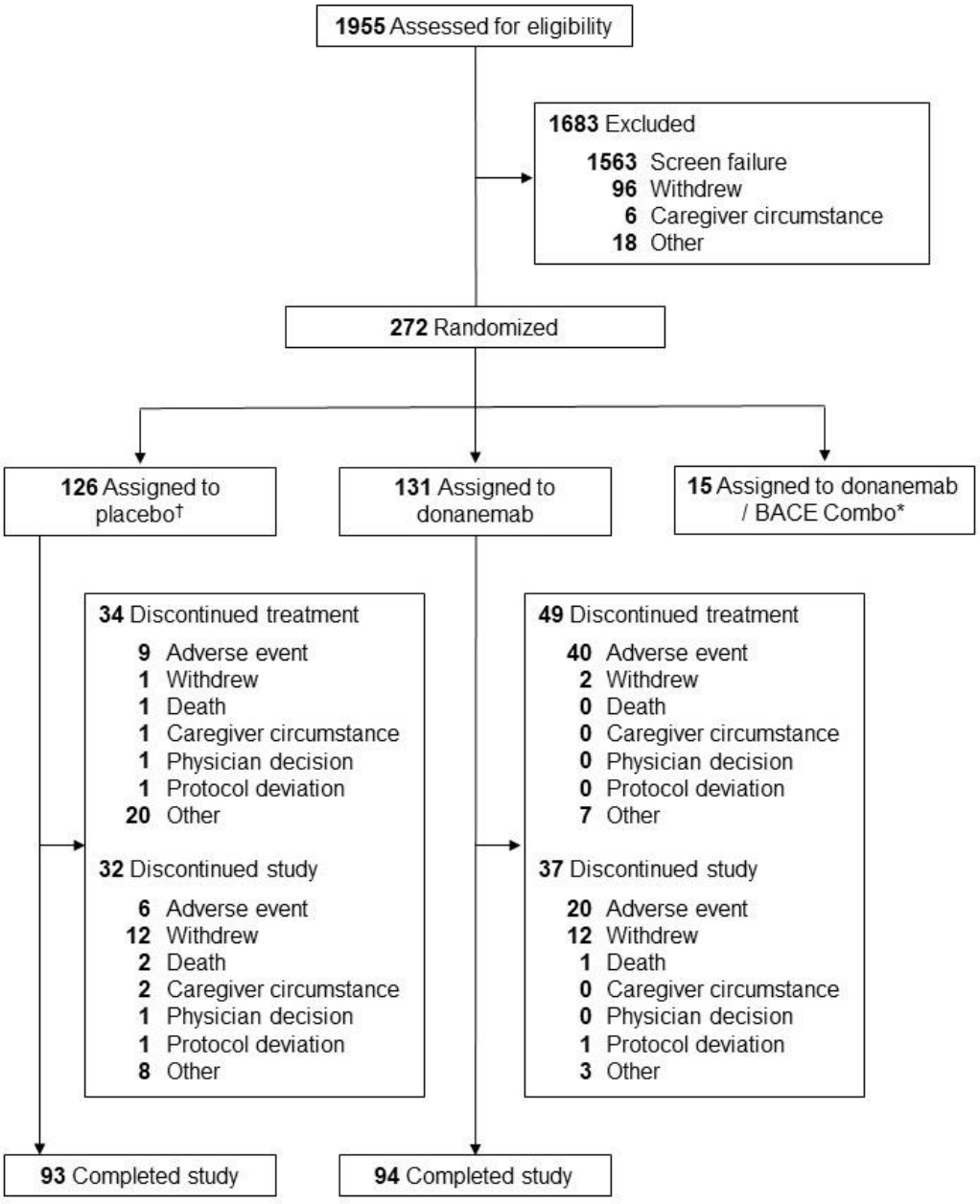
**Volumetric Magnetic Resonance Imaging (vMRI)**

The brain volumes were assessed by means of 3D T1 weighted, structural MRI scans at baseline and week 76. Global (whole brain volume and ventricular volume) and regional (hippocampal volume) vMRI measures were estimated.

**Integrated Alzheimer's Disease Rating Scale (iADRS)**

Details on the use of iADRS in TRAILBLAZER-ALZ have been previously reported [4]. Briefly, the iADRS score is a combination of results from ADAS-$Cog_{13}$ and ADCS-iADL measures. Scores range from 0 to 144. Lower scores indicate lower cognition and function [4,7]. Cognitive measurements were taken at baseline and weeks 12, 24, 36, 52, 64 and 76.

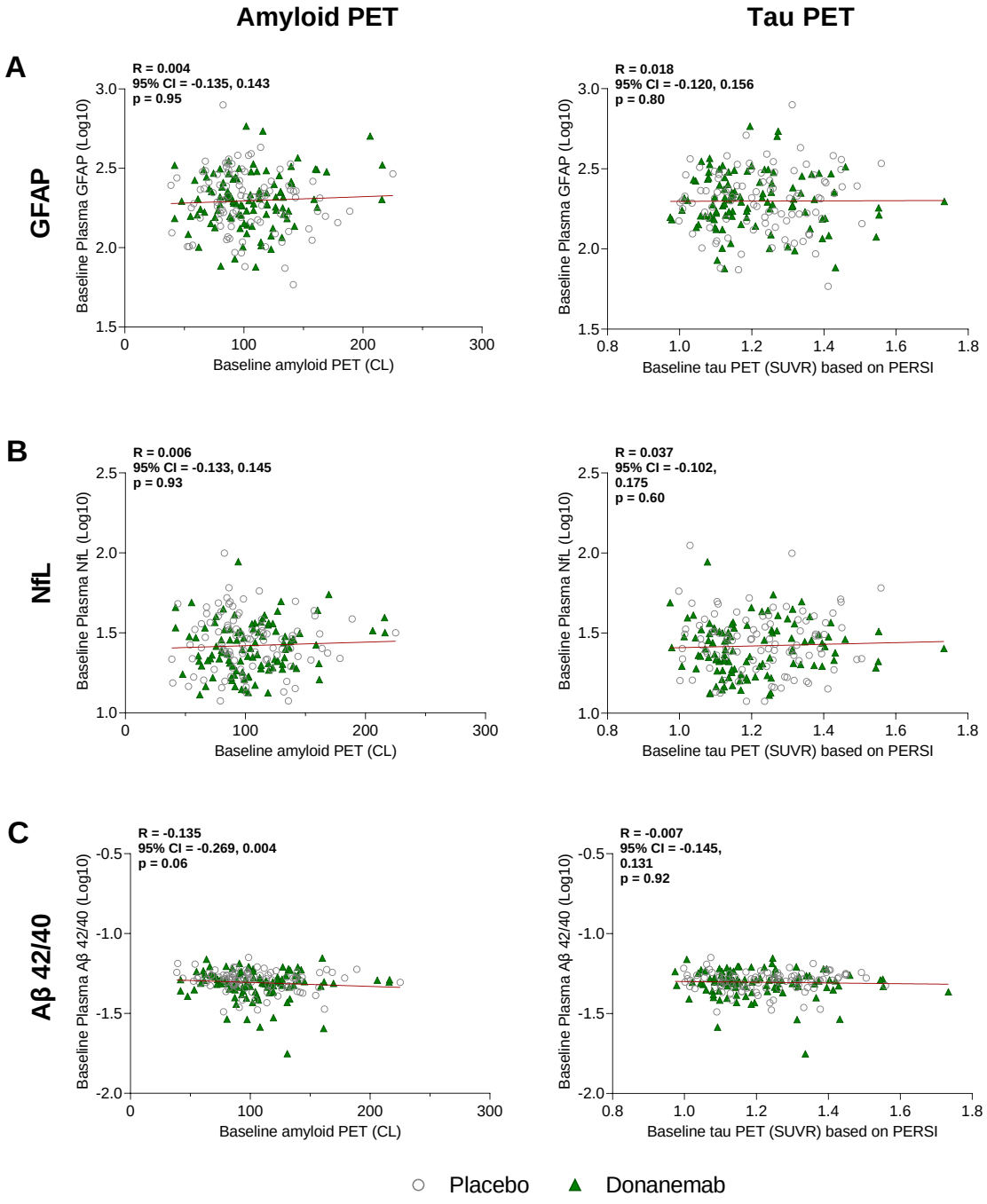
**eFigure 1. Participant flow diagram**




†One participant was randomized to placebo but discontinued before receiving an infusion.

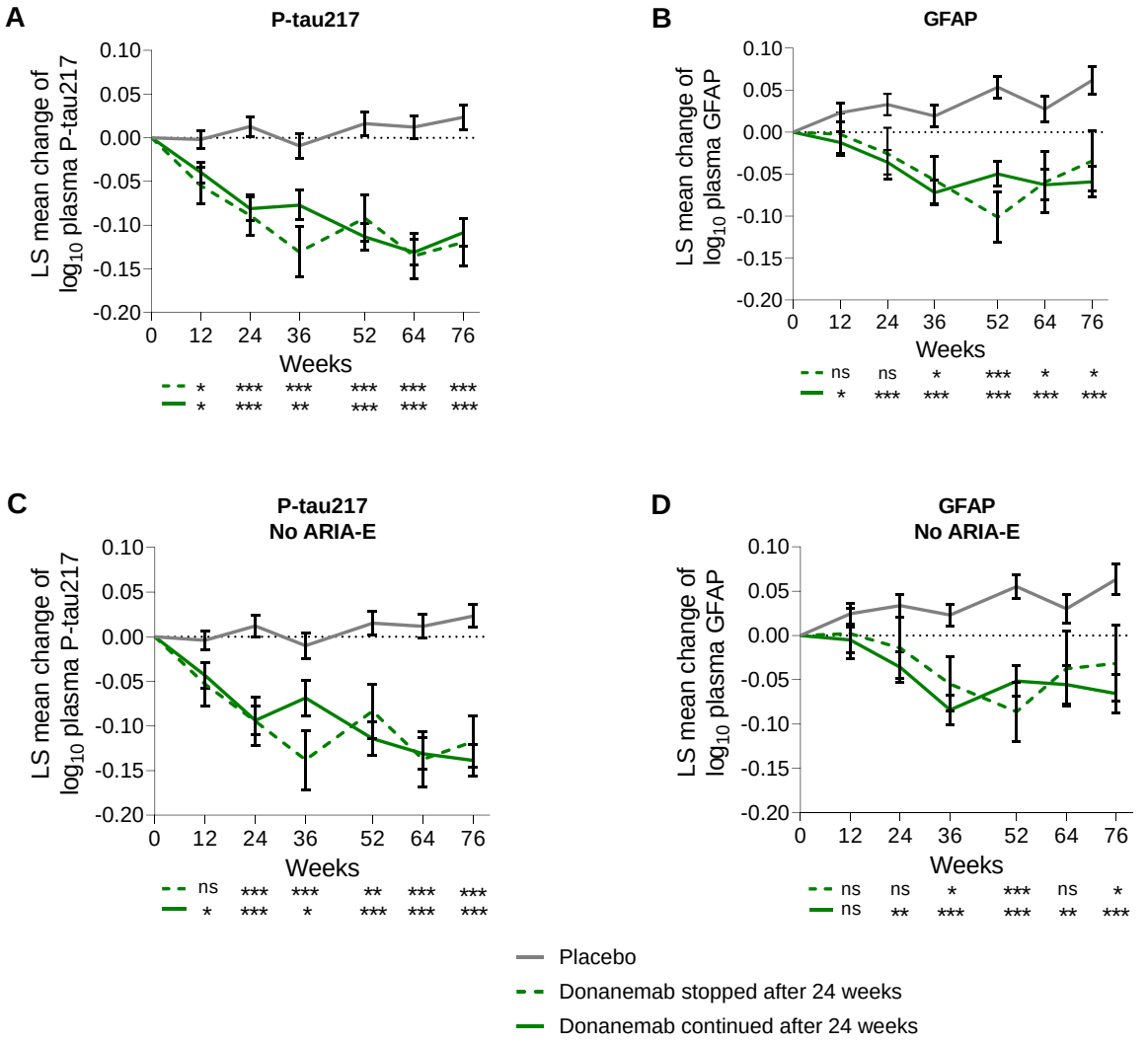
*Combination therapy was discontinued from the study in a protocol change.

**eFigure 2. Correlations between baseline plasma GFAP, NfL, Aβ 42/40, and baseline amyloid and tau PET endpoints**




Baseline amyloid PET and tau PET levels compared with baseline plasma levels of GFAP (A), NfL (B), and Aβ 42/40 (C). Plasma levels were log10 transformed. Placebo is represented by gray circles and donanemab by green triangles. Linear regression of all data points, regardless of treatment, shown in red. Spearman's rank was used for correlation coefficient. Amyloid PET: placebo N=97; donanemab N=102. Tau PET: placebo N=98; donanemab N=103. CI = confidence interval, CL = Centiloids, GFAP = glial fibrillary acidic protein p = p-value, PERSI = parametric estimation of reference signal intensity, PET = positron emission tomography, N = number of participants, NfL = neurofilament light chain, R = Spearman's rank correlation coefficient, SUVR = standardized uptake value ratio.

## eFigure 3. Change in plasma levels after discontinuing donanemab treatment




Least square mean change from baseline in plasma P-tau217 (A), GFAP (B), plasma P-tau217 excluding ARIA-E cases (C) and plasma GFAP excluding ARIA-E cases (D). Participants received placebo (gray line), stopped donanemab treatment at 24 weeks and switched to placebo due to amyloid levels <11 CL (dashed green line), or continued donanemab after 24 weeks (solid green line). Significance versus placebo is denoted below graph. No significant differences were observed between donanemab groups. Plasma values were log10 transformed. Error bars indicate standard error. Black dashed line indicates baseline.

P-tau 217 (A) placebo: week 12, N=107; week 24, N=108; week 36, N= 103; week 52, N=89; week 64, N=85; week 76, N=86.

P-tau 217 (A) donanemab stopped at 24 weeks: week 12, N=27; week 24, N=26; week 36, N= 25; week 52, N=22; week 64, N=20; week 76, N=22.

P-tau 217 (A) donanemab continued through 76 weeks: week 12, N=84; week 24, N=81; week 36, N= 74; week 52, N=67; week 64, N=63; week 76, N=63.

GFAP (B) placebo: week 12, N=82; week 24, N=89; week 36, N= 85; week 52, N=71; week 64, N=69; week 76, N=67.

GFAP (B) donanemab stopped at 24 weeks: week 12, N=18; week 24, N=15; week 36, N= 17; week 52, N=13; week 64, N=12; week 76, N=13.

GFAP (B) donanemab continued through 76 weeks: week 12, N=68; week 24, N=66; week 36, N= 61; week 52, N=56; week 64, N=50; week 76, N=55.

P-tau 217 no ARIA-E (C) placebo: week 12, N=106; week 24, N=107; week 36, N= 102; week 52, N=89; week 64, N=85; week 76, N=86.

P-tau 217 no ARIA-E (C) donanemab stopped at 24 weeks: week 12, N=22; week 24, N=21; week 36, N= 20; week 52, N=19; week 64, N=15; week 76, N=17.

P-tau 217 no ARIA-E (C) donanemab continued through 76 weeks: week 12, N=59; week 24, N=57; week 36, N= 54; week 52, N=50; week 64, N=46; week 76, N=45.

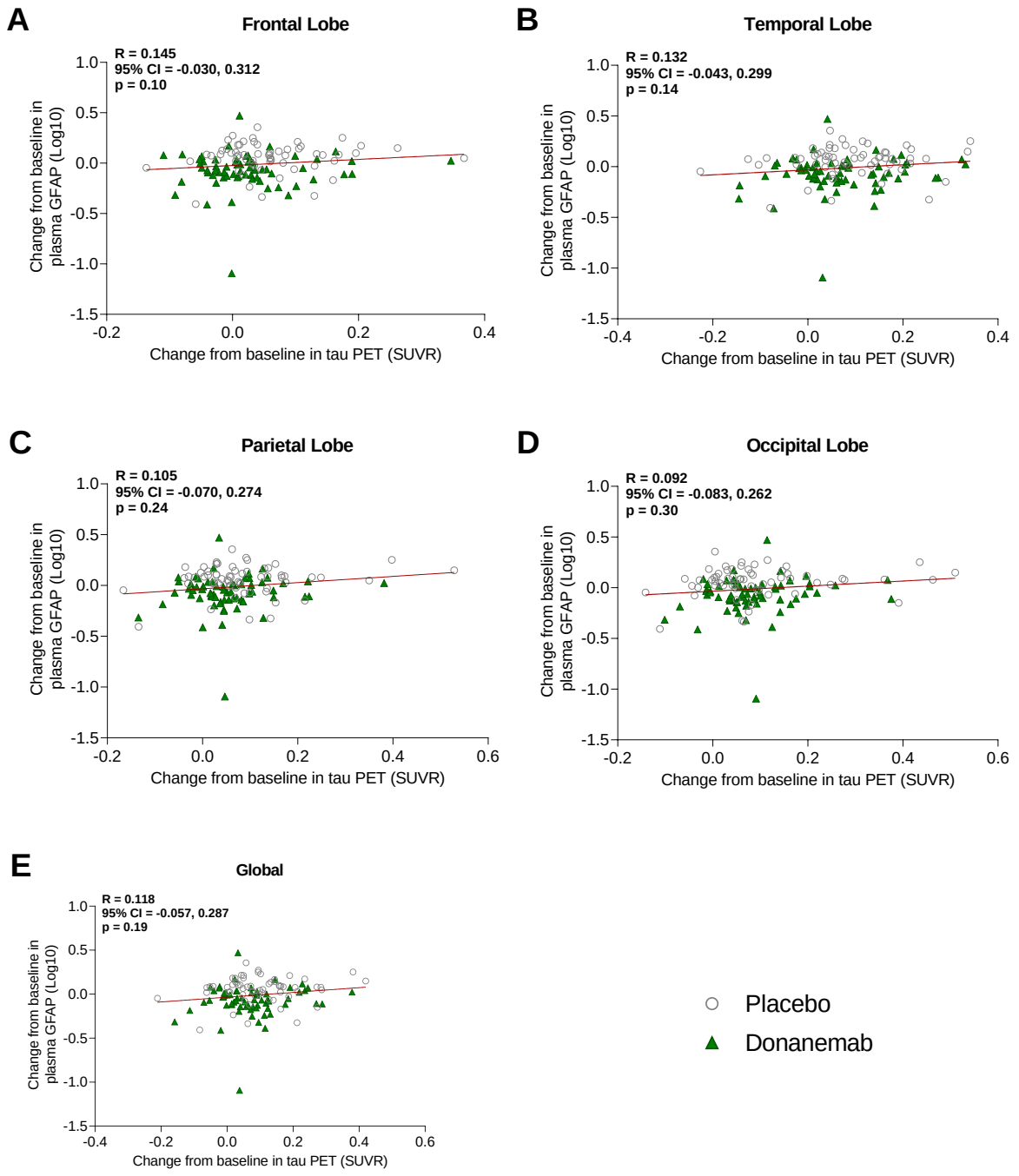
GFAP no ARIA-E (B) placebo: week 12, N=82; week 24, N=88; week 36, N= 84; week 52, N=71; week 64, N=69; week 76, N=67.

GFAP no ARIA-E (B) donanemab stopped at 24 weeks: week 12, N=14; week 24, N=12; week 36, N= 14; week 52, N=11; week 64, N=10; week 76, N=9.

GFAP no ARIA-E (B) donanemab continued through 76 weeks: week 12, N=50; week 24, N=47; week 36, N= 45; week 52, N=44; week 64, N=38; week 76, N=41.

(ARIA)-E = amyloid related imaging abnormalities-E, GFAP = glial fibrillary acidic protein, LS = least square, N = number of participants, ns = not significant, * p-value <0.05 versus placebo. ** p-value < 0.01 versus placebo.

## eFigure 4. Correlations of change in plasma GFAP with change in tau PET imaging




Change in tau PET levels compared with change in plasma GFAP levels from baseline to 76 weeks in frontal (A), temporal (B), parietal (C), and occipital (D) lobes as well as globally (E). Plasma levels were log10 transformed. Placebo is represented by gray circles and donanemab by green triangles. Linear regression of all data points, regardless of treatment, is shown in red. Spearman's rank was used for correlation coefficient. Placebo N=62; donanemab N=65. CI = confidence interval, N = number of participants, p = p-value, PET = positron emission tomography, R = Spearman's rank correlation coefficient, SUVR = standardized uptake value ratio.

## eTable 1. Baseline characteristics and biomarker levels

| | Placebo | Donanemab |
|---|---|---|
| **Baseline Characteristics** | | |
| Age (years) - N, mean (SD) | 120, 75.29 (5.438) | 125, 74.90 (5.504) |
| Sex N (%) | | |
| Female | 61 (50.8) | 64 (51.2) |
| Male | 59 (49.2) | 61 (48.8) |
| Race N (%) | | |
| American Indian or Alaska Native | 0 (0.00) | 2 (1.60) |
| Asian | 1 (0.83) | 1 (0.80) |
| Black or African American | 3 (2.50) | 5 (4.00) |
| Multiple | 0 (0.00) | 1 (0.80) |
| White | 116 (96.67) | 116 (92.80) |
| Ethnicity N (%) | | |
| Hispanic or Latino | 3 (2.5) | 4 (3.2) |
| Not Hispanic or Latino | 117 (97.5) | 121 (96.8) |
| Amyloid (CL)- N, mean (SD) | 112, 103.09 (33.841) | 121, 107.18 (33.938) |
| Global Tau (SUVR)- N, mean (SD) | 113, 1.22 (0.132) | 122, 1.21 (0.142) |
| MMSE- N, mean (SD) | 115, 23.77 (2.878) | 121, 23.56 (3.033) |
| iADRS- N, mean (SD) | 120, 106.06 (13.050) | 125 106.30 (12.730) |
| APOE ε4 N (%) | | |
| Carriers | 88 (73.9) | 92 (73.6) |
| non-carriers | 31 (26.1) | 33 (26.4) |
| P-tau217 (U/mL)- N, mean (SD) | 119, 0.42 (0.172) | 123, 0.41 (0.232) |
| GFAP (pg/mL)- N, mean (SD) | 103, 222.07 (103.406) | 104, 214.43 (88.891) |
| NfL (pg/mL)- N, mean (SD) | 119, 26.82 (24.847) | 121, 21.91 (8.491) |
| Aβ 42 (pg/mL)- N, mean (SD) | 103, 5.93 (1.382) | 104, 5.54 (1.395) |
| Aβ 40 (pg/mL)- N, mean (SD) | 103, 117.75 (29.559) | 104, 114.23 (28.395) |
| Aβ 42/40- N, mean (SD) | 103, 0.051 (0.0069) | 104, 0.049 (0.0089) |
| **Week 24** | | |
| P-tau217 (U/mL)- N, mean (SD) | 110, 0.45 (0.257) | 111, 0.34 (0.120) |
| GFAP (pg/mL)- N, mean (SD) | 102, 234.40 (103.238) | 98, 203.12 (90.661) |
| NfL (pg/mL)- n, mean (SD) | 111, 28.12 (25.906) | 110, 25.56 (11.390) |
| Aβ 42 (pg/mL)- N, mean (SD) | 102, 6.33 (1.657) | 98, 6.04 (1.500) |
| Aβ 40- N, mean (SD) | 102, 127.35 (36.264) | 98, 120.53 (31.935) |
| Aβ 42/40- N, mean (SD) | 102, 0.051 (0.0083) | 98, 0.051 (0.0096) |
| **Week 52** | | |
| P-tau217 (U/mL)- N, mean (SD) | 90, 0.45 (0.225) | 90, 0.32 (0.178) |
| GFAP (pg/mL)- N, mean (SD) | 78, 228.14 (76.810) | 82, 188.10 (80.199) |
| NfL (pg/mL)- n, mean (SD) | 93, 29.47 (30.087) | 90, 23.43 (11.193) |
| Aβ 42 (pg/mL)- N, mean (SD) | 78, 6.19 (1.735) | 82, 6.17 (1.552) |
| Aβ 40 (pg/mL)- N, mean (SD) | 78, 121.42 (40.220) | 82, 120.68 (27.122) |
| Aβ 42/40- N, mean (SD) | 78, 0.052 (0.0075) | 82, 0.052 (0.0094) |
| **Week 76** | | |
| P-tau217(U/mL)- N, mean (SD) | 87, 0.46 (0.245) | 86, 0.32 (0.167) |
| GFAP (pg/mL)- N, mean (SD) | 78, 242.25 (87.293) | 83, 189.99 (83.675) |
| NfL (pg/mL)- N, mean (SD) | 90, 26.05 (11.431) | 92, 23.86 (10.529) |
| Aβ 42 (pg/mL)- N, mean (SD) | 78, 6.65 (2.116) | 83, 6.31 (1.718) |
| Aβ 40 (pg/mL)- N, mean (SD) | 78, 132.30 (44.026) | 83, 123.68 (29.815) |
| Aβ 42/40- N, mean (SD) | 78, 0.051 (0.0090) | 83, 0.051 (0.0090) |

CL = Centiloids, GFAP = glial fibrillary acidic protein, iADRS = Integrated Alzheimer's Disease Rating Scale, MMSE = Mini–Mental State Examination, N = number of participants, NfL = neurofilament light chain, SD = standard deviation, SUVR = standardized uptake value ratio

**eTable 2. Plasma biomarker change from baseline**

| | | Placebo | Donanemab |
|---|---|---|---|
| **P-tau217 (U/mL) log10 transformed** | | | |
| Week 12 | LS Mean Change (SE), N | 0.00 (0.010), 113 | -0.04 (0.010), 119 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.04 (-0.07, -0.02) |
| | P-value vs. placebo | | 0.002 |
| Week 24 | LS Mean Change (SE), N | 0.01 (0.011), 110 | -0.09 (0.011), 110 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.10 (-0.13, -0.07) |
| | P-value vs. placebo | | <0.001 |
| Week 36 | LS Mean Change (SE), N | -0.01 (0.014), 103 | -0.09 (0.014), 100 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.08 (-0.12, -0.04) |
| | P-value vs. placebo | | <0.001 |
| Week 52 | LS Mean Change (SE), N | 0.02 (0.014), 89 | -0.11 (0.014), 90 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.12 (-0.16, -0.09) |
| | P-value vs. placebo | | <0.001 |
| Week 64 | LS Mean Change (SE), N | 0.01 (0.011), 85 | -0.13 (0.011), 84 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.14 (-0.17, -0.11) |
| | P-value vs. placebo | | <0.001 |
| Week 76 | LS Mean Change (SE), N | 0.03 (0.014), 86 | -0.11 (0.014), 86 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.14 (-0.18, -0.10) |
| | P-value vs. placebo | | <0.001 |
| **GFAP (pg/mL) log10 transformed** | | | |
| Week 12 | LS Mean Change (SE), N | 0.03 (0.011), 87 | -0.01 (0.011), 93 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.04 (-0.07, -0.01) |
| | P-value vs. placebo | | 0.01 |
| Week 24 | LS Mean Change (SE), N | 0.03 (0.012), 91 | -0.04 (0.013), 84 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.07 (-0.10, -0.03) |
| | P-value vs. placebo | | <0.001 |
| Week 36 | LS Mean Change (SE), N | 0.02 (0.012), 85 | -0.07 (0.013), 79 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.09 (-0.12, -0.06) |
| | P-value vs. placebo | | <0.001 |
| Week 52 | LS Mean Change (SE), N | 0.05 (0.013), 71 | -0.06 (0.013), 70 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.11 (-0.15, -0.08) |
| | P-value (vs. placebo) | | <0.001 |
| Week 64 | LS Mean Change (SE), N | 0.03 (0.015), 69 | -0.06 (0.016), 63 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.09 (-0.14, -0.05) |
| | P-value (vs. placebo) | | <0.001 |
| Week 76 | LS Mean Change (SE), N | 0.06 (0.016), 67 | -0.06 (0.016), 69 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.12 (-0.16, -0.07) |

| | | Placebo | Donanemab |
|---|---|---|---|
| | P-value (vs. placebo) | | <0.001 |
| **NfL (pg/mL) log10 transformed** | | | |
| Week 12 | LS Mean Change (SE), N | 0.03 (0.012), 87 | 0.03 (0.012), 93 |
| | LS Mean Diff (95% CI) vs. placebo | | 0.00 (-0.03, 0.04) |
| | P-value (vs. placebo) | | 0.89 |
| Week 24 | LS Mean Change (SE), N | 0.02 (0.011), 91 | 0.04 (0.012), 84 |
| | LS Mean Diff (95% CI) vs. placebo | | 0.02 (-0.01, 0.05) |
| | P-value (vs. placebo) | | 0.23 |
| Week 36 | LS Mean Change (SE), N | 0.02 (0.012), 85 | 0.02 (0.013), 79 |
| | LS Mean Diff (95% CI) vs. placebo | | 0.00 (-0.03, 0.04) |
| | P-value (vs. placebo) | | 0.97 |
| Week 52 | LS Mean Change (SE), N | 0.04 (0.015), 71 | 0.04 (0.015), 70 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.00 (-0.05, 0.04) |
| | P-value (vs. placebo) | | 0.86 |
| Week 64 | LS Mean Change (SE), N | 0.05 (0.015), 69 | 0.03 (0.016), 63 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.02 (-0.06, 0.03) |
| | P-value (vs. placebo) | | 0.44 |
| Week 76 | LS Mean Change (SE), N | 0.07 (0.015), 67 | 0.06 (0.015), 69 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.01 (-0.06, 0.03) |
| | P-value (vs. placebo) | | 0.49 |
| **Aβ 42/40 log10 transformed** | | | |
| Week 12 | LS Mean Change (SE), N | 0.00 (0.005), 87 | 0.00 (0.005), 93 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.00 (-0.02, 0.01) |
| | P-value (vs. placebo) | | 0.85 |
| Week 24 | LS Mean Change (SE), N | 0.00 (0.005), 91 | 0.01 (0.006), 84 |
| | LS Mean Diff (95% CI) vs. placebo | | 0.01 (-0.01, 0.02) |
| | P-value (vs. placebo) | | 0.36 |
| Week 36 | LS Mean Change (SE), N | 0.01 (0.006), 85 | 0.02 (0.006), 79 |
| | LS Mean Diff (95% CI) vs. placebo | | 0.02 (0.00, 0.03) |
| | P-value (vs. placebo) | | 0.04 |
| Week 52 | LS Mean Change (SE), N | 0.01 (0.008), 71 | 0.02 (0.008), 70 |
| | LS Mean Diff (95% CI) vs. placebo | | 0.00 (-0.02, 0.03) |
| | P-value (vs. placebo) | | 0.68 |
| Week 64 | LS Mean Change (SE), N | 0.01 (0.007), 69 | 0.01 (0.008), 63 |
| | LS Mean Diff (95% CI) vs. placebo | | 0.00 (-0.02, 0.02) |
| | P-value (vs. placebo) | | 0.88 |
| Week 76 | LS Mean Change (SE), N | 0.01 (0.007), 67 | 0.02 (0.007), 69 |
| | LS Mean Diff (95% CI) vs. placebo | | 0.01 (-0.01, 0.03) |
| | P-value (vs. placebo) | | 0.26 |

CI = confidence interval, Diff = difference, GFAP = glial fibrillary acidic protein, LS = least square, N = number of participants, NfL = neurofilament light chain, SE = standard error

**eTable 3. Plasma biomarker change from baseline after discontinuing donanemab treatment**

| | | Placebo | Donanemab Stopped after 24 weeks | Donanemab Continued after 24 weeks |
|---|---|---|---|---|
| **P-tau217 (U/mL) log10 transformed** | | | | |
| Week 12 | LS Mean Change (SE), N | -0.0020 (0.0104), 107 | -0.0550 (0.0210), 27 | -0.0397 (0.0118), 84 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.0530 (-0.0992, -0.0067) | -0.0377 (-0.0688, -0.0065) |
| | P-value vs. placebo | | 0.02 | 0.02 |
| | LS Mean Diff (95% CI) vs. stopped | | | -0.0153 (-0.0630, 0.0324) |
| | P-value vs. stopped | | | 0.53 |
| Week 24 | LS Mean Change (SE), N | 0.0128 (0.0114), 108 | -0.0888 (0.0233), 26 | -0.0811 (0.0131), 81 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.1016 (-0.1526, -0.0505) | -0.0939 (-0.1282, -0.0596) |
| | P-value vs. placebo | | <0.001 | <0.001 |
| | LS Mean Diff (95% CI) vs. stopped | | | -0.0076 (-0.0605, 0.0452) |
| | P-value vs. stopped | | | 0.78 |
| Week 36 | LS Mean Change (SE), N | -0.0093 (0.0142), 103 | -0.1308 (0.0289), 25 | -0.0772 (0.0168), 74 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.1215 (-0.1850, -0.0579) | -0.0679 (-0.1113, -0.0246) |
| | P-value vs. placebo | | <0.001 | 0.002 |
| | LS Mean Diff (95% CI) vs. stopped | | | -0.0535 (-0.1196, 0.0125) |
| | P-value vs. stopped | | | 0.11 |
| Week 52 | LS Mean Change (SE), N | 0.0161 (0.0133), 89 | -0.0920 (0.0269), 22 | -0.1134 (0.0153), 67 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.1081 (-0.1671, -0.0490) | -0.1295 (-0.1695, -0.0895) |
| | P-value vs. placebo | | <0.001 | <0.001 |
| | LS Mean Diff (95% CI) vs. stopped | | | 0.0214 (-0.0397, 0.0825) |
| | P-value vs. stopped | | | 0.49 |
| Week 64 | LS Mean Change (SE), N | 0.0122 (0.0127), 85 | -0.1353 (0.0262), 20 | -0.1311 (0.0147), 63 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.1475 (-0.2048, -0.0902) | -0.1433 (-0.1817, -0.1049) |
| | P-value vs. placebo | | <0.001 | <0.001 |
| | LS Mean Diff (95% CI) vs. stopped | | | -0.0042 (-0.0635, 0.0551) |
| | P-value vs. stopped | | | 0.89 |
| Week 76 | LS Mean Change (SE), N | 0.0234 (0.0138), 86 | -0.1196 (0.0275), 22 | -0.1087 (0.0161), 63 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.1430 (-0.2035, -0.0825) | -0.1321 (-0.1739, -0.0902) |
| | P-value vs. placebo | | <0.001 | <0.001 |
| | LS Mean Diff (95% CI) vs. stopped | | | -0.0109 (-0.0738, 0.0519) |
| | P-value vs. stopped | | | 0.73 |
| **GFAP (pg/mL) log10 transformed** | | | | |
| Week 12 | LS Mean Change (SE), N | 0.0231 (0.0114), 82 | -0.0025 (0.0250), 18 | -0.0124 (0.0126), 68 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.0256 (-0.0798, 0.0286) | -0.0355 (-0.0691, -0.0020) |
| | P-value vs. placebo | | 0.35 | 0.04 |
| | LS Mean Diff (95% CI) vs. stopped | | | 0.0099 (-0.0456, 0.0654) |

| | | Placebo | Donanemab Stopped after 24 weeks | Donanemab Continued after 24 weeks |
|---|---|---|---|---|
| | P-value vs. stopped | | | 0.72 |
| Week 24 | LS Mean Change (SE), N | 0.0328 (0.0127), 89 | -0.0257 (0.0309), 15 | -0.0360 (0.0147), 66 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.0585 (-0.1243, 0.0073) | -0.0688 (-0.1071, -0.0305) |
| | P-value vs. placebo | | 0.08 | <0.001 |
| | LS Mean Diff (95% CI) vs. stopped | | | 0.0103 (-0.0572, 0.0778) |
| | P-value vs. stopped | | | 0.76 |
| Week 36 | LS Mean Change (SE), N | 0.0190 (0.0126), 85 | -0.0574 (0.0282), 17 | -0.0721 (0.0146), 61 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.0763 (-0.1371, -0.0155) | -0.0910 (-0.1290, -0.0531) |
| | P-value vs. placebo | | 0.01 | <0.001 |
| | LS Mean Diff (95% CI) vs. stopped | | | 0.0147 (-0.0479, 0.0773 |
| | P-value vs. stopped | | | 0.64 |
| Week 52 | LS Mean Change (SE), N | 0.0534 (0.0130), 71 | -0.1013 (0.0298), 13 | -0.0499 (0.0147), 56 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.1548 (-0.2188, -0.0907) | -0.1033 (-0.1420, -0.0647) |
| | P-value vs. placebo | | <0.001 | <0.001 |
| | LS Mean Diff (95% CI) vs. stopped | | | -0.0514 (-0.1170, 0.0141) |
| | P-value vs. stopped | | | 0.12 |
| Week 64 | LS Mean Change (SE), N | 0.0274 (0.0155), 69 | -0.0596 (0.0366), 12 | -0.0628 (0.0182), 50 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.0870 (-0.1653, -0.0088) | -0.0902 (-0.1372, -0.0432) |
| | P-value vs. placebo | | 0.03 | <0.001 |
| | LS Mean Diff (95% CI) vs. stopped | | | 0.0032 (-0.0774, 0.0837) |
| | P-value vs. stopped | | | 0.94 |
| Week 76 | LS Mean Change (SE), N | 0.0618 (0.0163), 67 | -0.0343 (0.0358), 13 | -0.0593 (0.0181), 55 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.0961 (-0.1737, -0.0185) | -0.1211 (-0.1692, -0.0730) |
| | P-value vs. placebo | | 0.02 | <0.001 |
| | LS Mean Diff (95% CI) vs. stopped | | | 0.0250 (-0.0542, 0.1042) |
| | P-value vs. stopped | | | 0.53 |
| **P-tau217 (U/mL) log10 transformed no ARIA-E** | | | | |
| Week 12 | LS Mean Change (SE), N | -0.0040 (0.0109), 106 | -0.0533 (0.0241), 22 | -0.0434 (0.0146), 59 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.0493 (-0.1014, 0.0028) | -0.0394 (-0.0754, -0.0035) |
| | P-value vs. placebo | | 0.06 | 0.03 |
| | LS Mean Diff (95% CI) vs. stopped | | | -0.0099 (-0.0656, 0.0458) |
| | P-value vs. stopped | | | 0.73 |
| Week 24 | LS Mean Change (SE), N | 0.0120 (0.0119), 107 | -0.0947 (0.0268), 21 | -0.0938 (0.0163), 57 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.1066 (-0.1645, -0.0488) | -0.1057 (-0.1455, -0.0659) |
| | P-value vs. placebo | | <0.001 | <0.001 |
| | LS Mean Diff (95% CI) vs. stopped | | | -0.0009 (-0.0629, 0.0611 |
| | P-value vs. stopped | | | 0.98 |
| | LS Mean Change (SE), N | -0.0102 (0.0147), 102 | -0.1380 (0.0331, 20 | -0.0687 (0.0203), 54 |

| | | Placebo | Donanemab Stopped after 24 weeks | Donanemab Continued after 24 weeks |
|---|---|---|---|---|
| Week 36 | LS Mean Diff (95% CI) vs. placebo | | -0.1278 (-0.1991, -0.0565) | -0.0585 (-0.1079, -0.0091) |
| | P-value vs. placebo | | <0.001 | 0.02 |
| | LS Mean Diff (95% CI) vs. stopped | | | -0.0693 (-0.1459, 0.0073) |
| | P-value vs. stopped | | | 0.08 |
| Week 52 | LS Mean Change (SE), N | 0.0150 (0.0138), 89 | -0.0835 (0.0302), 19 | -0.1143 (0.0185), 50 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.0986 (-0.1641, -0.0330) | -0.1294 (-0.1749, -0.0838) |
| | P-value vs. placebo | | 0.003 | <0.001 |
| | LS Mean Diff (95% CI) vs. stopped | | | 0.0308 (-0.0392, 0.1008) |
| | P-value vs. stopped | | | 0.39 |
| Week 64 | LS Mean Change (SE), N | 0.0116 (0.0132), 85 | -0.1373 (0.0306), 15 | -0.1311 (0.0178), 46 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.1488 (-0.2146, -0.0831) | -0.1426 (-0.1864, -0.0989) |
| | P-value vs. placebo | | <0.001 | <0.001 |
| | LS Mean Diff (95% CI) vs. stopped | | | -0.0062 (-0.0762, 0.0638) |
| | P-value vs. stopped | | | 0.86 |
| Week 76 | LS Mean Change (SE), N | 0.0230 (0.0127), 86 | -0.1176 (0.0289), 17 | -0.1386 (0.0174), 45 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.1406 (-0.2028, -0.0784) | -0.1616 (-0.2042, -0.1190) |
| | P-value vs. placebo | | <0.001 | <0.001 |
| | LS Mean Diff (95% CI) vs. stopped | | | 0.0210 (-0.0457, 0.0877) |
| | P-value vs. stopped | | | 0.54 |
| **GFAP (pg/mL) log10 transformed no ARIA-E** | | | | |
| Week 12 | LS Mean Change (SE), N | 0.0244 (0.0114), 82 | 0.0019 (0.0284), 14 | -0.0051 (0.0149), 50 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.0226 (-0.0830, 0.0379) | -0.0296 (-0.0666, 0.0075) |
| | P-value vs. placebo | | 0.46 | 0.12 |
| | LS Mean Diff (95% CI) vs. stopped | | | 0.0070 (-0.0564, 0.0704) |
| | P-value vs. stopped | | | 0.83 |
| Week 24 | LS Mean Change (SE), N | 0.0337 (0.0128), 88 | -0.0143 (0.0344), 12 | -0.0360 (0.0174), 47 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.0480 (-0.1204, 0.0244) | -0.0698 (-0.1124, -0.0272) |
| | P-value vs. placebo | | 0.19 | 0.002 |
| | LS Mean Diff (95% CI) vs. stopped | | | 0.0217 (-0.0544, 0.0979) |
| | P-value vs. stopped | | | 0.57 |
| Week 36 | LS Mean Change (SE), N | 0.0231 (0.0125), 84 | -0.0548 (0.0310), 14 | -0.0841 (0.0170), 45 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.0780 (-0.1440, -0.0120) | -0.1072 (-0.1489, -0.0656) |
| | P-value vs. placebo | | 0.02 | <0.001 |
| | LS Mean Diff (95% CI) vs. stopped | | | 0.0292 (-0.0406, 0.0990) |
| | P-value vs. stopped | | | 0.41 |
| Week 52 | LS Mean Change (SE), N | 0.0551 (0.0132), 71 | -0.0862 (0.0333), 11 | -0.0516 (0.0171), 44 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.1412 (-0.2119, -0.0706) | -0.1067 (-0.1493, -0.0640) |
| | P-value vs. placebo | | <0.001 | <0.001 |

| | | Placebo | Donanemab Stopped after 24 weeks | Donanemab Continued after 24 weeks |
|---|---|---|---|---|
| | LS Mean Diff (95% CI) vs. stopped | | | -0.0346 (-0.1085, 0.0394) |
| | P-value vs. stopped | | | 0.36 |
| Week 64 | LS Mean Change (SE), N | 0.0302 (0.0160), 69 | -0.0375 (0.0422), 10 | -0.0555 (0.0216), 38 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.0677 (-0.1566, 0.0212) | -0.0857 (-0.1387, -0.0327) |
| | P-value vs. placebo | | 0.14 | 0.002 |
| | LS Mean Diff (95% CI) vs. stopped | | | 0.0180 (-0.0754, 0.1115) |
| | P-value vs. stopped | | | 0.70 |
| Week 76 | LS Mean Change (SE), N | 0.0635 (0.0169), 67 | -0.0316 (0.0430), 9 | -0.0657 (0.0219), 41 |
| | LS Mean Diff (95% CI) vs. placebo | | -0.0951 (-0.1862, -0.0040) | -0.1292 (-0.1838, -0.0745) |
| | P-value vs. placebo | | 0.04 | <0.001 |
| | LS Mean Diff (95% CI) vs. stopped | | | 0.0341 (-0.0611, 0.1292) |
| | P-value vs. stopped | | | 0.48 |

CI = confidence interval, Diff = difference, GFAP = glial fibrillary acidic protein, LS = least square, N = number of participants, NfL = neurofilament light chain, SE = standard error

## eTable 4. Correlations of plasma biomarkers with vMRI

| | Baseline | 76-Week Change |
|---|---|---|
| **P-tau217:** (R (95% CI), p) | N=239 | N=152 |
| Whole brain volume | -0.0777 (-0.2034, 0.0505), 0.23 | 0.1359 (-0.0255, 0.2903), 0.10 |
| Ventricular volume | -0.0199 (-0.1473, 0.1080), 0.76 | -0.1006 (-0.2573, 0.0612), 0.22 |
| Hippocampal volume | -0.0544 (-0.1809, 0.0738), 0.41 | 0.0875 (-0.0744, 0.2448), 0.29 |
| **GFAP:** (R (95%CI), p) | N=204 | N=118 |
| Whole brain volume | 0.0951 (-0.0439, 0.2304), 0.18 | 0.0615 (-0.1230, 0.2419), 0.51 |
| Ventricular volume | 0.0457 (-0.0932, 0.1830), 0.52 | 0.0532 (-0.1312, 0.2340), 0.57 |
| Hippocampal volume | 0.1209 (-0.0178, 0.2551), 0.09 | 0.0183 (-0.1653, 0.2008), 0.85 |
| **NfL:** (R (95% CI), p) | N=237 | N=157 |
| Whole brain volume | 0.0121 (-0.1163, 0.1401), 0.85 | -0.1710 (-0.3205, -0.0132), 0.03 |
| Ventricular volume | 0.0828 (-0.0459, 0.2089), 0.21 | 0.1207 (-0.0382, 0.2737), 0.14 |
| Hippocampal volume | -0.0992 (-0.2246, 0.0294), 0.13 | -0.1454 (-0.2967, 0.0131), 0.07 |
| **Aβ 42/40:** (R (95%CI), p) | N=204 | N=118 |
| Whole brain volume | -0.0339 (-0.1715, 0.1050), 0.63 | -0.1599 (-0.3332, 0.0239), 0.09 |
| Ventricular volume | 0.0605 (-0.0785, 0.1973), 0.39 | 0.0379 (-0.1463, 0.2195), 0.69 |
| Hippocampal volume | 0.0312 (-0.1077, 0.1689), 0.66 | -0.0032 (-0.1862, 0.1800), 0.97 |

CI = confidence interval, GFAP = glial fibrillary acidic protein, N = number of participants, NfL = neurofilament light chain, p = p-value, R = Spearman's rank correlation coefficient.

## eTable 5. Correlations of plasma biomarkers with iADRS

| | Baseline | 76-Week Change |
|---|---|---|
| **P-tau217** (N, R, (95% CI), p) | 241, -0.0838, (-0.2087, 0.0439), 0.20 | 170, -0.0397, (-0.1904, 0.1129), 0.61 |
| **GFAP** (N, R, (95% CI), p) | 206, -0.0477, (-0.1842, 0.0906), 0.50 | 135, -0.0759, (-0.2436, 0.0962), 0.39 |
| **NfL** (N, R, (95% CI), p) | 239, -0.1096, (-0.2340, 0.0184), 0.09 | 177, -0.1823, (-0.3223, -0.0345), 0.02 |
| **Aβ 42/40** (N, R, (95% CI), p) | 206, 0.1032, (-0.0350, 0.2375), 0.14 | 135, -0.0761, (-0.2438, 0.0960), 0.38 |

CI = confidence interval, GFAP = glial fibrillary acidic protein, N = number of participants, NfL = neurofilament light chain, p = p-value, R = Spearman's rank correlation coefficient.

**eTable 6. Effect of plasma biomarker change at weeks 24 on iADRS MCID**

| | P-tau217 | GFAP |
|---|---|---|
| | Coefficient estimate (SE), (95% CI), p-value | |
| **Baseline iADRS** | -0.0011 (0.0095), (-0.0197, 0.0175), 0.91 | 0.0013 (0.0099), (-0.0180, 0.0207), 0.89 |
| **Baseline biomarker** | 0.6849 (0.4954), (-0.2861, 1.6559), 0.17 | 0.0009 (0.0014), (-0.0019, 0.0036), 0.54 |
| **Age** | 0.0309 (0.0210), (-0.0103, 0.0721), 0.14 | 0.0365 (0.0270), (-0.0163, 0.0893), 0.18 |
| **Biomarker change** | 0.7042 (0.3480), (0.0222, 1.3862), 0.04 | -0.0015 (0.0018), (-0.0051, 0.0021), 0.41 |

MCID: 5-point or more decrease for mild cognitive impairment and 9-point or more decrease for mild AD
P-values are computed using the following GEE model
MCID = baseline iADRS value, baseline parameter value, age, parameter value/dist = binomial link = logit lrci
Abbreviation: AD = Alzheimer's disease, CI = confidence interval, GEE = general estimating equation, GFAP = glial fibrillary acidic protein, iADRS = Integrated Alzheimer's Disease Rating Scale, MCID = minimal clinically important difference, SE = standard error

**eReferences**


1. Joshi AD, Pontecorvo MJ, Lu M, Skovronsky DM, Mintun MA, Devous MD, Sr. A Semiautomated Method for Quantification of F 18 Florbetapir PET Images. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine.* 2015;56(11):1736-1741.

2. Navitsky M, Joshi AD, Kennedy I, et al. Standardization of amyloid quantitation with florbetapir standardized uptake value ratios to the Centiloid scale. *Alzheimer's & dementia : the journal of the Alzheimer's Association.* 2018;14(12):1565-1571.

3. Devous MD, Sr., Joshi AD, Navitsky M, et al. Test-Retest Reproducibility for the Tau PET Imaging Agent Flortaucipir F 18. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine.* 2018;59(6):937-943.

4. Mintun MA, Lo AC, Duggan Evans C, et al. Donanemab in Early Alzheimer's Disease. *N Engl J Med.* 2021;384(18):1691-1704.

5. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage.* 2002;15(1):273-289.

6. Pontecorvo MJ, Devous MD, Sr., Navitsky M, et al. Relationships between flortaucipir PET tau binding and amyloid burden, clinical diagnosis, age and cognition. *Brain.* 2017;140(3):748-763.

7. Wessels AM, Siemers ER, Yu P, et al. A Combined Measure of Cognition and Function for Clinical Trials: The Integrated Alzheimer's Disease Rating Scale (iADRS). *The journal of prevention of Alzheimer's disease.* 2015;2(4):227-241.