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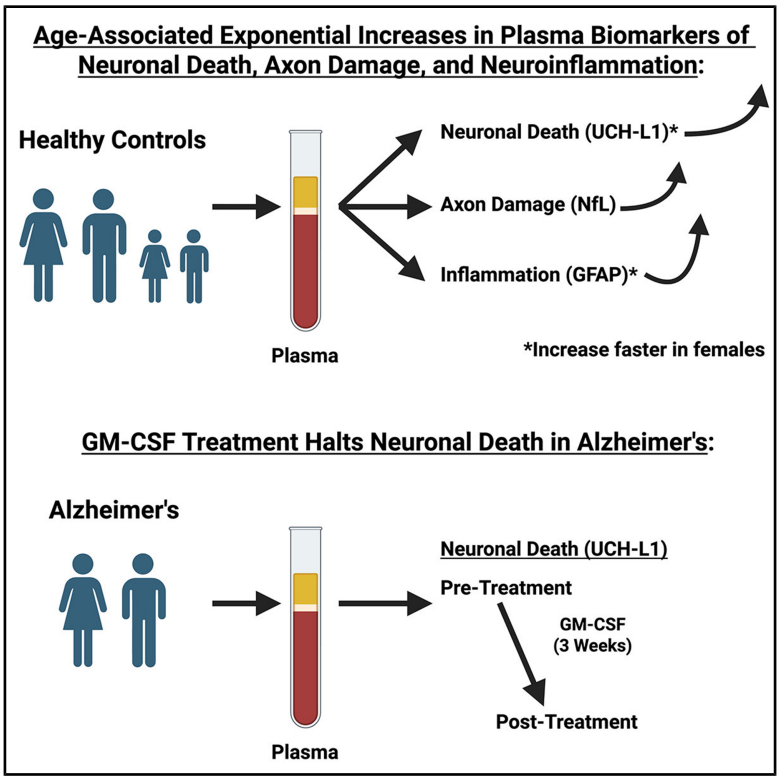
Blood measure of neuronal death is exponentially higher with age, especially in females, and halted in Alzheimer's disease by GM-CSF treatment

Graphical abstract



Highlights

- Plasma measures of neuronal death/damage (UCH-L1, NfL) rise exponentially from age 2
- A plasma measure of brain inflammation (GFAP) rises exponentially from age 40
- Women exhibit faster exponential rises in plasma UCH-L1 and GFAP than men
- Promising Alzheimer drug GM-CSF greatly reduces neuronal death in patients with AD, AD rats

Authors

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In brief

Sillau, Coughlan, Ahmed, and colleagues (University of Colorado Anschutz) found that cross-sectional neuronal death/damage (UCH-L1/NfL) plasma measures rise exponentially from age 2. Brain inflammation (plasma GFAP) rises exponentially from age 40, especially in females. A 3-week Alzheimer's trial with cytokine GM-CSF improves cognition (MMSE) and neuropathology (plasma Aβ and Tau) and reduces neuronal death (plasma UCH-L1).

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Blood measure of neuronal death is exponentially higher with age, especially in females, and halted in Alzheimer's disease by GM-CSF treatment

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SUMMARY

Aging increases the risk of neurodegeneration, cognitive decline, and Alzheimer's disease (AD). We report that plasma concentrations of ubiquitin C-terminal hydrolase-L1 (UCH-L1) and neurofilament light (NfL) become exponentially higher from ages 2 to 85 in cross-sectional samples, serving as neuronal death/damage biomarkers across the lifespan. UCH-L1 concentrations rise faster in females, who exhibit increased AD risk. Glial fibrillary acidic protein (GFAP) concentrations increase exponentially after age 40, especially in females. Age-adjusted UCH-L1, NfL, and GFAP plasma concentrations are greatly elevated in mildly cognitively impaired participants. Treatment of human AD trial participants with granulocyte-macrophage colony-stimulating factor (GM-CSF/sargramostim) apparently halts neuronal cell death: UCH-L1 biomarker concentrations are reduced to those of 5-year-old healthy controls. GM-CSF treatment also reduces neuronal apoptosis and astrogliosis in a rat model of AD. An exponential increase in neurodegeneration with age, accelerated by astrogliosis/inflammation, may underlie the contribution of aging to cognitive decline and AD and can be halted by GM-CSF/sargramostim treatment.

INTRODUCTION

Increasing age is the greatest risk factor for "natural" age-associated cognitive decline (AACD) and, especially in females, for developing Alzheimer's disease (AD), but the mechanisms underlying these connections are unknown.[1–10] Neuronal loss (apoptosis) and brain atrophy accompany AD and are associated with cognitive deficits.[11–15] Neuronal apoptosis also increases with age in mice and humans, although gray matter atrophy is less clearly associated with normal aging.[15–19] Increased inflammation is also correlated with aging and AD pathogenesis (termed "inflammaging"), but whether inflammation causes or is a response to neurodegeneration, or both, is also unknown.[1,8,20–23] For example, early studies of the inflammatory disease rheumatoid arthritis (RA) found that patients exhibited a reduced risk of developing AD, which was attributed to their use of non-steroidal anti-inflammatory drugs (NSAIDs).[24] However, clinical trials showed no NSAID benefit to participants with either AD or mild cognitive impairment (MCI), and studies of other inflammatory diseases, such as periodontitis, detected an increase in AD risk.[21,24,25] Evidently, the role of inflammation in aging and neurodegenerative disease is complex.

The identification of blood biomarkers of brain damage is essential for advancing the development of therapies for AD and AACD.[22,26–29] Here, we first studied whether ubiquitin C-terminal hydrolase-L1 (UCH-L1) and neurofilament light (NfL) are potential biomarkers of neurodegeneration during aging, building on numerous reports that their plasma concentrations are well correlated with neuronal damage in neurodegenerative disease, including AD, traumatic brain injury (TBI), and white matter disorders.[30–42] In Creutzfeldt-Jacob disease, plasma UCH-L1 and NfL concentrations are greatly increased, and higher levels of UCH-L1 predict faster decline.[43] In Parkinson's disease (PD), plasma UCH-L1 and NfL levels are increased and correlate with cognitive decline.[44,45]

Although UCH-L1 was initially discovered and named as a ubiquitin C-terminal hydrolase, it is likely that this is not its primary function, as cells and animals lacking UCH-L1 are not

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defective in the ubiquitin-proteosome system.[30,46] Indeed, UCH-L1 is primarily a brain protein, making up 1%–5% of total protein in neurons, with some expression in endocrine tissue. Indeed, only brain damage and disease result in increased blood UCH-L1 concentrations, while diabetes results in no change in blood UCH-L1, nor does hypertension, cerebrovascular disease, dyslipidemia, or depression.[30,36] Suggestions for the neuronal functions of UCH-L1 include the regulation of energy metabolism and mitochondrial fusion, antioxidant activity, synaptic activity, and tau phosphorylation.[31,46–48] Indeed, in the human AD brain and in AD mouse model brains, UCH-L1 protein levels are reduced due to neuronal loss/damage.[31,49]

NfL is a neuronal protein and an essential component of axons. Traumatic or disease-associated damage to axon tracks leads to the release of NfL into the cerebrospinal fluid (CSF) and plasma, where it can be detected as a biomarker of axonal damage in numerous neurodegenerative diseases, TBI, and aging.[30,39–41] Glial fibrillary acidic protein (GFAP) is upregulated in activated astrocytes, and its increased concentration in CSF or plasma is a marker/proxy of reactive gliosis/inflammation in aging, neurodegenerative disease, and TBI.[22,35,38,50]

Here, we assessed cross-sectional plasma concentrations of UCH-L1 and NfL across the lifespan and found that these measures of neuronal loss and damage become exponentially higher starting from childhood. Plasma concentrations of GFAP become exponentially higher starting at age 40. Finally, we studied the effect on plasma concentrations of UCH-L1 when treating patients with AD with granulocyte-macrophage colony-stimulating factor (GM-CSF)/CSF2/sargramostim, a long-approved drug for stimulating immune stem cells, which we previously found to improve a measure of cognition and plasma biomarkers of neurodegeneration in a double-blind, randomized, placebo-controlled, phase 2 clinical trial.[51] GM-CSF treatment reduces plasma UCH-L1 concentrations in participants with AD to the very low levels normally observed in early childhood. GM-CSF treatment also reduces the high levels of neuronal apoptosis and astrogliosis in the hippocampi of aged TgF344-AD rats, a model of AD.

RESULTS

Plasma concentrations of UCH-L1 are exponentially higher with advancing age from early childhood

Plasma concentrations of UCH-L1, a measure of neuronal cell loss, were assessed cross-sectionally in 317 healthy control participants between ages 2 and 85 from three observational studies (see STAR Methods; Tables S5 and S6). The assessments were determined using the very sensitive Quanterix SIMOA platform, which was also used previously to assess plasma biomarkers in the sargramostim/GM-CSF AD trial (see discussion in STAR Methods; Table S7).[51] As shown in Figure 1A, plasma UCH-L1 concentrations are exponentially higher across the entire age spectrum, from an estimated 6.22 pg/mL at age 2 to approximately 15.56 pg/mL at age 85 (estimated change per year = 1.110%, $p = 5.504 \times 10^{-8}$). Graphical inspection of the data and comparison to a spline fit (Figures S1–S3) indicate that a log-linear relationship of UCH-L1 concentrations with age is an excellent fit (Pearson correlation estimate [replicates log averaged = 0.30]), which is equivalent to an exponential relationship on the original scale (Figures 1A and 1B) (estimated change per year = 1.110%, 95% confidence interval [CI]: [0.716%, 1.505%], $p = 5.504 \times 10^{-8}$). Interestingly, most of the age-associated increase in plasma concentrations of UCH-L1 occurs in females (Figure 1C; estimated female change per year = 1.448% [95% CI: (0.942%, 1.957%); $p = 3.635 \times 10^{-8}$]; estimated male change per year = 0.582% [95% CI: (−0.036%, 1.203%), $p = 0.0650$]; and estimate of the gender ratio of ratios = 0.99146, [95% CI: (0.98362, 0.99936), $p = 0.0342$]). The log plots in Figure 1D and the spline analyses (Figure S1) support the conclusion that the plasma concentration of UCH-L1 increases exponentially with age in males and females. Although the rate of exponential increase in males is slower, there is no evidence against the functional form (log-linear) we chose for regression. Our exponential curves are a linear regression fit on the logarithmic transform of UCH-L1. For the age range of 40–83 years, the area under the curve (AUC) average for the expected UCH-L1 values in males was statistically significantly less than the expected values in females (ratio estimate = 0.792, 95% CI: [0.628, 0.997], $p = 0.0472$). Because the age effects in the model are linear in the log plot, the AUC average is equivalent to comparing the expected value for males to that for females at the midpoint of the range, 61.5 years (see also Figure S1; Tables S7 and S8).

Plasma concentrations of NfL are exponentially higher with advancing age from early childhood

Plasma concentrations of NfL are commonly used to assess neuron/axon damage and the risk of future cognitive decline in AD, MCI, and other neurodegenerative diseases and have been found to increase with age.[28,39,41,52,53] Therefore, we examined the effects of age and gender on plasma concentrations of NfL in the 317 healthy control participants. As shown in Figures 2A and 2B and Table S7, plasma concentrations of NfL were exponentially higher with increasing age in healthy control participants ($p < 2.220 \times 10^{-16}$), with the slope of the log-transformed curve being greater than that observed for UCH-L1 with increasing age, such that the estimated change per year = 2.469% per year (95% CI: [2.225%, 2.714%], $p < 2.220 \times 10^{-16}$). Of note, the 95% CIs for the NfL and UCH-L1 rates of increase are non-overlapping, suggesting a statistically significant difference (alpha = 0.05) between the two. The exponential curve of NfL plasma concentrations with age showed a trend of being steeper for males than for females (ratio of ratios = 1.00487, 95% CI: [1.00000, 1.00976], $p = 0.0502$), with an estimated difference per year of 2.775% (95% CI: [2.388%, 3.164%], $p < 2.220 \times 10^{-16}$) for males compared to an estimated difference per year of 2.277% (95% CI: [1.965%, 2.590%], $p < 2.220 \times 10^{-16}$) for females (Figure 2C). Spline fits show that the log-transformed data are linear (Figure S1).

Plasma concentrations of GFAP are exponentially higher from age 40

The log-linear relationship between plasma concentration and age found for UCH-L1 and NfL was not appropriate for describing the age-associated changes in plasma GFAP in the 317 healthy control participants. Specifically, spline modeling

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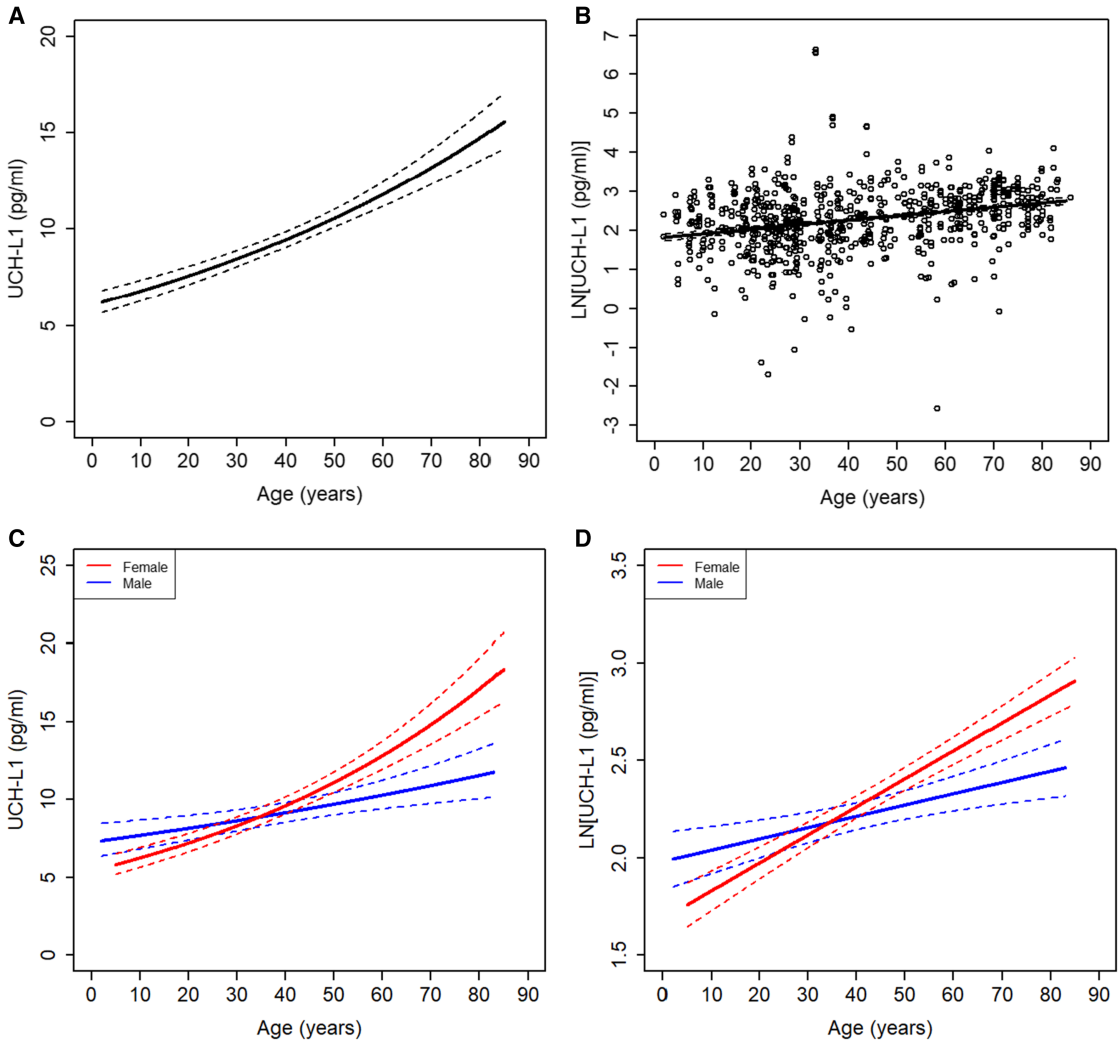

Figure 1. Plasma UCH-L1 concentrations are higher with advancing age in healthy control participants, especially in females

Concentrations of UCH-L1 in plasma from 314 healthy control participants, spanning ages 2–85 from the Crnic Institute Human Trisome Project (n = 103), the CUACC Bio-AD longitudinal observational study (n = 69), or the MS healthy controls biomarker study (n = 145) (3 participants lacked usable UCH-L1 data) were compared to the age of the participant. The assessment of the plasma concentration of each biological sample was replicated 2–3 times, and all such replicates were used in the analysis; see STAR Methods.

(A) Association between absolute UCH-L1 concentrations and age and the point-wise standard errors.

(B and C) UCH-L1 log plot with point-wise standard errors. Plasma UCH-L1 levels are exponentially higher with age across the lifespan (estimated change per year = 1.110%, $p = 5.504 \times 10^{-8}$). The effect of gender on the curve is shown in (C), which indicates that most of the promotion effect of age on plasma UCH-L1 concentrations is driven by females (estimated female change per year = 1.448% [$p = 3.635 \times 10^{-8}$] compared to estimated male change per year = 0.582% [$p = 0.0650$]; gender difference $p = 0.0342$).

(D) The effect of gender on the UCH-L1 log plot with point-wise standard errors. For the age range of 40–83 years, the area under the curve (AUC) average for the expected UCH-L1 values in males was statistically significantly less than the expected values in females (p value = 0.0472).

See also Figure S1 and Tables S7 and S8.

of the plasma levels of GFAP (replicates averaged) passes the deviance test for comparing it to the null hypothesis of linear ($p < 2.2 \times 10^{-16}$). GFAP levels are lower across the age range from ages 2 to 25, stay constant, and rise exponentially from approximately age 40 (Figure 3A; Table S8). Relatively high levels of plasma GFAP in children have been reported previously.[41] The U-shaped spline plot is apparent for both genders (Figures 3B and 3C). To quantify the age slopes, we approximated the relationships between log GFAP and age with a piece-wise linear model, with a knot at age 30 (Figure 3D). The estimated change per year for females less than 30 years old = −3.156% (95% CI: [−4.474%, −1.820%], $p < 5.908 \times 10^{-6}$), the estimated change per year for females greater than or equal to 30 years old = 3.110% (95% CI: [2.630%, 3.592%], $p < 2.220 \times 10^{-16}$), the estimated change per year for males less than 30 years old = −3.368% (95% CI: [−4.748%,

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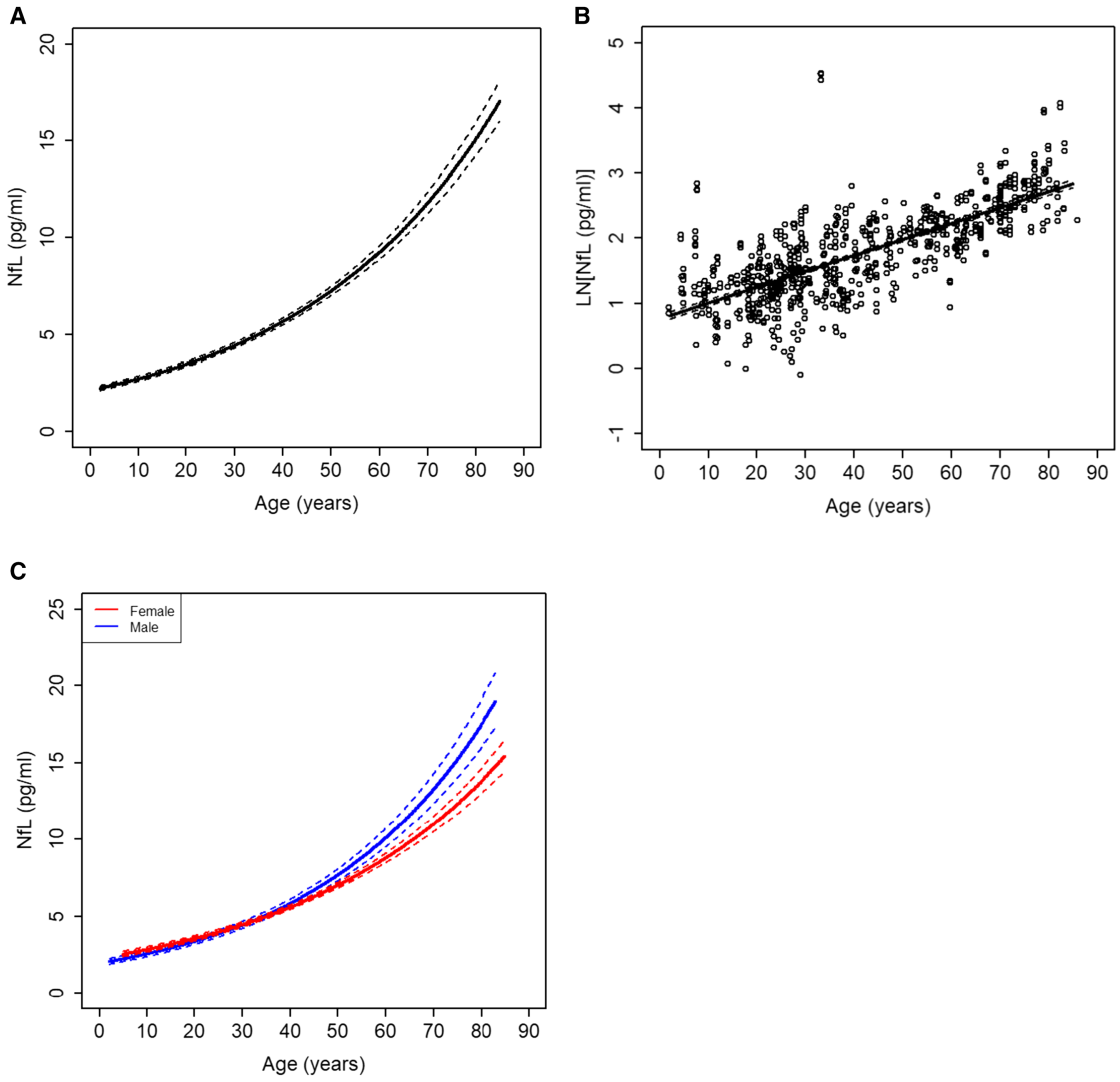

Figure 2. Plasma concentrations of NfL are exponentially higher with advancing age in healthy control participants

(A and B) Concentrations of NfL in plasma samples from 317 healthy control participants spanning ages 2–85 were compared to the age and gender of the donor. Plasma concentrations of NfL are exponentially higher with age across the entire lifespan. The assessment of each biological sample was replicated 2–3 times, and all such replicates were used in the analysis; see STAR Methods. The associations between absolute NfL concentrations and age and the point-wise standard errors are shown in (A), and the log plot is shown in (B) (estimated change = 2.469% per year [$p < 2.220 \times 10^{-16}$]).

(C) Comparison of males and females: estimated change per year was 2.775% ($p < 2.220 \times 10^{-16}$) in males compared to 2.277% per year ($p < 2.220 \times 10^{-16}$) in females. The exponential rate of change determined cross-sectionally was marginally statistically non-significantly greater in males (2.775% per year) than in females (2.277% per year) ($p = 0.0502$). When comparing the log-linear fit to splines for the association of NfL with age and gender in healthy controls (replicates averaged), the graph suggests that the rate of increase accelerates somewhat with older age, but a log-linear relationship is still an excellent approximation for all healthy controls (Figure S1; Tables S7 and S9) and when males and females are examined separately (Figure S1).

−1.967%], $p = 4.107 \times 10^{-6}$), and the estimated change per year for males greater than or equal to 30 years old = 2.899% (95% CI: [2.332%, 3.469%], $p = 2.220 \times 10^{-16}$). Within gender, the pre- and post-age 30 age slopes differed significantly ($p = 1.571 \times 10^{-12}$ and 1.798×10^{-11} for females and males, respectively). Within age groups, the differences in gender-age slope were not significant. The GFAP AUCs were higher in females than males overall from ages 2 to 83 (ratio = 0.843, 95% CI: [0.752, 0.946], $p = 0.0037$) and from ages 30 to 83 (ratio = 0.819, 95% CI: [0.717, 0.935], $p = 0.0033$) (see also Table S8). These findings indicate that plasma markers of astrogliosis/neuroinflammation (as measured by GFAP) temporally follow and thus are likely a reaction to the earlier and ongoing age-associated neurodegeneration, with females exhibiting higher plasma markers of astrogliosis and inflammation at all ages.

Correlation analyses of plasma measures of age-associated brain degeneration

The plasma measures of neurodegeneration (UCH-L1 and NfL) and astrogliosis (GFAP) for all healthy control participants are

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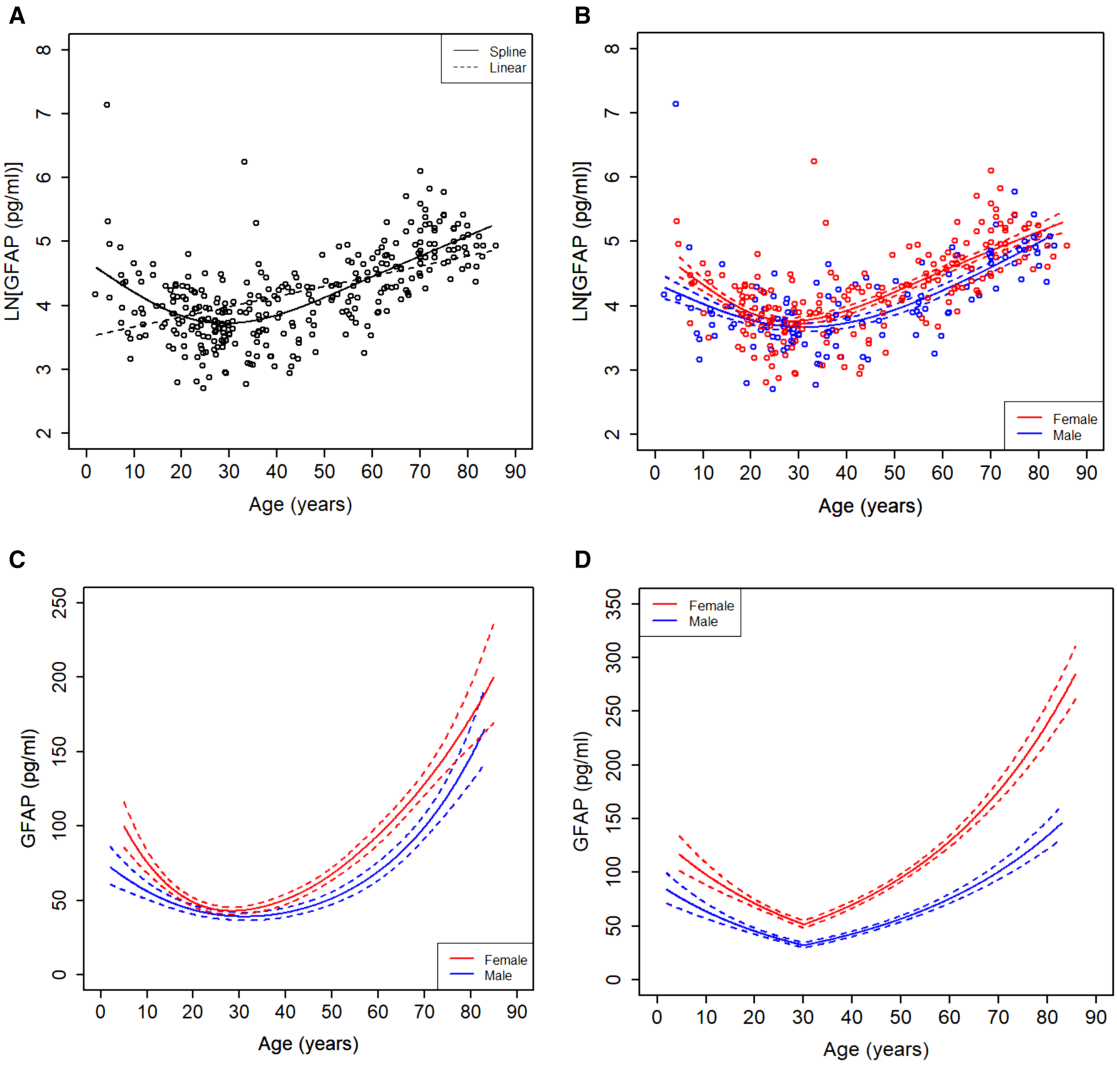

Figure 3. Plasma concentrations of GFAP are exponentially higher with age in healthy control participants after age 40

Plasma concentrations of GFAP in 317 healthy control participants spanning ages 2–85 were compared to the age and gender of the donor.

(A) Comparison of the log-linear fit to splines for the biomarker association with age in the healthy controls (replicates averaged). The deviance test for comparing the spline model to the null hypothesis of linear: $p < 2.2 \times 10^{-16}$, with the graph suggesting a U-shape, with GFAP concentrations accelerating after age 40. A spline plot by gender in the healthy controls (replicates averaged) also suggests a U-shape, with GFAP concentrations accelerating significantly after age 40 (Tables S7 and S10). The assessment of each biological sample was replicated 2–3 times, and all such replicates were used in the analysis; see STAR Methods.

(B–D) Deviation from linearity is evidenced in the absolute spline plot (C). To quantitate the relationships between log GFAP and age, a piece-wise linear model was constructed with a knot at age 30 (D) for both genders (estimated female change per year for less than 30 years old = −3.156% [$p < 5.908 \times 10^{-6}$]; estimated female change per year for greater than or equal to 30 years old = 3.110% [$p < 2.220 \times 10^{-16}$]; estimated male change per year for less than 30 years old = −3.368% [$p = 4.107 \times 10^{-6}$]; estimated male change per year for greater than or equal to 30 years old = 2.899% [$p = 2.220 \times 10^{-16}$]). Within gender, the pre- and post-age 30 age slopes differed significantly ($p = 1.571 \times 10^{-12}$ and 1.798×10^{-11} for females and males, respectively). Within age groups, the differences in gender-age slope were not significant. The GFAP AUCs were higher in females than males overall from ages 2 to 83 ($p = 0.0037$) and from ages 30 to 83 ($p = 0.0033$) (see also Table S10).

highly correlated with each other (UCH-L1:NfL, UCH-L1:GFAP, and NfL:GFAP, $p < 10^{-11}$) (Figure S2). This finding provides some evidence that UCH-L1 is indeed a proxy marker of neuronal death/damage, like the established marker NfL, and that brain aging across the lifespan reflects potentially parallel increases in neurodegeneration, both cellular and axonal, and astrogliosis/inflammation.

Comparisons of plasma measures of age-associated brain neurodegeneration and inflammation in MCI due to AD, mild-to-moderate AD, and healthy controls

AD dementia and its precursor, MCI due to AD, are both strongly associated with age and are accompanied by neurodegeneration and astrogliosis in the brain.[22] Having established full age curves for plasma markers of neurodegeneration

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(UCH-L1 and NfL) and astrogliosis/inflammation (GFAP), we compared these to the age-associated concentrations of NfL, GFAP, and UCH-L1 in plasma samples from the 32 participants with MCI due to AD from the Bio-AD study[22] and in plasma samples from the 36 participants with mild-to-moderate AD from our sargramostim/GM-CSF AD trial at baseline (before any placebo or GM-CSF intervention)[51] (Figure S4). A diagnosis of MCI or mild-to-moderate AD was associated with higher overall levels of both NfL (Figure S4A) and GFAP (Figure S4B) compared to age-matched healthy control (HC) participants at 73.7 years, which is the mean age for the MCI participants (MCI/HC estimate age of 73.7: NfL, ratio estimate = 1.497, $p = 5.923 \times 10^{-6}$ and GFAP, ratio estimate = 1.798, $p = 7.816 \times 10^{-7}$; AD/HC estimated age of 73.7: NfL, ratio estimate = 1.878, $p = 6.728 \times 10^{-7}$ and GFAP, ratio estimate = 1.391, $p = 0.0018$). Plasma concentrations of NfL were marginally non-significantly higher in participants with mild-to-moderate AD than in participants with MCI at 73.7 years, while GFAP concentrations were significantly lower (AD/MCI estimate age of 73.7: NfL, ratio estimate = 1.254 and $p = 0.0680$ and GFAP, ratio estimate = 0.774 and $p = 0.0464$), as expected from previous studies.[22,39] Interestingly, the plasma concentration of NfL showed age-associated higher levels in the participants with mild-to-moderate AD (2.714% per year, $p = 0.0122$) (Figure S4A), whereas GFAP plasma concentrations did not show age-associated higher levels in the participants with mild-to-moderate AD (−0.030% per year, $p = 0.9761$) (Figure S4B). Plasma concentrations of both NfL (Figure S4A) and GFAP (Figure S4B) showed age-associated higher levels for MCI (NfL: 4.138% per year, $p = 0.0017$; GFAP: 3.466% per year, $p = 0.0398$). There were no statistically significant differences among the age slopes for healthy controls, patients with AD, and patients with MCI for NfL. The age effect in GFAP for healthy controls greater than 30 years old was statistically significantly different than that for patients with mild-to-moderate AD ($p = 0.0056$).

UCH-L1 concentrations in plasma from the participants with MCI due to AD are higher overall than in healthy control participants at the mean age for the participants with MCI, at 73.7 years (ratio estimate = 1.559, $p = 0.0001$), whereas the plasma UCH-L1 concentrations of the participants with mild-to-moderate AD at the baseline visit (before any placebo or GM-CSF intervention) are approximately the same as those of the healthy control participants (ratio estimate = 1.042, $p = 0.7072$) (Figure S4C). UCH-L1 concentrations in MCI were higher than in mild-to-moderate AD at 73.7 years (ratio estimate = 1.497, $p = 0.0007$). UCH-L1 plasma concentrations increased for participants with mild-to-moderate AD by an estimated 2.420% per year of age ($p = 0.0054$), while the estimated age-associated increase for MCI is marginally statistically non-significant (estimate = 2.499% per year, $p = 0.0941$), possibly because it is underpowered with the modest sample size. There were no statistically significant differences among the age slopes for healthy controls, patients with mild-to-moderate AD, and patients with MCI for UCH-L1 (interaction test $p = 0.2040$).

An exploratory receiver operating characteristic (ROC) analysis was carried out (Table S11) to determine the discriminatory capacity of a model combining UCH-L1, NfL, and GFAP and age, compared to age alone. The AUCs for the full model were 0.8121 (MCI:HC), 0.8792 (MCI:AD), and 0.8622 (AD:HC), which are all higher than the AUC of age alone ($p < 0.001$).

Treatment of AD trial participants with sargramostim/GM-CSF lowers the UCH-L1 measure of neuronal loss to that of healthy controls many decades younger

In addition to identifying potential mechanisms of brain aging, age-associated biomarkers may be used to assess the efficacy of interventions that may slow or halt the aging process. We have previously discovered GM-CSF/sargramostim as a potential treatment for AD, whose likely mechanisms of action may include targeting the aging process in the brain. Specifically, based on early studies showing that patients with RA had a reduced risk of developing AD, we hypothesized that this protection might be due to a physiological reaction against RA's associated inflammation, with the beneficial side effect of reducing the risk of AD, which exhibits brain inflammation. We tested our hypothesis and found that treatment of a mouse model of AD with GM-CSF, an immune-system-stimulating/modulating cytokine that stimulates the proliferation of phagocytes in both the bone marrow and the brain and is upregulated in the plasma of patients with RA, reduced brain amyloid levels by half, increased brain synaptophysin levels, and restored memory to normal after a few weeks of subcutaneous administration.[54] GM-CSF treatment also improves the impaired cognition and reduced neuronal function in aged wild-type (WT) mice, indicating that the beneficial effects of GM-CSF on brain function are general and not exclusively derived from targeting AD pathology.[54,55] These findings in AD and aged mice have been confirmed by others.[56,57]

Building on this foundation, we completed a phase 2, double-blind, randomized, placebo-controlled trial of human recombinant GM-CSF (sargramostim) (250 μg/m^2/day subcutaneous injection, 5 days/week for 3 weeks) in participants with mild-to-moderate AD.[51] Treatment with sargramostim led to improved scores in the mini-mental state examination (MMSE) by almost two points (compared to baseline and to placebo) and moved the concentrations of AD-associated plasma biomarkers—Aβ40 and total tau—toward normal.[51] Interestingly, the largest change in a measure of AD neuropathology at the end of treatment was in the plasma UCH-L1 concentrations, which had decreased in the sargramostim-treated group by 40% compared to baseline ($p = 0.0017$) and by 42% compared to placebo ($p = 0.0019$).[51] Now, we can compare these results to the age curves for UCH-L1 in the healthy control participants shown in Figure 1 to determine how effective sargramostim/GM-CSF treatment was in reducing this measure of neuronal loss.

The plasma concentrations of UCH-L1 in 18 participants with mild-to-moderate AD at baseline and at the end of treatment with sargramostim/GM-CSF are plotted together with the data from healthy control participants in Figure 4A and show that the absolute values of plasma UCH-L1 are greatly reduced after sargramostim/GM-CSF treatment (ratio estimate = 0.497, $p = 0.0008$). Indeed, sargramostim/GM-CSF treatment reduces the concentrations of UCH-L1 in the plasma of trial participants to an average level far below those of similarly aged healthy control participants (ratio estimate = 0.502, $p = 0.0019$) and equivalent to

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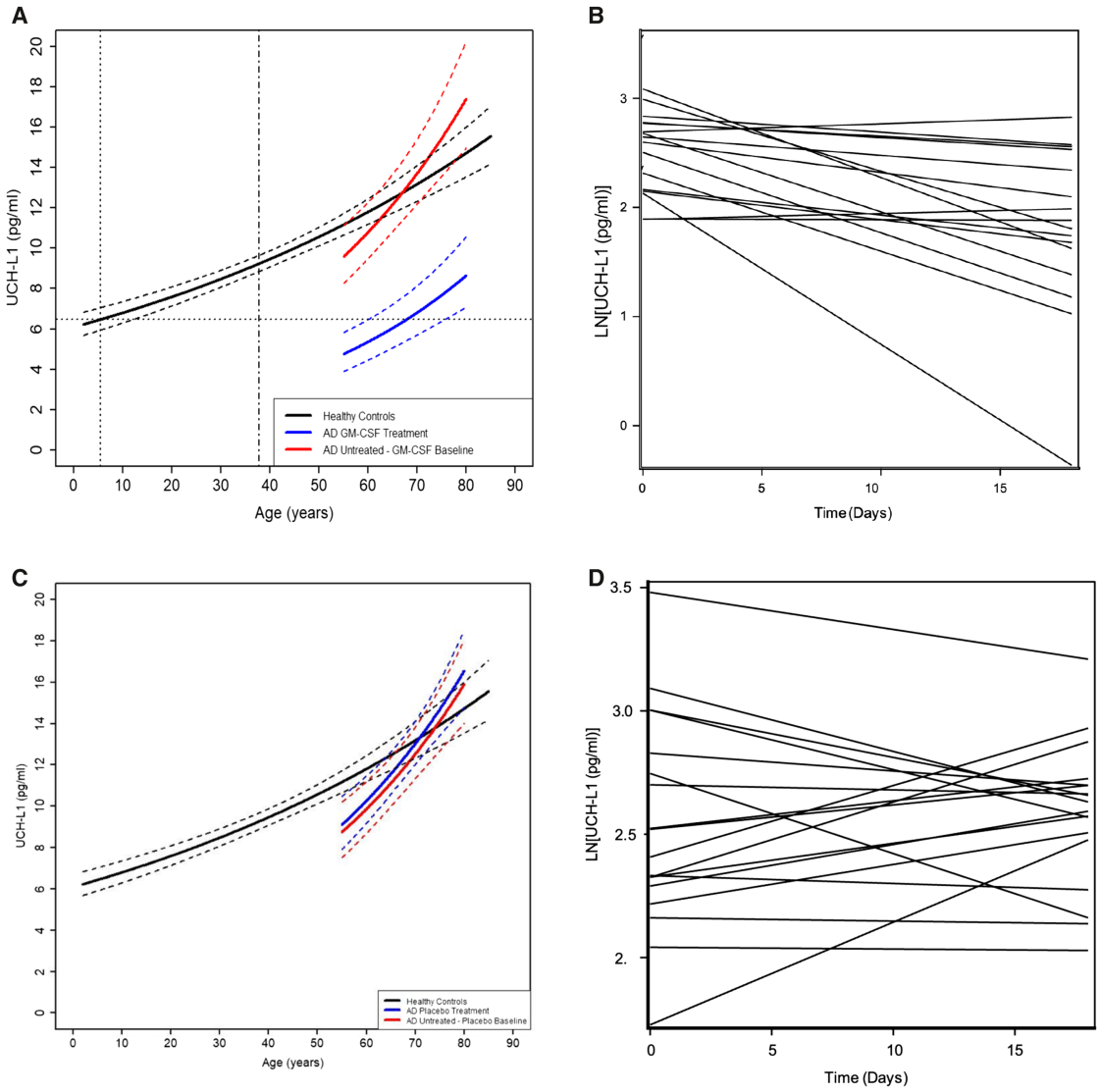

Figure 4. Treatment of participants with mild-to-moderate AD with sargramostim/GM-CSF reduces plasma UCH-L1 measure of neuronal loss to levels equivalent to those of healthy controls many decades younger

(A) Association plots and point-wise standard errors are shown for the 18 participants with mild-to-moderate AD from the phase 2, double-blind, randomized, placebo-controlled trial with recombinant human GM-CSF (the sargramostim/GM-CSF AD trial) at baseline before they were treated with sargramostim/GM-CSF (AD untreated—GM-CSF baseline, $n = 18$) or after they were treated with sargramostim/GM-CSF (AD GM-CSF treatment, $n = 18$), together with the correlation curve for healthy control participants from Figure 1. The assessment of each biological sample was replicated 2–3 times, and all such replicates were used in the analysis; see STAR Methods. The comparison shows that treatment of participants with mild-to-moderate AD with sargramostim/GM-CSF leads to absolute UCH-L1 values that are greatly reduced compared to their starting (baseline) levels (ratio estimate = 0.497; $p = 0.0008$). Furthermore, sargramostim/GM-CSF treatment reduces plasma UCH-L1 concentrations to levels far below those expected of similarly aged healthy control participants (ratio estimate = 0.502, $p = 0.0019$ at 67.8 years, the mean age for the sargramostim/GM-CSF-treated patients with mild-to-moderate AD). The average plasma UCH-L1 level of the participants with AD equals the level expected of a 5.4-year-old healthy control participant (horizontal dotted line). The reduced plasma UCH-L1 levels associated with GM-CSF treatment of participants with AD are statistically significantly lower than those of all healthy control participants above approximately age 37.8 (vertical dot-dash line) but are statistically indistinguishable ($p > 0.05$) from healthy control participants younger than age 37.8.

(B) A spaghetti plot of plasma UCH-L1 measures before and after GM-CSF treatment of participants with AD.

(C) Plot of 18 baseline and placebo-treated participants with AD together with the correlation curve for healthy control participants from Figure 1.

(D) Spaghetti plot of plasma UCH-L1 measures before and after placebo treatment of participants with AD.

those found in healthy control participants six decades younger (Figure 4A). For example, a 67.8-year-old participant with mild-to-moderate AD treated with sargramostim/GM-CSF, corresponding to the mean age for this group, would have had a plasma UCH-L1 concentration of 6.47 pg/mL (geometric mean, 95% CI: [4.45, 9.39], not baseline calibrated—sargramostim/GM-CSF baseline only), which is equivalent to that expected of a 5.4-year-old healthy control participant, based on our

cross-sectional data in Figure 1. The spaghetti plot of each participant from baseline to the end of treatment is shown in Figure 4B. The lack of any effect of placebo treatment is shown in Figures 4C and 4D.

GM-CSF treatment reduces neuronal cell death/apoptosis in the CA1, CA3, and dentate gyrus/hilus regions of an AD rat model

To investigate the mechanism by which GM-CSF treatment reduces plasma concentrations of UCH-L1, a measure of neuronal death, we examined aged (18- to 20-month-old) TgF344-AD rats, a model of AD that shows the complete brain pathology of human AD (amyloid and tau deposition and neuronal loss).[58] TgF344-AD rats were treated with recombinant rat GM-CSF or saline placebo for 5 weeks, and their brains and those of age-matched WT control F344 rats were assessed by immunohistochemical staining for caspase-3, a marker of apoptosis that is increased in humans and animal models with AD and during aging.[50,59] As shown in Figure 5, aged TgF344-AD rats (treated with placebo/saline) exhibited large numbers of caspase-3-positive cells in the CA1, CA3, and dentate gyrus/hilus regions of the hippocampus compared to WT F344 rats. GM-CSF treatment of aged TgF344-AD rats significantly reduced the elevated number of caspase-3-positive cells compared to placebo-treated TgF344-AD rats, almost reaching the low number observed in the untreated WT F344 rats. Notably, the vast majority of caspase-3-positive cells in the TgF344-AD rats were neurons (90%–95%) based on co-staining for the MAP2 neuronal marker (see STAR Methods).

GM-CSF treatment reverses astrogliosis in the CA1 and dentate gyrus/hilus of an AD rat model

In addition to being associated with normal human aging, MCI, and AD, as shown above, higher plasma concentrations of GFAP are also associated with lower measures of some cognitive attributes and with defects in white matter microstructure.[22] Furthermore, astrogliosis is increased in the brain of the Dp16 mouse model of Down syndrome, which is reversed by GM-CSF treatment.[55] To determine the effect of GM-CSF treatment on astrogliosis in the AD brain, hippocampal sections of the aged male TgF344-AD rats treated with GM-CSF or placebo/saline injection for 5 weeks were assessed for patterns of activated astrocytes by immunohistochemical staining for GFAP together with DAPI co-staining. Quantitative analyses of the percentage of area of GFAP-positive astrocytes showed a significant increase in the CA1, CA3, and dentate gyrus/hilus regions of saline-treated TgF344-AD rats compared to age-matched WT rats. The percentage of area of GFAP staining was significantly reduced in the CA1 and dentate gyrus/hilus regions, but was not statistically significantly reduced in the CA3 region, in GM-CSF-treated TgF344-AD rats compared to saline-treated TgF344-AD rats (Figure 6).

DISCUSSION

The identification of biomarkers of brain aging is an area of active investigation. For example, blood biomarkers of aging in multiple organs, including the brain, have been reported recently, focusing on function rather than cell loss/damage, and a study in marmosets showed that serum UCH-L1, NfL, and GFAP concentrations are higher in late age compared to adult age.[26,60] However, the limited age range of the individuals in these previous studies did not yield insights into when brain aging begins, which we now report. Although a recent multi-omics aging study showed that many molecules rise exponentially from age 44,[61] except for NfL,[41] no previous studies[22,26,27,62] have shown an exponential age-associated elevation in plasma markers of brain damage across the life span.

Although postmortem analyses provide information about accumulated damage, the plasma concentrations of the biomarkers that we evaluated, UCH-L1, NfL, and GFAP, may be measures of current active damage in the brain, possibly of neuronal cell loss, axon damage, and astrogliosis, respectively. This hypothesis is supported by multiple studies of acute brain injury, which show that the half-lives of these proteins in the blood are short (~24 h for NfL and GFAP but only 8.5 h for UCH-L1) and that brain neuronal loss and gliosis after TBI correlate with the plasma levels of UCH-L1 and GFAP.[33,63,64] Thus, the increases we observed cross-sectionally over many decades are unlikely to be due to accumulation and may instead serve as measures of processes currently underway in the brain that are enhanced with increased age. Interestingly, the numerical density of normal diploid neurons remained stable in a cross-sectional autopsy study of brains from cognitively normal individuals and individuals across various stages of the MCI-AD process. In contrast, the proportion of abnormal neurons destined for imminent apoptosis, as assessed by single-cell chromosome aneuploidy, is low in cognitively normal individuals, highest (~30%) in preclinical and mild AD, and declines greatly in the late stages of AD when accumulated cell loss is highest[65] (for a discussion, see Potter et al.[66]). Indeed, some 95% of the final neuron loss in AD brains can be calculated to be due to the loss of aneuploid neurons.[65] Similarly, although plasma levels of UCH-L1, NfL, and GFAP are increased in patients with early PD, most neuronal loss occurs before clinical motor or cognitive symptoms appear, and plasma UCH-L1 levels in participants with more advanced PD, especially in those with dementia, are indeed lower than in participants with early PD or even normal controls because, although the accumulated loss is high, the rate of loss has decreased.[44,45]

An exponential increase in any product of a biological process implies the existence of at least one positive feedback loop that accelerates the process. The exponential increases with age of the cross-sectionally assessed plasma markers of the rates of brain degeneration, first of neuron loss (UCH-L1) and axon damage (NfL) and then of astrogliosis/neuroinflammation (GFAP), and their strong correlations with each other imply the existence of such a positive feedback loop in the process of brain aging. Although speculative, neuron loss and axon damage evident via plasma biomarkers from early childhood could induce gliosis/inflammation to phagocytose the resulting debris and initiate the inflammaging cascade, resulting in a vicious cycle of more neuronal damage and death and more inflammation.

Interestingly, the positive feedback loop involving neuronal loss/damage and inflammation in brain aging that the data imply includes the same components as an already-established

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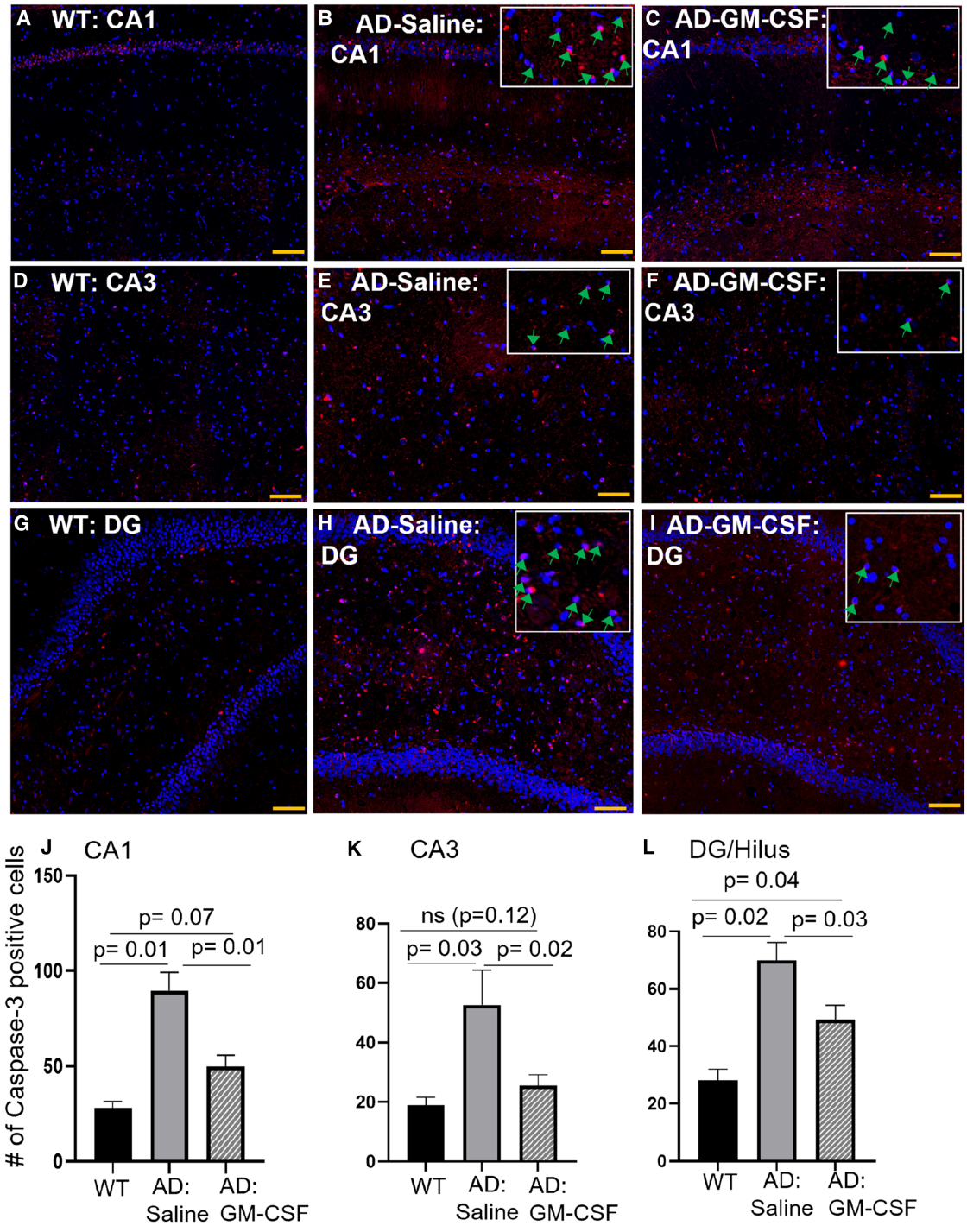

Figure 5. Treatment with GM-CSF reduces neuronal cell death in the CA1, CA3, and dentate gyrus/hilus in a rat model of AD

Aged male TgF344-AD rats (18–20 months), treated with GM-CSF or placebo (saline) injection for 5 weeks, were assessed for neuronal cell death by immunohistochemical staining for caspase-3 (red) together with DAPI staining (blue), followed by blinded counting of caspase-3-positive cells. Arrowheads in the insets indicate caspase-3-positive cells. The numbers of caspase-3-positive cells were determined in the CA1 (A–C), CA3 (D–F), and dentate gyrus/hilus (G–I) regions of the hippocampus in age-matched F344 male wild-type (WT) rats (A, D, and G), TgF344-AD rats injected with saline for 5 weeks (B, E, and H), and TgF344-AD rats treated with GM-CSF for 5 weeks (C, F, and I). Quantitative analyses showed a significantly higher number of caspase-3-positive cells in all three hippocampal regions of TgF344-AD rats injected with saline compared to age-matched WT rats, which decreased significantly with GM-CSF treatment (*p* values are as indicated: J–L). For each bar, data are represented as mean ± SEM (*p* values are as indicated; see STAR Methods). Scale bar: 100 mm (20× magnification). All experiments were performed 2–6 times (technical replicates) with similar results (biological replicates: $n = 5$ for WT rats, $n = 7$ for Tg344-AD rats injected with saline, and $n = 7$ for Tg344-AD rats treated with GM-CSF).

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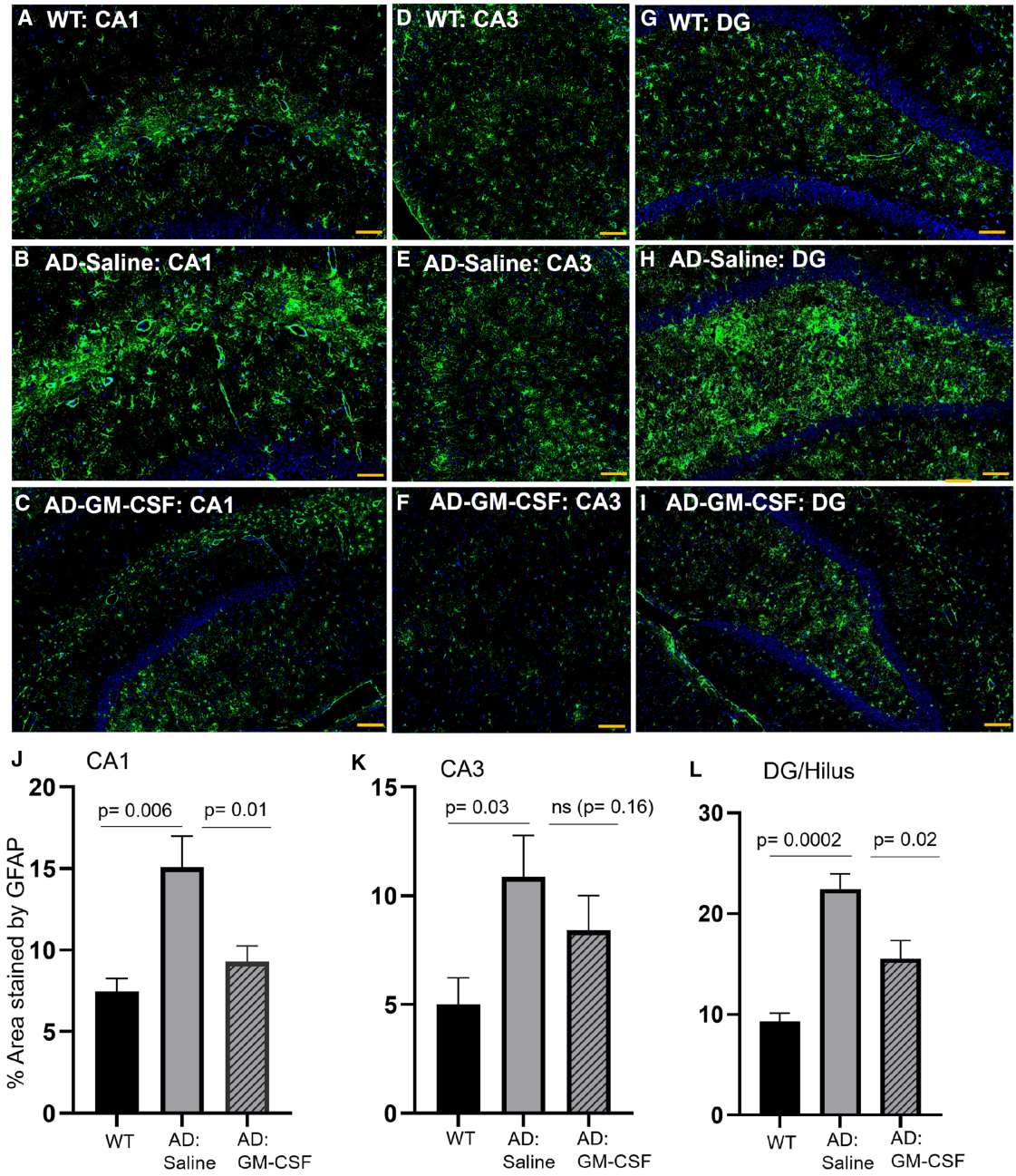

Figure 6. Treatment with GM-CSF reduces astrogliosis in a rat model of AD

Brain sections of the aged male TgF344-AD rats treated with GM-CSF or placebo (saline) injection for 5 weeks were also assessed for patterns of GFAP-positive astrocytes by immunohistochemical staining for GFAP together with DAPI staining, followed by blinded analyses of the expression patterns of the percentage of area stained for GFAP-positive astrocytes: the CA1 (A–C), CA3 (D–F), and dentate gyrus/hilus (G–I) regions of the hippocampus in age-matched F344 male wild-type (WT) rats (A, D, and G), TgF344-AD rats injected with saline for 5 weeks (B, E, and H), and TgF344-AD rats treated with GM-CSF for 5 weeks (C, F, and I). Quantitative analyses showed a significantly increased GFAP % area staining in the CA1, CA3, and dentate gyrus/hilus regions of saline-treated TgF344-AD rats compared to age-matched WT rats (*p* values are as indicated: J–L). In contrast, the GFAP % area staining was significantly reduced in the CA1 and dentate gyrus/hilus regions, but not statistically significantly in the CA3 region, in GM-CSF-treated TgF344-AD rats compared to saline-treated TgF344-AD rats (*p* values as indicated: J–L). For each bar, data are represented as the mean ± SEM for separate groups of mice. Scale bar: 200 mm (20× magnification). All experiments were repeated 2–6 times (technical replicates) with similar results (n = 5–8 rats/group; biological replicates).

See STAR Methods.

positive feedback loop in the development of AD. Specifically, gliosis/neuroinflammation in AD increases the expression of cytokines that lead through several steps to increased production of Aβ peptides and their apoE-dependent polymerization into neurotoxic oligomers and filaments. The Aβ oligomers, in turn, further increase neuroinflammation through further activation of microglia and astrocytes.[20,21,67–69] Positive feedback loops in pathogenic pathways are ideal targets for therapeutic intervention, as illustrated by repurposing GM-CSF for treatment of AD.

In sum, our findings allow several conclusions to be drawn.

(1) The exponential increases in the concentrations of two plasma biomarkers of current neuronal damage, UCH-L1 and NfL, with increasing age in healthy control participants, indicate that normal brain aging is a lifelong process that becomes behaviorally apparent only later, as the accumulated neuronal damage overcomes neurogenesis, functional redundancy, and resiliency in some individuals but not all.[70]

(2) The higher average UCH-L1 concentrations in plasma in participants with MCI compared to healthy control participants provide potential support for an additional "N" measure for the AD amyloid/tau/neurodegeneration (A/T/N) assessment tool, which should be validated in future studies. Based on cross-sectional data, the finding that the elevations in the concentrations of the plasma biomarkers of neuronal damage, UCH-L1 and NfL, in healthy control participants occur earlier than the increase in astrogliosis (GFAP), which accelerates significantly after age 40, potentially indicates that astrogliosis and its attendant inflammation (inflammaging) are likely to be both a response to and an accelerator of the age-related neuronal damage in a positive feedback loop rather than an initiating cause. The higher age-associated plasma concentrations of UCH-L1 and GFAP in females should be further explored, as greater rates of neuronal cell loss and astrogliosis may explain or contribute to their increased risk for age-associated neurodegenerative diseases such as AD.

(3) The standard errors of the mean for the concentration versus age curves for all three biomarkers are very tight across the lifespan, and together and separately, these biomarkers are able to identify individuals with MCI, thus potentially predicting future disease. However, there is also variance between individuals, which may reflect increased risk for or resilience to the development of AD, MCI, or AACD caused by genetic variants such as carrying an *APOE4* allele,[68] lifestyle, environment, or other as-yet unidentified factors.[10]

In contrast to other potential treatments for AD, sargramostim/GM-CSF is also beneficial for preventing normal AACD in mouse models and for treating many neurological injuries and diseases that do not have associated AD pathology, for example, in animal models of Down syndrome, stroke, TBI, and PD[71–74]; in humans with chemobrain[75]; and in a preliminary clinical trial in participants with PD.[76] Its broad therapeutic applicability may be related to the ability of GM-CSF to cross the blood-brain barrier, to be both neuroprotective and anti-apoptotic, to stimulate arteriogenesis and blood flow, to promote axon preservation/regeneration and neuronal plasticity, and to induce the proliferation of neural stem cells[74,77–82] (for more references and discussion, see Ahmed et al.[21]). In the TgF344-AD rat model of AD, GM-CSF treatment reduced both neuronal death and astrogliosis, based on caspase-3 and GFAP staining, respectively, in the brain after 5 weeks of treatment, whereas sargramostim/GM-CSF treatment of participants with AD in the phase 2 trial led to reduced plasma concentrations of UCH-L1 but not of NfL or GFAP, likely due to the very short treatment period of 3 weeks in the human trial and the short half-life of plasma UCH-L1 compared to NfL and GFAP.[83]

The finding that 3 weeks of sargramostim/GM-CSF treatment of participants with mild-to-moderate AD in a phase 2, double-blind, randomized, placebo-controlled trial led to improved performance on a cognitive measure (almost 2 points on MMSE) and changes in plasma levels of markers of AD neuropathology (Aβ40, total tau, and UCH-L1), but no ARIA,[51] together with the findings reported here, allow the following additional conclusions to be drawn.

(4) A reduction in plasma UCH-L1 may be a sensitive biomarker for testing the efficacy of many AD treatments, including therapeutic changes in lifestyle.

Our finding that GM-CSF treatment reduced the elevated neuronal apoptosis and gliosis in the hippocampus in the TgF344-AD rat model of AD to levels that were close to those of age-matched WT control rats suggests that the ability of GM-CSF to improve cognition as measured by MMSE, to partially normalize the concentrations of amyloid and tau plasma biomarkers, and to greatly reduce the plasma concentrations of the biomarker of neurodegeneration, UCH-L1, in participants with AD[51] is likely due to a reduction in the number of apoptotic neurons in the brain. Indeed, GM-CSF has been shown to suppress apoptosis, and we and others have found that apoptotic/damaged brain neurons in neurodegenerative diseases, including in AD, are often aneuploid and that aneuploidy leads to apoptosis, suggesting that GM-CSF may prevent this process.[4,66,84–91] Furthermore, cells naturally undergo senescence with increased age, and their removal with "senolytic" molecules can reverse some features of aging, including improving cognition in animal models of AD.[67,92–94] Similarly, through its ability to stimulate the production and activity of innate immune phagocytes (i.e., microglia) in the brain,[54] GM-CSF treatment may enhance the removal of damaged, apoptotic, and senescent neurons, thus allowing the remaining neurons to function more effectively. In sum, our mechanistic studies in TgF344-AD rats are consistent with the following conclusion.

(5) GM-CSF may have an anti-apoptotic and/or senolytic activity on neurons that may underlie its ability to prevent neuronal loss and reverse cognitive decline due to neurodegenerative disease or normal aging.

In participants with mild-to-moderate AD, the benefits of GM-CSF treatment in improving cognition and reducing plasma markers of neuropathology are evidently temporary, as markers

of neuropathology return to almost pre-treatment levels by 45 days after sargramostim/GM-CSF treatment is halted, yet there is still a statistically significant benefit to cognition, as measured by MMSE, at 45 days post-treatment compared to the placebo.[51] Treatment of healthy aged individuals with sargramostim/GM-CSF may reduce age-associated neuronal damage and reverse AACD most effectively with continuous application or may similarly confer a more long-lived benefit after treatment is halted.

Limitations of the study

(1) Patients with MCI due to AD were identified by clinical diagnosis, not confirmed with CSF markers or positron emission tomography (PET). There were no cutoffs used. Thus, we could not categorize participants into A/T/N positivity. Although plasma AD biomarkers are increasingly used, future studies to confirm our findings would benefit from an analysis of CSF samples or from the use of mass spectrometry to analyze plasma biomarkers.

(2) Combined data were obtained from cross-sectional/baseline assessments from different studies with different inclusion/exclusion criteria. Caution is warranted in interpreting the data as indicating longitudinal *change* in outcomes, as we cannot rule out cohort effects and resulting bias. Future studies should assess these biomarkers longitudinally across the lifespan and/or pool studies with harmonized recruitment/enrollment techniques to confirm the results.

(3) High CVs in UCHL-1 measures may impact the clinical application of these findings, with cutoffs, as well as replication of results.

RESOURCE AVAILABILITY

Lead contact

Requests for further information, resources, and reagents should be directed to and will be fulfilled by the lead contact, Huntington Potter (huntington.potter@cuanschutz.edu).

Materials availability

No new materials have been generated in the project.

There are restrictions to the availability of human plasma samples due to the lack of an external centralized repository for their distribution and our need to maintain the stock. We are glad to share plasma samples with reasonable compensation by requestor for its processing and shipping.

All unique/stable reagents generated in this study are available from the lead contact with a completed materials transfer agreement.

Data and code availability

- All data are included in the manuscript and supplemental information and are available on Mendeley Data: https://data.mendeley.com/datasets/wrx5pbhr6z/1.
- No custom code was developed for or used in any of the studies.
- Any additional information required to reanalyze the data reported in this work paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization, H.P., S.H.S., N.E., C.C., M.M.A., and H.J.C.; methodology, S.H.S., C.C., M.M.A., K.N., and B.M.B.; investigation, S.H.S., C.C., M.M.A., K.N., P.A., M.D.G., B.M.B., and J.M.E.; supervision, H.P. and J.M.E.; writing – original draft, H.P.; and writing – review & editing, H.J.C., S.H.S., C.C., M.M.A., B.M.B., H.P., and T.D.B.

DECLARATION OF INTERESTS

H.P., T.D.B., C.C., M.M.A., and S.H.S. are inventors on several non-licensed US patents or pending applications owned by the University of South Florida or the University of Colorado and related to this research.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

No AI or AI-assisted technologies were used in the writing process..

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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  - Sex/gender influence
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- METHOD DETAILS
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- ADDITIONAL RESOURCES

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xcrm.2025.102525.

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REFERENCES

1. Trollor, J.N., Smith, E., Agars, E., Kuan, S.A., Baune, B.T., Campbell, L., Samaras, K., Crawford, J., Lux, O., Kochan, N.A., et al. (2012). The association between systemic inflammation and cognitive performance in the elderly: the Sydney Memory and Ageing Study. Age (Dordr) *34*, 1295–1308. https://doi.org/10.1007/s11357-011-9301-x.

2. Singh, P.P., Demmitt, B.A., Nath, R.D., and Brunet, A. (2019). The Genetics of Aging: A Vertebrate Perspective. Cell *177*, 200–220. https://doi.org/10.1016/j.cell.2019.02.038.

3. Shcherbakov, D., Nigri, M., Akbergenov, R., Brilkova, M., Mantovani, M., Petit, P.I., Grimm, A., Karol, A.A., Teo, Y., Sanchón, A.C., et al. (2022). Premature aging in mice with error-prone protein synthesis. Sci. Adv. *8*, eabl9051. https://doi.org/10.1126/sciadv.abl9051.

4. Potter, H. (1991). Review and hypothesis: Alzheimer disease and Down syndrome–chromosome 21 nondisjunction may underlie both disorders. Am. J. Hum. Genet. *48*, 1192–1200.

5. Mattson, M.P., Longo, V.D., and Harvie, M. (2017). Impact of intermittent fasting on health and disease processes. Ageing Res. Rev. *39*, 46–58. https://doi.org/10.1016/j.arr.2016.10.005.

6. Maegawa, S., Lu, Y., Tahara, T., Lee, J.T., Madzo, J., Liang, S., Jelinek, J., Colman, R.J., and Issa, J.P.J. (2017). Caloric restriction delays age-related methylation drift. Nat. Commun. *8*, 539. https://doi.org/10.1038/s41467-017-00607-3.

7. Lalli, M.A., Bettcher, B.M., Arcila, M.L., Garcia, G., Guzman, C., Madrigal, L., Ramirez, L., Acosta-Uribe, J., Baena, A., Wojta, K.J., et al. (2015). Whole-genome sequencing suggests a chemokine gene cluster that modifies age at onset in familial Alzheimer's disease. Mol. Psychiatry *20*, 1294–1300. https://doi.org/10.1038/mp.2015.131.

8. Frasca, D., and Blomberg, B.B. (2016). Inflammaging decreases adaptive and innate immune responses in mice and humans. Biogerontology *17*, 7–19. https://doi.org/10.1007/s10522-015-9578-8.

9. Bettcher, B.M., Watson, C.L., Walsh, C.M., Lobach, I.V., Neuhaus, J., Miller, J.W., Green, R., Patel, N., Dutt, S., Busovaca, E., et al. (2014). Interleukin-6, age, and corpus callosum integrity. PLoS One *9*, e106521. https://doi.org/10.1371/journal.pone.0106521.

10. Boyle, P.A., Yu, L., Wilson, R.S., Leurgans, S.E., Schneider, J.A., and Bennett, D.A. (2018). Person-specific contribution of neuropathologies to cognitive loss in old age. Ann. Neurol. *83*, 74–83. https://doi.org/10.1002/ana.25123.

11. Risacher, S.L., Anderson, W.H., Charil, A., Castelluccio, P.F., Shcherbinin, S., Saykin, A.J., and Schwarz, A.J.; Alzheimer's Disease Neuroimaging Initiative (2017). Alzheimer disease brain atrophy subtypes are associated with cognition and rate of decline. Neurology *89*, 2176–2186. https://doi.org/10.1212/wnl.0000000000004670.

12. Duara, R., Loewenstein, D.A., Potter, E., Appel, J., Greig, M.T., Urs, R., Shen, Q., Raj, A., Small, B., Barker, W., et al. (2008). Medial temporal lobe atrophy on MRI scans and the diagnosis of Alzheimer disease. Neurology *71*, 1986–1992. https://doi.org/10.1212/01.wnl.0000336925.79704.9f.

13. Hyman, B.T., Van Hoesen, G.W., Damasio, A.R., and Barnes, C.L. (1984). Alzheimer's disease: cell-specific pathology isolates the hippocampal formation. Science *225*, 1168–1170. https://doi.org/10.1126/science.6474172.

14. Alves, F., Kalinowski, P., and Ayton, S. (2023). Accelerated Brain Volume Loss Caused by Anti-β-Amyloid Drugs: A Systematic Review and Meta-analysis. Neurology *100*, e2114–e2124. https://doi.org/10.1212/wnl.0000000000207156.

15. Means, J.C., Gerdes, B.C., Kaja, S., Sumien, N., Payne, A.J., Stark, D.A., Borden, P.K., Price, J.L., and Koulen, P. (2016). Caspase-3-Dependent Proteolytic Cleavage of Tau Causes Neurofibrillary Tangles and Results in Cognitive Impairment During Normal Aging. Neurochem. Res. *41*, 2278–2288. https://doi.org/10.1007/s11064-016-1942-9.

16. Markov, N.T., Lindbergh, C.A., Staffaroni, A.M., Perez, K., Stevens, M., Nguyen, K., Murad, N.F., Fonseca, C., Campisi, J., Kramer, J., and Furman, D. (2022). Age-related brain atrophy is not a homogenous process: Different functional brain networks associate differentially with aging and blood factors. Proc. Natl. Acad. Sci. USA *119*, e2207181119. https://doi.org/10.1073/pnas.2207181119.

17. Resnick, S.M., Pham, D.L., Kraut, M.A., Zonderman, A.B., and Davatzikos, C. (2003). Longitudinal magnetic resonance imaging studies of older adults: a shrinking brain. J. Neurosci. *23*, 3295–3301. https://doi.org/10.1523/jneurosci.23-08-03295.2003.

18. Scahill, R.I., Frost, C., Jenkins, R., Whitwell, J.L., Rossor, M.N., and Fox, N.C. (2003). A longitudinal study of brain volume changes in normal aging using serial registered magnetic resonance imaging. Arch. Neurol. *60*, 989–994. https://doi.org/10.1001/archneur.60.7.989.

19. Zheng, Z., Liu, Y., Wang, Z., Yin, H., Zhang, D., and Yang, J. (2024). Evaluating age-and gender-related changes in brain volumes in normal adult using synthetic magnetic resonance imaging. Brain Behav. *14*, e3619. https://doi.org/10.1002/brb3.3619.

20. Potter, H. (2001). Beyond beta protein—the essential role of inflammation in Alzheimer Amyloid formation. In Inflammatory Events in Neurodegeneration, S.C. Bondy and A. Cambell, eds. (Prominent Press).

21. Ahmed, M.M., Johnson, N.R., Boyd, T.D., Coughlan, C., Chial, H.J., and Potter, H. (2021). Innate Immune System Activation and Neuroinflammation in Down Syndrome and Neurodegeneration: Therapeutic Targets or Partners? Front. Aging Neurosci. *13*, 718426. https://doi.org/10.3389/fnagi.2021.718426.

22. Bettcher, B.M., Olson, K.E., Carlson, N.E., McConnell, B.V., Boyd, T., Adame, V., Solano, D.A., Anton, P., Markham, N., Thaker, A.A., et al. (2021). Astrogliosis and episodic memory in late life: higher GFAP is related to worse memory and white matter microstructure in healthy aging and Alzheimer's disease. Neurobiol. Aging *103*, 68–77. https://doi.org/10.1016/j.neurobiolaging.2021.02.012.

23. Fulop, T., Larbi, A., Dupuis, G., Le Page, A., Frost, E.H., Cohen, A.A., Witkowski, J.M., and Franceschi, C. (2017). Immunosenescence and Inflamm-Aging As Two Sides of the Same Coin: Friends or Foes? Front. Immunol. *8*, 1960. https://doi.org/10.3389/fimmu.2017.01960.

24. McGeer, P.L., Schulzer, M., and McGeer, E.G. (1996). Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. Neurology *47*, 425–432. https://doi.org/10.1212/wnl.47.2.425.

25. Zhou, M., Xu, R., Kaelber, D.C., and Gurney, M.E. (2020). Tumor Necrosis Factor (TNF) blocking agents are associated with lower risk for Alzheimer's disease in patients with rheumatoid arthritis and psoriasis. PLoS One *15*, e0229819. https://doi.org/10.1371/journal.pone.0229819.

26. Oh, H.S.H., Rutledge, J., Nachun, D., Pálovics, R., Abiose, O., Moran-Losada, P., Channappa, D., Urey, D.Y., Kim, K., Sung, Y.J., et al. (2023). Organ aging signatures in the plasma proteome track health and disease. Nature *624*, 164–172. https://doi.org/10.1038/s41586-023-06802-1.

27. Zecca, C., Pasculli, G., Tortelli, R., Dell'Abate, M.T., Capozzo, R., Barulli, M.R., Barone, R., Accogli, M., Arima, S., Pollice, A., et al. (2021). The Role of Age on Beta-Amyloid(1-42) Plasma Levels in Healthy Subjects. Front. Aging Neurosci. *13*, 698571. https://doi.org/10.3389/fnagi.2021.698571.

28. Shir, D., Mielke, M.M., Hofrenning, E.I., Lesnick, T.G., Knopman, D.S., Petersen, R.C., Jack, C.R., Algeciras-Schimnich, A., Vemuri, P., and Graff-Radford, J. (2023). Associations of Neurodegeneration Biomarkers in Cerebrospinal Fluid with Markers of Alzheimer's Disease and Vascular Pathology. J. Alzheimers Dis. *92*, 887–898. https://doi.org/10.3233/jad-221015.

29. Morgan, A.R., Touchard, S., Leckey, C., O'Hagan, C., Nevado-Holgado, A.J., NIMA Consortium; Barkhof, F., Bertram, L., Blin, O., Bos, I., et al. (2019). Inflammatory biomarkers in Alzheimer's disease plasma. Alzheimer's Dement. *15*, 776–787. https://doi.org/10.1016/j.jalz.2019.03.007.

30. Wang, K.K., Yang, Z., Sarkis, G., Torres, I., and Raghavan, V. (2017). Ubiquitin C-terminal hydrolase-L1 (UCH-L1) as a therapeutic and diagnostic target in neurodegeneration, neurotrauma and neuro-injuries. Expert Opin. Ther. Targets *21*, 627–638. https://doi.org/10.1080/14728222.2017.1321635.

31. Butterfield, D.A. (2021). Ubiquitin carboxyl-terminal hydrolase L-1 in brain: Focus on its oxidative/nitrosative modification and role in brains of subjects with Alzheimer disease and mild cognitive impairment. Free Radic. Biol. Med. *177*, 278–286. https://doi.org/10.1016/j.freeradbiomed.2021.10.036.

32. López-Avalos, M.D., Duvivier-Kali, V.F., Xu, G., Bonner-Weir, S., Sharma, A., and Weir, G.C. (2006). Evidence for a role of the ubiquitin-proteasome pathway in pancreatic islets. Diabetes *55*, 1223–1231. https://doi.org/10.2337/db05-0450.

33. Papa, L., Brophy, G.M., Welch, R.D., Lewis, L.M., Braga, C.F., Tan, C.N., Ameli, N.J., Lopez, M.A., Haeussler, C.A., Mendez Giordano, D.I., et al. (2016). Time Course and Diagnostic Accuracy of Glial and Neuronal Blood Biomarkers GFAP and UCH-L1 in a Large Cohort of Trauma Patients With and Without Mild Traumatic Brain Injury. JAMA Neurol. *73*, 551–560. https://doi.org/10.1001/jamaneurol.2016.0039.

34. Papa, L., Lewis, L.M., Silvestri, S., Falk, J.L., Giordano, P., Brophy, G.M., Demery, J.A., Liu, M.C., Mo, J., Akinyi, L., et al. (2012). Serum levels of ubiquitin C-terminal hydrolase distinguish mild traumatic brain injury from trauma controls and are elevated in mild and moderate traumatic brain injury patients with intracranial lesions and neurosurgical intervention. J. Trauma Acute Care Surg. *72*, 1335–1344. https://doi.org/10.1097/TA.0b013e3182491e3d.

35. Tabor, J.B., Penner, L.C., Galarneau, J.M., Josafatow, N., Cooper, J., Ghodsi, M., Huang, J., Fraser, D.D., Smirl, J., Esser, M.J., et al. (2024). Plasma Biomarkers of Traumatic Brain Injury in Adolescents With Sport-Related Concussion. JAMA Netw. Open *7*, e2431959. https://doi.org/10.1001/jamanetworkopen.2024.31959.

36. Li, Y., Sun, Y., Li, J., Wang, Z., Lin, Y., Tang, L., Xia, D., Zheng, T., Yang, X., Sha, L., and Sun, C.K. (2015). Changes of ubiquitin C-terminal hydrolase-L1 levels in serum and urine of patients with white matter lesions. J. Neurol. Sci. *357*, 215–221. https://doi.org/10.1016/j.jns.2015.07.033.

37. Ohrfelt, A., Johansson, P., Wallin, A., Andreasson, U., Zetterberg, H., Blennow, K., and Svensson, J. (2016). Increased Cerebrospinal Fluid Levels of Ubiquitin Carboxyl-Terminal Hydrolase L1 in Patients with Alzheimer's Disease. Dement. Geriatr. Cogn. Dis. Extra *6*, 283–294. https://doi.org/10.1159/000447239.

38. Korley, F.K., Jain, S., Sun, X., Puccio, A.M., Yue, J.K., Gardner, R.C., Wang, K.K.W., Okonkwo, D.O., Yuh, E.L., Mukherjee, P., et al. (2022). Prognostic value of day-of-injury plasma GFAP and UCH-L1 concentrations for predicting functional recovery after traumatic brain injury in patients from the US TRACK-TBI cohort: an observational cohort study. Lancet Neurol. *21*, 803–813. https://doi.org/10.1016/s1474-4422(22)00256-3.

39. Graff-Radford, J., Mielke, M.M., Hofrenning, E.I., Kouri, N., Lesnick, T.G., Moloney, C.M., Rabinstein, A., Cabrera-Rodriguez, J.N., Rothberg, D.M., Przybelski, S.A., et al. (2022). Association of plasma biomarkers of amyloid and neurodegeneration with cerebrovascular disease and Alzheimer's disease. Neurobiol. Aging *119*, 1–7. https://doi.org/10.1016/j.neurobiolaging.2022.07.006.

40. Brum, W.S., Ashton, N.J., Simrén, J., di Molfetta, G., Karikari, T.K., Benedet, A.L., Zimmer, E.R., Lantero-Rodriguez, J., Montoliu-Gaya, L., Jeromin, A., et al. (2024). Biological variation estimates of Alzheimer's disease plasma biomarkers in healthy individuals. Alzheimer's Dement. *2*, 1284–1297. https://doi.org/10.1002/alz.13518.

41. Benkert, P., Meier, S., Schaedelin, S., Manouchehrinia, A., Yaldizli, Ö., Maceski, A., Oechtering, J., Achtnichts, L., Conen, D., Derfuss, T., et al. (2022). Serum neurofilament light chain for individual prognostication of disease activity in people with multiple sclerosis: a retrospective modelling and validation study. Lancet Neurol. *21*, 246–257. https://doi.org/10.1016/s1474-4422(22)00009-6.

42. Zhang, M., Chen, M.Y., Wang, S.L., Ding, X.M., Yang, R., Li, J., and Jiang, G.H. (2022). Association of Ubiquitin C-Terminal Hydrolase-L1 (Uch-L1) serum levels with cognition and brain energy metabolism. Eur. Rev. Med. Pharmacol. Sci. *26*, 3656–3663. https://doi.org/10.26355/eurrev_202205_28861.

43. Naranjo, L., Sarto, J., Nos, C., Alcolea, D., Rodríguez-Baz, I., Navalpotro-Gómez, I., Fernández-Lebrero, A., Bertrán-Recasens, B., Erro, M.E., Pelayo-Negro, A.L., et al. (2025). Isolated CSF RT-QuIC positivity associates with a less aggressive disease course and decreased levels of neuronal/glial damage biomarkers in patients with sporadic Creutzfeldt-Jakob disease. J. Neurol. *272*, 198. https://doi.org/10.1007/s00415-024-12757-8.

44. Dong, L., Chang, Q., Ma, J., Liu, C., Guo, D., Li, X., Yang, D., Fan, Y., Liang, K., Li, D., and Gu, Q. (2023). Associations of blood UCH-L1 and NfL levels with cognitive dysfunction in Parkinson's disease patients. Neurosci. Lett. *804*, 137219. https://doi.org/10.1016/j.neulet.2023.137219.

45. Youssef, P., Hughes, L., Kim, W.S., Halliday, G.M., Lewis, S.J.G., Cooper, A., and Dzamko, N. (2023). Evaluation of plasma levels of NFL, GFAP, UCHL1 and tau as Parkinson's disease biomarkers using multiplexed single molecule counting. Sci. Rep. *13*, 5217. https://doi.org/10.1038/s41598-023-32480-0.

46. Liu, H., Povysheva, N., Rose, M.E., Mi, Z., Banton, J.S., Li, W., Chen, F., Reay, D.P., Barrionuevo, G., Zhang, F., and Graham, S.H. (2019). Role of UCHL1 in axonal injury and functional recovery after cerebral ischemia. Proc. Natl. Acad. Sci. USA *116*, 4643–4650. https://doi.org/10.1073/pnas.1821282116.

47. Smith, D.L., Pozueta, J., Gong, B., Arancio, O., and Shelanski, M. (2009). Reversal of long-term dendritic spine alterations in Alzheimer disease models. Proc. Natl. Acad. Sci. USA *106*, 16877–16882. https://doi.org/10.1073/pnas.0908706106.

48. Xie, M., Han, Y., Yu, Q., Wang, X., Wang, S., and Liao, X. (2016). UCH-L1 Inhibition Decreases the Microtubule-Binding Function of Tau Protein. J. Alzheimers Dis. *49*, 353–363. https://doi.org/10.3233/jad-150032.

49. Fu, Y., Zhao, D., Pan, B., Wang, J., Cui, Y., Shi, F., Wang, C., Yin, X., Zhou, X., and Yang, L. (2015). Proteomic Analysis of Protein Expression Throughout Disease Progression in a Mouse Model of Alzheimer's Disease. J. Alzheimers Dis. *47*, 915–926. https://doi.org/10.3233/jad-150312.

50. Kashon, M.L., Ross, G.W., O'Callaghan, J.P., Miller, D.B., Petrovitch, H., Burchfiel, C.M., Sharp, D.S., Markesbery, W.R., Davis, D.G., Hardman, J., et al. (2004). Associations of cortical astrogliosis with cognitive performance and dementia status. J Alzheimers Dis *6*, 595–604. https://doi.org/10.3233/jad-2004-6604.

51. Potter, H., Woodcock, J.H., Boyd, T.D., Coughlan, C.M., O'Shaughnessy, J.R., Borges, M.T., Thaker, A.A., Raj, B.A., Adamszuk, K., Scott, D., et al. (2021). Safety and efficacy of sargramostim (GM-CSF) in the treatment of Alzheimer's disease. Alzheimer's Dement. *7*, e12158. https://doi.org/10.1002/trc2.12158.

52. Araya, P., Kinning, K.T., Coughlan, C., Smith, K.P., Granrath, R.E., Enriquez-Estrada, B.A., Worek, K., Sullivan, K.D., Rachubinski, A.L., Wolter-Warmerdam, K., et al. (2022). IGF1 deficiency integrates stunted growth and neurodegeneration in Down syndrome. Cell Rep. *41*, 111883. https://doi.org/10.1016/j.celrep.2022.111883.

53. Dong, Y., Hou, T., Li, Y., Liu, R., Cong, L., Liu, K., Liu, C., Han, X., Ren, Y., Tang, S., et al. (2023). Plasma Amyloid-β, Total Tau, and Neurofilament Light Chain Across the Alzheimer's Disease Clinical Spectrum: A Population-Based Study. J. Alzheimers Dis. *96*, 845–858. https://doi.org/10.3233/jad-230932.

54. Boyd, T.D., Bennett, S.P., Mori, T., Governatori, N., Runfeldt, M., Norden, M., Padmanabhan, J., Neame, P., Wefes, I., Sanchez-Ramos, J., et al. (2010). GM-CSF upregulated in rheumatoid arthritis reverses cognitive impairment and amyloidosis in Alzheimer mice. J. Alzheimers Dis. *21*, 507–518. https://doi.org/10.3233/jad-2010-091471.

55. Ahmed, M.M., Wang, A.C.J., Elos, M., Chial, H.J., Sillau, S., Solano, D.A., Coughlan, C., Aghili, L., Anton, P., Markham, N., et al. (2022). The innate immune system stimulating cytokine GM-CSF improves learning/memory and interneuron and astrocyte brain pathology in Dp16 Down syndrome mice and improves learning/memory in wild-type mice. Neurobiol. Dis. *168*, 105694. https://doi.org/10.1016/j.nbd.2022.105694.

56. Kiyota, T., Machhi, J., Lu, Y., Dyavarshetty, B., Nemati, M., Yokoyama, I., Mosley, R.L., and Gendelman, H.E. (2018). Granulocyte-macrophage colony-stimulating factor neuroprotective activities in Alzheimer's disease mice. J. Neuroimmunol. *319*, 80–92. https://doi.org/10.1016/j.jneuroim.2018.03.009.

57. Castellano, J.M., Mosher, K.I., Abbey, R.J., McBride, A.A., James, M.L., Berdnik, D., Shen, J.C., Zou, B., Xie, X.S., Tingle, M., et al. (2017). Human umbilical cord plasma proteins revitalize hippocampal function in aged mice. Nature *544*, 488–492. https://doi.org/10.1038/nature22067.

58. Cohen, R.M., Rezai-Zadeh, K., Weitz, T.M., Rentsendorj, A., Gate, D., Spivak, I., Bholat, Y., Vasilevko, V., Glabe, C.G., Breunig, J.J., et al. (2013). A transgenic Alzheimer rat with plaques, tau pathology, behavioral impairment, oligomeric aβ, and frank neuronal loss. J. Neurosci. *33*, 6245–6256. https://doi.org/10.1523/jneurosci.3672-12.2013.

59. Rohn, T.T., and Head, E. (2008). Caspase activation in Alzheimer's disease: early to rise and late to bed. Rev. Neurosci. *19*, 383–393. https://doi.org/10.1515/revneuro.2008.19.6.383.

60. Phillips, K.A., Lopez, M., Bartling-John, E., Meredith, R., Buteau, A., Alvarez, A., and Ross, C.N. (2024). Serum biomarkers associated with aging and neurodegeneration in common marmosets (Callithrix jacchus). Neurosci. Lett. *819*, 137569. https://doi.org/10.1016/j.neulet.2023.137569.

61. Shen, X., Wang, C., Zhou, X., Zhou, W., Hornburg, D., Wu, S., and Snyder, M.P. (2024). Nonlinear dynamics of multi-omics profiles during human aging. Nat. Aging *4*, 1619–1634. https://doi.org/10.1038/s43587-024-00692-2.

62. Phillips, J.C. (2019). Why Aβ42 Is Much More Toxic than Aβ40. ACS Chem. Neurosci. *10*, 2843–2847. https://doi.org/10.1021/acschemneuro.9b00068.

63. Hicks, C., Dhiman, A., Barrymore, C., and Goswami, T. (2022). Traumatic Brain Injury Biomarkers, Simulations and Kinetics. Bioengineering (Basel) *9*, 612. https://doi.org/10.3390/bioengineering9110612.

64. Huang, X.J., Glushakova, O., Mondello, S., Van, K., Hayes, R.L., and Lyeth, B.G. (2015). Acute Temporal Profiles of Serum Levels of UCH-L1 and GFAP and Relationships to Neuronal and Astroglial Pathology following Traumatic Brain Injury in Rats. J. Neurotrauma *32*, 1179–1189. https://doi.org/10.1089/neu.2015.3873.

65. Arendt, T., Brückner, M.K., Mosch, B., and Lösche, A. (2010). Selective cell death of hyperploid neurons in Alzheimer's disease. Am. J. Pathol. *177*, 15–20. https://doi.org/10.2353/ajpath.2010.090955.

66. Potter, H., Chial, H.J., Caneus, J., Elos, M., Elder, N., Borysov, S., and Granic, A. (2019). Chromosome Instability and Mosaic Aneuploidy in Neurodegenerative and Neurodevelopmental Disorders. Front. Genet. *10*, 1092. https://doi.org/10.3389/fgene.2019.01092.

67. Zhang, P., Kishimoto, Y., Grammatikakis, I., Gottimukkala, K., Cutler, R.G., Zhang, S., Abdelmohsen, K., Bohr, V.A., Misra Sen, J., Gorospe, M., and Mattson, M.P. (2019). Senolytic therapy alleviates Aβ-associated oligodendrocyte progenitor cell senescence and cognitive deficits in an Alzheimer's disease model. Nat. Neurosci. *22*, 719–728. https://doi.org/10.1038/s41593-019-0372-9.

68. Potter, H., and Wisniewski, T. (2012). Apolipoprotein e: essential catalyst of the Alzheimer amyloid cascade. Int. J. Alzheimers Dis. *2012*, 489428. https://doi.org/10.1155/2012/489428.

69. Rogers, J.T., Leiter, L.M., McPhee, J., Cahill, C.M., Zhan, S.S., Potter, H., and Nilsson, L.N. (1999). Translation of the alzheimer amyloid precursor protein mRNA is up-regulated by interleukin-1 through 5'-untranslated region sequences. J. Biol. Chem. *274*, 6421–6431. https://doi.org/10.1074/jbc.274.10.6421.

70. Gage, F.H. (2019). Adult neurogenesis in mammals. Science *364*, 827–828. https://doi.org/10.1126/science.aav6885.

71. Kim, N.K., Choi, B.H., Huang, X., Snyder, B.J., Bukhari, S., Kong, T.H., Park, H., Park, H.C., Park, S.R., and Ha, Y. (2009). Granulocyte-macrophage colony-stimulating factor promotes survival of dopaminergic neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced murine Parkinson's disease model. Eur. J. Neurosci. *29*, 891–900. https://doi.org/10.1111/j.1460-9568.2009.06653.x.

72. Kelso, M.L., Elliott, B.R., Haverland, N.A., Mosley, R.L., and Gendelman, H.E. (2015). Granulocyte-macrophage colony stimulating factor exerts protective and immunomodulatory effects in cortical trauma. J. Neuroimmunol. *278*, 162–173. https://doi.org/10.1016/j.jneuroim.2014.11.002.

73. Kong, T., Choi, J.K., Park, H., Choi, B.H., Snyder, B.J., Bukhari, S., Kim, N.K., Huang, X., Park, S.R., Park, H.C., and Ha, Y. (2009). Reduction in programmed cell death and improvement in functional outcome of transient focal cerebral ischemia after administration of granulocyte-macrophage colony-stimulating factor in rats. Laboratory investigation. J. Neurosurg. *111*, 155–163. https://doi.org/10.3171/2008.12.JNS08172.

74. Schneider, U.C., Schilling, L., Schroeck, H., Nebe, C.T., Vajkoczy, P., and Woitzik, J. (2007). Granulocyte-macrophage colony-stimulating factor-induced vessel growth restores cerebral blood supply after bilateral carotid artery occlusion. Stroke *38*, 1320–1328. https://doi.org/10.1161/01.STR.0000259707.43496.71.

75. Jim, H.S., Boyd, T.D., Booth-Jones, M., Pidala, J., and Potter, H. (2012). Granulocyte Macrophage Colony Stimulating Factor Treatment is Associated with Improved Cognition in Cancer Patients. Brain Disord. Ther. *1*, 1000101. https://doi.org/10.4172/bdt.1000101.

76. Olson, K.E., Abdelmoaty, M.M., Namminga, K.L., Lu, Y., Obaro, H., Santamaria, P., Mosley, R.L., and Gendelman, H.E. (2023). An open-label multiyear study of sargramostim-treated Parkinson's disease patients examining drug safety, tolerability, and immune biomarkers from limited case numbers. Transl. Neurodegener. *12*, 26. https://doi.org/10.1186/s40035-023-00361-1.

77. Krieger, M., Both, M., Kranig, S.A., Pitzer, C., Klugmann, M., Vogt, G., Draguhn, A., and Schneider, A. (2012). The hematopoietic cytokine granulocyte-macrophage colony stimulating factor is important for cognitive functions. Sci. Rep. *2*, 697. https://doi.org/10.1038/srep00697.

78. Buschmann, I.R., Busch, H.J., Mies, G., and Hossmann, K.A. (2003). Therapeutic induction of arteriogenesis in hypoperfused rat brain via granulocyte-macrophage colony-stimulating factor. Circulation *108*, 610–615. https://doi.org/10.1161/01.CIR.0000074209.17561.99.

79. Choi, J.K., Kim, K.H., Park, H., Park, S.R., and Choi, B.H. (2011). Granulocyte macrophage-colony stimulating factor shows anti-apoptotic activity in neural progenitor cells via JAK/STAT5-Bcl-2 pathway. Apoptosis *16*, 127–134. https://doi.org/10.1007/s10495-010-0552-2.

80. Huang, X., Choi, J.K., Park, S.R., Ha, Y., Park, H., Yoon, S.H., Park, H.C., Park, J.O., and Choi, B.H. (2007). GM-CSF inhibits apoptosis of neural cells via regulating the expression of apoptosis-related proteins. Neurosci. Res. *58*, 50–57. https://doi.org/10.1016/j.neures.2007.01.015.

81. Krüger, C., Laage, R., Pitzer, C., Schäbitz, W.R., and Schneider, A. (2007). The hematopoietic factor GM-CSF (granulocyte-macrophage colony-stimulating factor) promotes neuronal differentiation of adult neural stem cells in vitro. BMC Neurosci. *8*, 88. https://doi.org/10.1186/1471-2202-8-88.

82. Altendahl, M., Cotter, D.L., Staffaroni, A.M., Wolf, A., Mumford, P., Cobigo, Y., Casaletto, K., Elahi, F., Ruoff, L., Javed, S., et al. (2020). REM sleep is associated with white matter integrity in cognitively healthy, older adults. PLoS One *15*, e0235395. https://doi.org/10.1371/journal.pone.0235395.

83. Wu, Y.C., Wen, Q., Thukral, R., Yang, H.C., Gill, J.M., Gao, S., Lane, K.A., Meier, T.B., Riggen, L.D., Harezlak, J., et al. (2023). Longitudinal Associations Between Blood Biomarkers and White Matter MRI in Sport-Related Concussion: A Study of the NCAA-DoD CARE Consortium. Neurology *101*, e189–e201. https://doi.org/10.1212/wnl.0000000000207389.

84. Boeras, D.I., Granic, A., Padmanabhan, J., Crespo, N.C., Rojiani, A.M., and Potter, H. (2008). Alzheimer's presenilin 1 causes chromosome missegregation and aneuploidy. Neurobiol. Aging *29*, 319–328. https://doi.org/10.1016/j.neurobiolaging.2006.10.027.

85. Borysov, S.I., Granic, A., Padmanabhan, J., Walczak, C.E., and Potter, H. (2011). Alzheimer Aβ disrupts the mitotic spindle and directly inhibits mitotic microtubule motors. Cell Cycle *10*, 1397–1410. https://doi.org/10.4161/cc.10.9.15478.

86. Caneus, J., Granic, A., Rademakers, R., Dickson, D.W., Coughlan, C.M., Chial, H.J., and Potter, H. (2018). Mitotic defects lead to neuronal aneuploidy and apoptosis in frontotemporal lobar degeneration caused by MAPT mutations. Mol. Biol. Cell *29*, 575–586. https://doi.org/10.1091/mbc.E17-01-0031.

87. Freund, R.K., Gibson, E.S., Potter, H., and Dell'Acqua, M.L. (2016). Inhibition of the Motor Protein Eg5/Kinesin-5 in Amyloid β-Mediated Impairment of Hippocampal Long-Term Potentiation and Dendritic Spine Loss. Mol. Pharmacol. *89*, 552–559. https://doi.org/10.1124/mol.115.103085.

88. Granic, A., Padmanabhan, J., Norden, M., and Potter, H. (2010). Alzheimer Abeta peptide induces chromosome mis-segregation and aneuploidy, including trisomy 21: requirement for tau and APP. Mol. Biol. Cell *21*, 511–520. https://doi.org/10.1091/mbc.e09-10-0850.

89. Granic, A., and Potter, H. (2013). Mitotic spindle defects and chromosome mis-segregation induced by LDL/cholesterol-implications for Niemann-Pick C1, Alzheimer's disease, and atherosclerosis. PLoS One *8*, e60718. https://doi.org/10.1371/journal.pone.0060718.

90. Lucero, E.M., Freund, R.K., Smith, A., Johnson, N.R., Dooling, B., Sullivan, E., Prikhodko, O., Ahmed, M.M., Bennett, D.A., Hohman, T.J., et al. (2022). Increased KIF11/kinesin-5 expression offsets Alzheimer Aβ-mediated toxicity and cognitive dysfunction. iScience *25*, 105288. https://doi.org/10.1016/j.isci.2022.105288.

91. Potter, H. (2016). Beyond Trisomy 21: Phenotypic Variability in People with Down Syndrome Explained by Further Chromosome Mis-segregation and Mosaic Aneuploidy. J. Down Syndr. Chromosom. Abnorm. *2*, 109. https://doi.org/10.4172/2472-1115.1000109.

92. Gorgoulis, V., Adams, P.D., Alimonti, A., Bennett, D.C., Bischof, O., Bishop, C., Campisi, J., Collado, M., Evangelou, K., Ferbeyre, G., et al. (2019). Cellular Senescence: Defining a Path Forward. Cell *179*, 813–827. https://doi.org/10.1016/j.cell.2019.10.005.

93. Herdy, J.R., Traxler, L., Agarwal, R.K., Karbacher, L., Schlachetzki, J.C.M., Boehnke, L., Zangwill, D., Galasko, D., Glass, C.K., Mertens, J., and Gage, F.H. (2022). Increased post-mitotic senescence in aged human neurons is a pathological feature of Alzheimer's disease. Cell Stem Cell *29*, 1637–1652.e6. https://doi.org/10.1016/j.stem.2022.11.010.

94. Chaib, S., Tchkonia, T., and Kirkland, J.L. (2022). Cellular senescence and senolytics: the path to the clinic. Nat. Med. *28*, 1556–1568. https://doi.org/10.1038/s41591-022-01923-y.

95. Mannix, R., Borglund, E., Monashefsky, A., Master, C., Corwin, D., Badawy, M., Thomas, D.G., and Reisner, A. (2024). Age-Dependent Differences in Blood Levels of Glial Fibrillary Acidic Protein but Not Ubiquitin Carboxy-Terminal Hydrolase L1 in Children. Neurology *103*, e209651. https://doi.org/10.1212/wnl.0000000000209651.

96. Wang, Y. (2011). Smoothing Splines: Methods and Applications (CRC Press: Taylor & Francis Group. A Chapman & Hall Book).

97. Czeiter, E., Amrein, K., Gravesteijn, B.Y., Lecky, F., Menon, D.K., Mondello, S., Newcombe, V.F.J., Richter, S., Steyerberg, E.W., Vyvere, T.V., et al. (2020). Blood biomarkers on admission in acute traumatic brain injury: Relations to severity, CT findings and care path in the CENTER-TBI study. EBioMedicine *56*, 102785. https://doi.org/10.1016/j.ebiom.2020.102785.

98. Bazarian, J.J., Biberthaler, P., Welch, R.D., Lewis, L.M., Barzo, P., Bogner-Flatz, V., Gunnar Brolinson, P., Büki, A., Chen, J.Y., Christenson, R.H., et al. (2018). Serum GFAP and UCH-L1 for prediction of absence of intracranial injuries on head CT (ALERT-TBI): a multicentre observational study. Lancet Neurol. *17*, 782–789. https://doi.org/10.1016/s1474-4422(18)30231-x.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Chicken anti rat-MAP2	Phosphosolutions	Cat # 1100-MAPT; RRID: AB_2492141
Rabbit anti-GFAP antibody	Abcam	Cat #7260; RRID: AB_305808
Rabbit anti-Caspase-3 antibody	Cell Signaling Technology	Cat # 9662; RRID: AB_331439
Biological samples		
Human plasma samples	This paper	Unpublished MS controls, published AD samples[22,51] and controls from Human Trisome Project[52]
Chemicals, peptides, and recombinant proteins		
Rat GM-CSF	Fuji film	Cat # 300-03
Critical commercial assays		
SIMOA®, SR-X Analyzer	Quanterix	SIMOA®, SR-X Analyzer Neuro-4-Plex B
Experimental models: Organisms/strains		
Transgenic rat model of AD	Terrence Town	TgF344-AD rat
Wild-type leukinelittermate rats	Terrence Town	F344 rat
Software and algorithms		
SAS 9.4 and R	**SAS® Academic Software**	SAS 9.4 and R

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Participants: All participants signed informed consent forms approved for each study by the Colorado multi-institutional review board (COMIRB numbers below)

We analyzed healthy control plasma samples from three different studies. Healthy control participants (n = 317) who were part of the Crnic Institute Human Trisome Project (HTP, n = 103; age range: 2–61 years; 54% female), the University of Colorado Alzheimer's and Cognition Center (CUACC) Bio-AD longitudinal observational study (n = 69; age range: 53–83; 70% female), or the multiple sclerosis (MS) healthy controls biomarker study (termed Nair) (n = 145; age range: 16–86; 64% female; 3 participants lacked usable UCH-L1 data). See demographics (including gender) and inclusion/exclusion criteria in Tables S4 and S5. Information on ancestry, race, and ethnicity were not collected unless as indicated in the demographics. The HTP is focused on studying biomarkers and clinical features of people with Down syndrome (DS) and includes typical control participants without DS, whose average biomarker values in three age groups for NfL, UCH-L1, and GFAP were published previously,[52] and the CUACC Bio-AD study is focused on studying the effects of inflammation on the development of AD.[22] The Nair MS biomarker study is investigating biomarkers associated with MS and includes healthy control participants. Together, these three healthy control cohorts span ages 2–85.

Using three community-dwelling healthy control cohorts that are diverse and heterogeneous with a wide range of ages adds confidence to the cross-sectional measures of the plasma biomarkers. If different populations had different levels and slopes of a marker with age, then pooling them would be averaging them and would cause a deviation away from linearity across the full age range, with parametric linear fit trying to smooth it out. However, there is no appreciable deviation from linearity for UCH-L1 or NfL when examined with the spline fits (Figure S1). GFAP measures across age are more complex, increasing exponentially only after age 40.

Participants assessed as having mild cognitive impairment (MCI) due to AD were part of the CUACC Bio-AD longitudinal observational study (n = 45) and were diagnosed based on an interdisciplinary consensus conference with review of cognitive testing, neurological examination, clinical dementia rating scale (CDR), and brain MRI. Participants with mild-to-moderate AD were from our published Phase II, double-blind, randomized, placebo-controlled trial[51] with recombinant human GM-CSF (the "sargramostim/GM-CSF AD trial"). We include available plasma samples taken at the baseline visit prior to treatment with sargramostim/GM-CSF or placebo (n = 36) as the cohort of AD individuals, as well as available plasma samples taken at the end of three weeks of treatment with either sargramostim/GM-CSF (n = 18) or placebo (n = 18) to provide information on the effect of GM-CSF treatment on the plasma levels of UCH-L1. Some plasma samples were unavailable for measurements of NfL and GFAP concentrations.

Sex/gender influence

Gender was determined by self-report or physician report. Gender was associated with different results in the age-associated biomarker curves with females showing a higher and more rapid increase with age of UCH-L1 and GFAP.

Sample size and randomization

Samples from all available participants from the three observational (HTP controls, n = 103); the MS study controls (n = 145), the BioAD (n = 69). Participants assessed as having mild cognitive impairment (MCI) due to AD were part of the CUACC Bio-AD longitudinal observational study (n = 45), and the one interventional (GM-CSF/sargramostim) study (n = 43) were included in the analysis. For the interventional (GM-CSF/sargramostim) study, participants were assigned random ID numbers by a random number generator and assigned to either drug or placebo treatment randomly as described.[51]

Human subjects institutional approval and oversight

All human subjects research was approved by the Colorado Multiple Institutional Review Board (COMIRB). Specifically, we analyzed healthy control plasma samples from three different studies. Healthy control participants (n = 317) who were part of the Crnic Institute Human Trisome Project (HTP, n = 103; age range: 2–61 years; 54% female) (COMIRB #15–2170; NCT02864108; Dr. Joaquin Espinosa), the University of Colorado Alzheimer's and Cognition Center (CUACC) Bio-AD longitudinal observational study (n = 69; age range: 53–83; 70% female) (COMIRB #15–1774; Dr. Brianne Bettcher), or the multiple sclerosis (MS) healthy controls biomarker study (termed Nair) (n = 145; age range: 16–86; 64% female; 3 participants lacked usable UCH-L1 data) (COMIRB #21–3703; Dr. Kavita Nair). The Colorado Multiple Institutional Review Board also approved the Pilot Phase 2 Trial of the Safety & Efficacy of GM-CSF (Leukine) in the Treatment of Alzheimer's Disease (COMIRB # 12–1273; NCT01409915; Dr. Huntington Potter) from which plasma biomarker data on AD participants before and after treatment with GM-CSF/sargramostim are reported here. The biomarker data reported on the participants with mild cognitive impairment were part of the University of Colorado Alzheimer's and Cognition Center BioAD project approved by the COMIRB (COMIRB #15–1774; Dr. Brianne Bettcher).

Animal model institutional permission and oversight

The TgF344-AD rat was developed as a model of AD by inserting transgenes that express the Swedish mutant human *APP* (*APPsw*) and mutant human presenilin 1 (*PSEN1 delta E9*) genes that cause familial AD.[58] As a result, and due to the fact that the rat MAPT (Tau) gene resembles the human version, the TgF344-AD rats overexpress human Aβ peptide and develop the full complement of human AD brain pathology: amyloid deposits, p-Tau-positive neurofibrillary tangles, and neuronal loss. Untreated age-matched wild-type (WT) F344 rats were used as controls. All rats were 18–20 months of age and only males were analyzed to reduce the effect of hormonal cycling.

All animal research was approved by the University of Colorado Institutional Animal Care and Use Committee (IACUC#00878).

METHOD DETAILS

Measurement of plasma biomarker concentrations

Concentrations of UCH-L1, GFAP, and NfL in plasma samples were assessed in healthy control participants and in participants with MCI due to AD using published methods,[22,51] specifically with the Quanterix single molecule array, or SIMOA, SR-X Analyzer system and the Neuro-4-Plex B kits. Concentrations of UCH-L1, GFAP, and NfL in the samples from mild-to-moderate AD participants in the sargramostim/GM-CSF AD trial were determined in our previously published manuscript using the same methods.[51]

The low pg sensitivity of the SIMOA platform is important for our studies. Plasma concentrations reported for UCH-L1 in the literature vary according to the exact population studied and the method of measurement used. For example, Papa and colleagues[34] used an in-house developed ELISA assay that shows ~83 pg/mL for all controls, which includes normal and 'trauma' controls that are twice as high. A data table is missing in that manuscript, but the graph looks like 70 pg/mL for normal controls, which corresponds to hospitalized patients without TBI rather than to the type of healthy community dwelling control participants that we used. The very best commercial ELISA assay has a lower detection limit of ~37 pg/mL, whereas the SIMOA platform is much more sensitive (LOD 1.9 pg/mL; LLOQ 9.36 pg/mL), which likely explains our lower measured levels.

Similarly, Mannix and colleagues[95] reported that children <18 years show UCH-L1 plasma concentrations of 150 pg/mL; they used the Alinity i TBI test from Abbott, which is designed to measure the highly elevated plasma concentrations of UCH-L1 that follows TBI and is not validated for measuring low levels in healthy controls. Again, the SIMOA platform is much more sensitive.

Rat AD model

The 18- to 20-month-old TgF344-AD male rats were injected subcutaneously with GM-CSF (83.3 μg/kg/day; 5 days/week; n = 7) or with saline (200 μL/day; 5 days/week; n = 7) for 24 injections total over 32 days. On day 32, the rats were anesthetized with sodium pentobarbital, perfused intracardially with PBS for 5–7 min, and the brains were removed rapidly. The right hemisphere was immersed in freshly prepared 4% paraformaldehyde (PFA) in PBS for 24 h at 4°C. After fixing with PFA, 4 μm-thick paraffin-embedded hippocampal brain sections were mounted on glass slides and processed for immunohistochemistry for the apoptosis marker Caspase-3 (Rabbit anti-Caspase-3 antibody; Cell Signaling Technology; Cat# 9662; dilution 1:100) or for GFAP (Rabbit anti-GFAP antibody; Abcam, MA, USA; Cat#7260; dilution 1:200) and co-stained with DAPI. A detailed protocol for immunohistochemistry and imaging is described in.[55] We assessed total GFAP staining by ImageJ analysis and visually counted the numbers of Caspase-3-positive cells in the CA1, CA3, and dentate gyrus/hilus regions of the hippocampus (blinded as to genotype and treatment). Specifically, for counting the Caspase-3-positive cells in hippocampal subregions, we used hippocampal sections with similar

septo-temporal areas to compare between groups. Therefore, when we examined the Caspase-3-positive cells in the hippocampus, all of our images were taken from similar regions for all rats. We repeated 2-6 sections/rat, counted Caspase-3-positive cells from each section, and then the mean of these repeated values was used as one value for each rat and used for statistical analysis considering with the number of technical replicates (section/rat). For determining the proportion of Caspase-3-positive cells that were neurons, co-staining for MAPT was performed (Chicken anti-MAP2 antibody; PhosphoSolutions, CO, USA; Cat# 1100-MAP2; dilution 1:500) and the coded slides were examined visually. Specifically, two random aged WT mice were selected for this analysis, shown below.

Caspase-3-positive cells that were also MAPT2-positive:
CA1 = 95.48% and 93.39%
CA3 = 92.25%
DG = 94.20% and 91.86%

QUANTIFICATION AND STATISTICAL ANALYSES

Data were analyzed using mixed model regression, with unstructured error covariance on repeated measures, for the effects of biomarkers, disease status, and treatment on the logarithmic transforms of the plasma biomarkers of UCH-L1, GFAP, and NfL. Although data from healthy control participants and participants with MCI due to AD were cross-sectional, the framework of mixed model regression could still be adapted, as regression with independent data is merely a simplified version of regression with correlated data. Healthy control, MCI due to AD, mild-to-moderate AD at baseline, mild-to-moderate AD treated with placebo/saline, and mild-to-moderate AD treated with sargramostim/GM-CSF were allowed different covariance matrices. Because the biomarker data was measured using 2 or 3 replicates, all of the observations were used, instead of averaging replicates and weighting. The age effects were modeled as log-linear, with separate age slopes and intercepts for healthy control, MCI due to AD, and mild-to-moderate AD. The intercept for mild-to-moderate AD depended on the treatment x study time, but a common age slope was assumed for all mild-to-moderate AD participants, and independent of treatment effects. The log linearity assumption was checked graphically, and by comparing to spline fits.[96] It was apparent that log linearity was a reasonable fit for the UCH-L1 and NfL data, but not for the GFAP data, which was subjected to spine analysis. Piece-wise log-linear regression models were considered if deviation from pure log-linearity was judged important. Interactions with gender were also considered. Linear combinations of model parameters were estimated, along with 95% confidence intervals, back transformed, and tested. Histograms of the Coefficient of Variation among replicates found some biomarker measures obtained with the SIMOA, SR-X Analyzer system, particularly UCH-L1, for example, can have Coefficients of Variance higher than 20%, as has also been found in UCH-L1 assessments after TBI using various assays, indicating that the variance is an inherent feature of the measure and not due, for example, to unreliable outliers. For example, a histogram of the UCH-L1 coefficients of variance of the healthy controls is shown in supplemental information, Figure S3.

There are known limitations to using biomarkers with high CVs in analyses, with most studies utilizing less than 20% cutoffs at the higher end. The primary concern is that, for example, if analysis of the same sample only twice yields two markedly different values, there are understandable concerns for reliability of measurement (and clinical applicability for an individual). Variability of such measures limits their utility for assessing individuals from a small number of replicates, but the laws of large numbers still allow us to estimate the average with a medium to large sample, when the purpose is to assess an overall effect of age or intervention. For cross-sectional comparisons between large cohorts, the average or log average of all individual participant's replicate measures is often used, as we have done here, rather than first averaging an individual's few measurements. Measures of UCH-L1 often show high variance,[33,38,63,97] but UCH-L1 levels were FDA approved in 2018 as part of a measure of brain damage after TBI.[98] Because some of our cohorts had two replicates for each participant (healthy controls and participants with MCI due to AD from the Bio-AD study) and some (healthy controls from the HTP, sargramostim/GM-CSF AD trial participants with mild-to-moderate AD, and healthy controls from the MS study) had mostly three replicates for each participant, and some of the biomarkers, especially UCH-L1, have high variability among replicates, we decided in the modeling to use each replicate as a separate measure in the main analysis, rather than using the averages of the individual participants' replicate measures. The variation among replicates was modeled by introducing an additional noise term into the model. A common variance for the replicate noise term was assumed across all treatments because of software option limitations. Model predicted values of the response, along with pointwise standard errors, were plotted. All data calculations are provided in Tables S6, S7, S8, S9 and S10. As a test of confidence in the results, the averages of the participants' replicate measures for the biomarkers were also modeled, weighted by the number of replicates, and the conclusions of age-associated exponential increases in the three biomarkers, UCH-L1, NfL, and GFAP, and the effect of GM-CSF treatment on reducing UCH-L1 levels were obtained. The overall results and conclusions were the same. Univariate statistical significance was set at alpha = 0.05, two-sided, for all tests unless otherwise stated. Statistics were computed using SAS 9.4 and R 4.1.3. There are remaining limitations to using this approach in terms of both within-site and cross-site replication of findings, and we acknowledge that it is challenging to move a biomarker into clinical practice for AD with high CVs, although it has been done for UCH-L1 in TBI, and multiple replicate measurements will provide higher confidence that the average is a valid measure of the biomarker plasma concentration in an individual.

The number of Caspase-3-positive cells in the TgF344-AD rat model were analyzed separately for each region using negative binomial count models with a log link, robust standard error method, and weighting by the number of technical replicates. Score tests

were used for *p* values. A protective omnibus test for group differences was performed, followed by pairwise contrasts. The protective omnibus method for controlling multiple testing permits the pairwise contrasts to be performed univariately, provided that the omnibus test is statistically significant, which it is for each hippocampal subregion. To avoid the potential distorting effect of assessing cell numbers by the area, in this study, we used similar septo-temporal axes of brain sections and compared counted cell numbers between groups. Because individual cells are counted in several sections across similar regions of the whole of the tissue regions of interest and not per square millimeter, atrophy is not the problem that would arise from total tissue expression measures by immunohistochemistry, which might better be adjusted by using stereology.

The statistical analyses for GFAP protein expression were performed by using a negative binomial count model with a log link, robust standard error method, and weighting by the number of technical replicates (Figure 6). Score tests were used for *p* values. An omnibus test for group differences was performed, followed by pairwise contrasts. The protective omnibus method for controlling multiple testing permits the pairwise contrasts to be performed univariately, provided the omnibus test is statistically significant, which is for each hippocampal subregion.

The ability of the biomarkers to discriminate between health controls, MCI, and AD was investigated fitting logistic regression models, with logarithmically transformed biomarkers and age as explanatory variables, and the group as the outcome. For each model, subsamples were used to impose a common age range for the groups. Explanatory power was assessed with receiver operating characteristic (ROC) curves, showing the tradeoff between sensitivity and specificity, and the area under the curve.

ADDITIONAL RESOURCES

Pilot Phase 2 Trial of the Safety & Efficacy of GM-CSF (Leukine) in the Treatment of Alzheimer's Disease (COMIRB # 12–1273; NCT01409915; https://www.clinicaltrials.gov/study/NCT01409915).

Crnic Institute Human Trisome Project (COMIRB #15–2170; NCT02864108; https://clinicaltrials.gov/expert-search?term=NCT02864108).

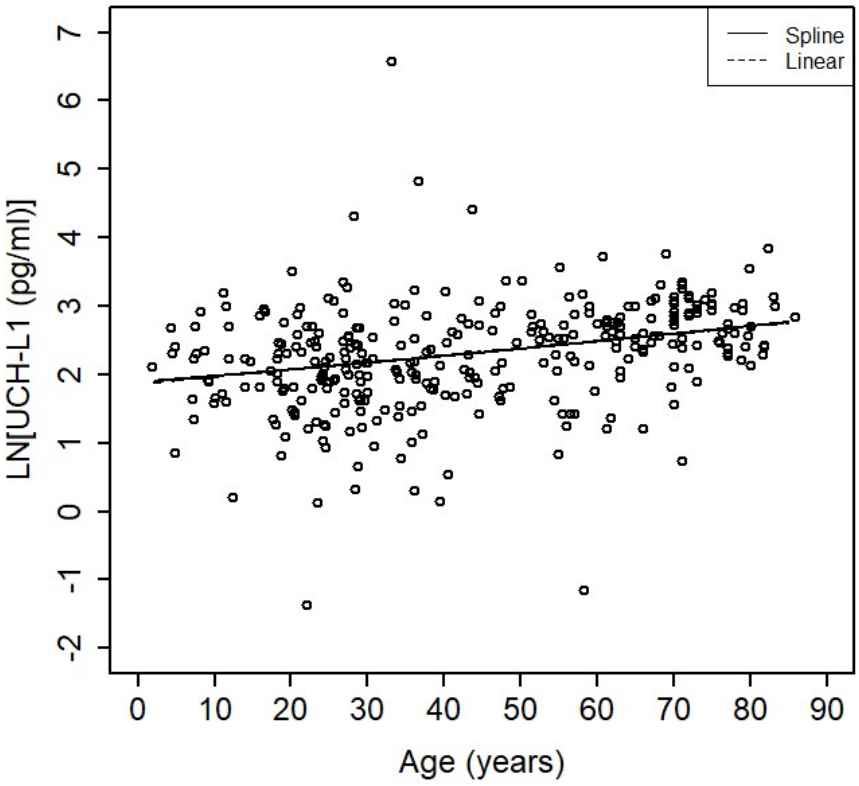
Cell Reports Medicine, Volume *7*

Supplemental information

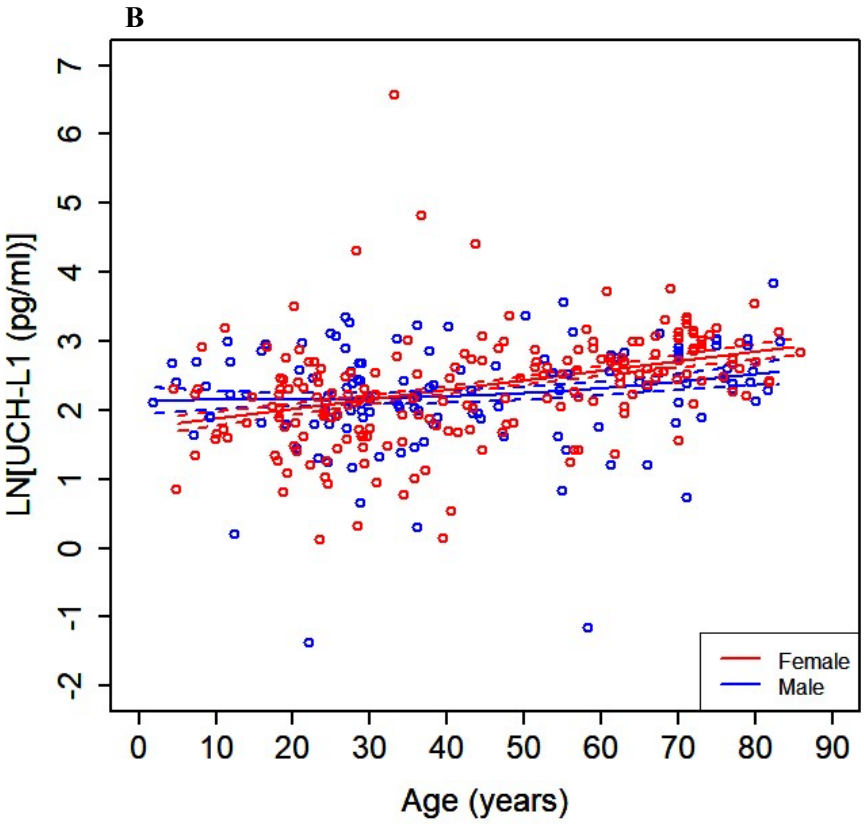
Blood measure of neuronal death is exponentially higher with age, especially in females, and halted in Alzheimer's disease by GM-CSF treatment

Stefan H. Sillau, Christina Coughlan, Md. Mahiuddin Ahmed, Kavita Nair, Paula Araya, Matthew D. Galbraith, Alanna Ritchie, Athena Ching-Jung Wang, Mihret T. Elos, Brianne M. Bettcher, Joaquin M. Espinosa, Heidi J. Chial, Neill Epperson, Timothy D. Boyd, and Huntington Potter

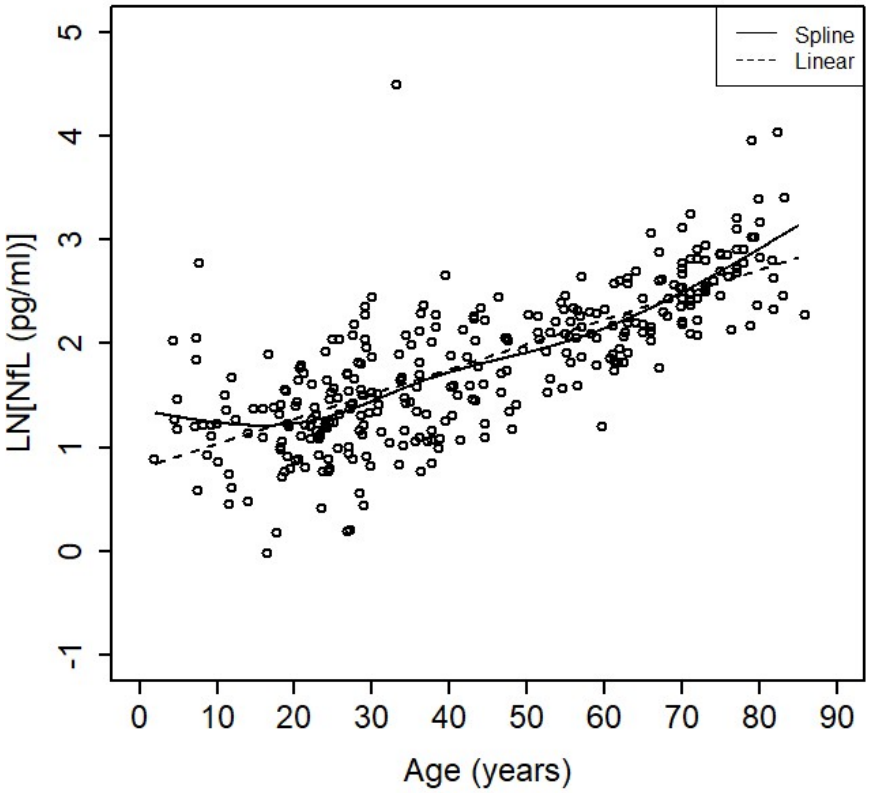
A


Spline
Linear
LN[UCH-L1 (pg/ml)]
-2
-1
0
1
2
3
4
5
6
7
0
10
20
30
40
50
60
70
80
90
Age (years)

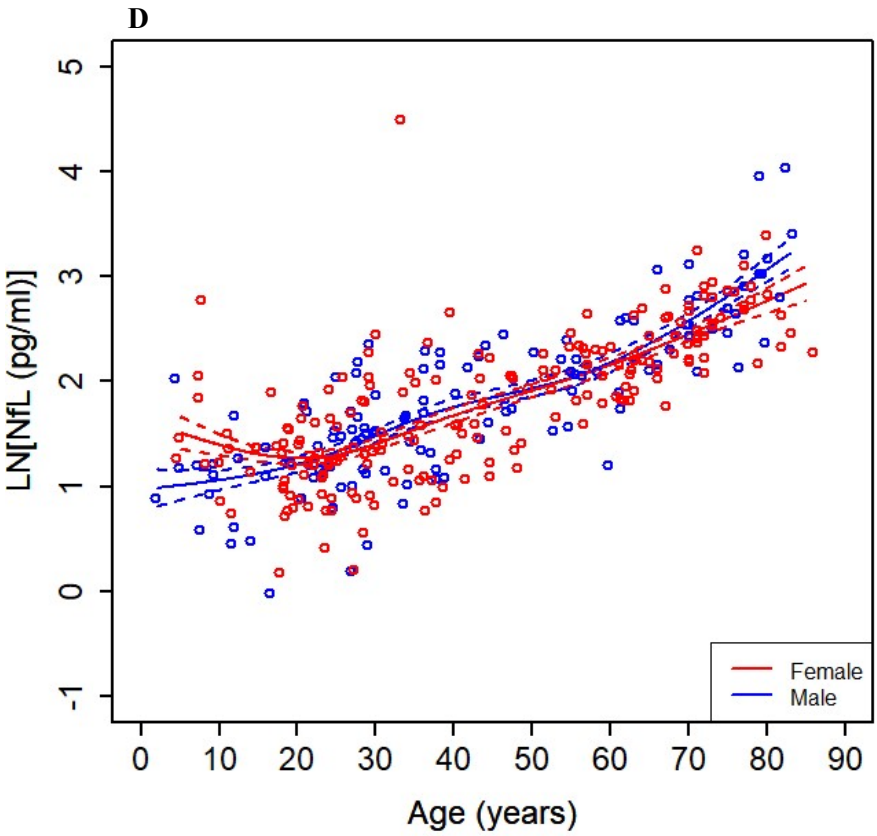
B


Female
Male
LN[UCH-L1 (pg/ml)]
-2
-1
0
1
2
3
4
5
6
7
0
10
20
30
40
50
60
70
80
90
Age (years)

C


Spline
Linear
LN[NfL (pg/ml)]
Age (years)

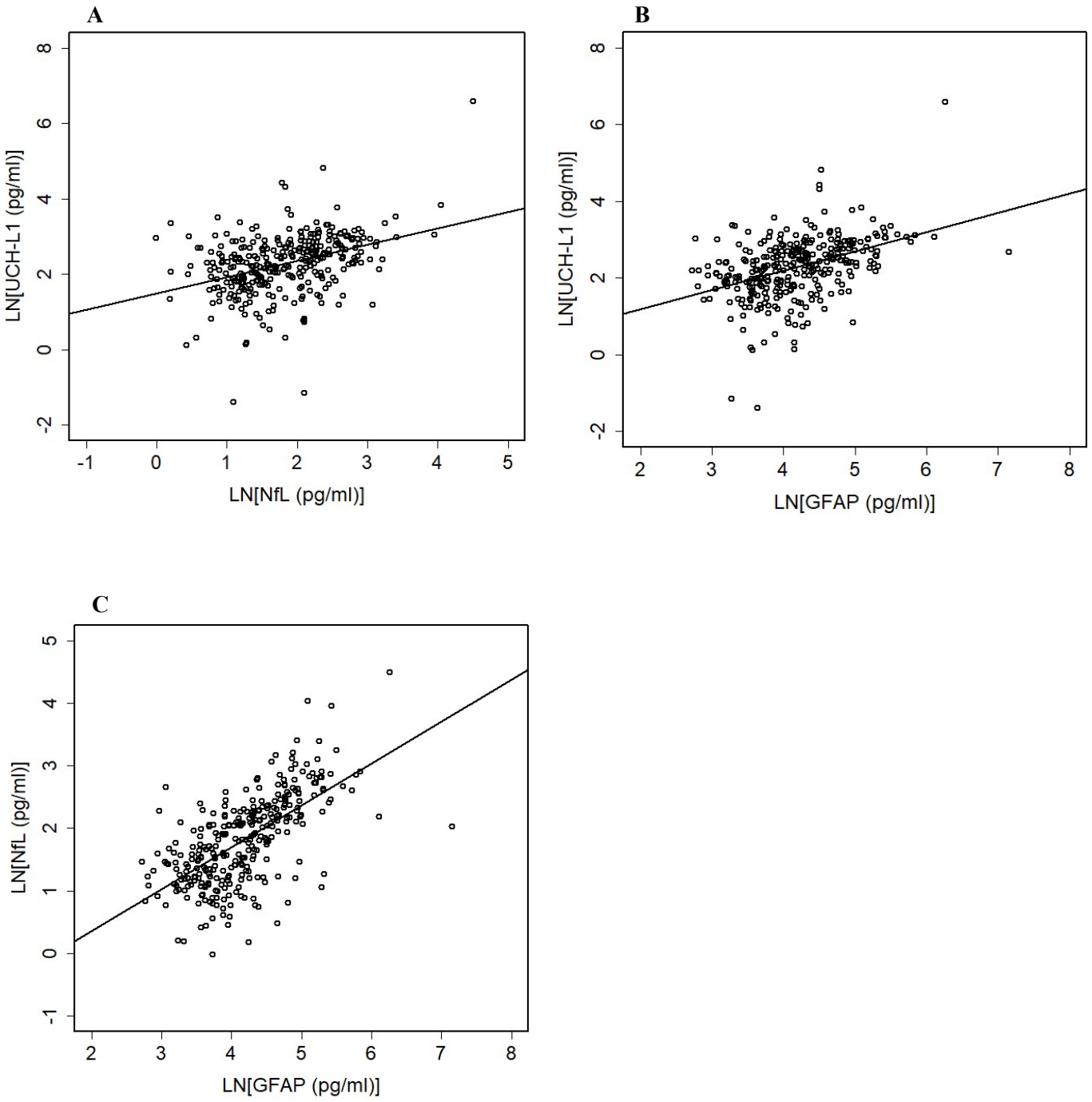
D


Female
Male
LN[NfL (pg/ml)]
Age (years)

Supplementary Figure S1 (related to Figures 1-3). Comparing log linear fit to splines for biomarker association with age and sex in healthy controls (replicates averaged) for:

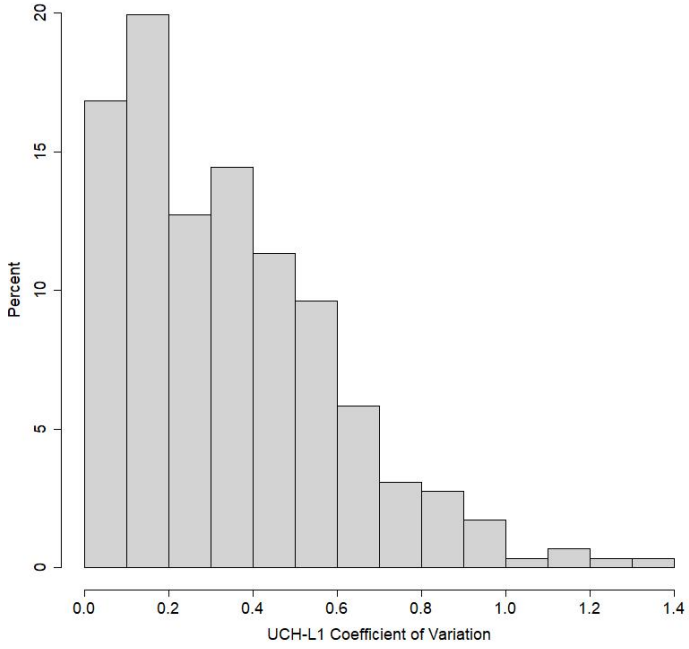
UCH-L1 (A): Deviance test for comparing spline model to the null hypothesis of linear: p value = 0.199. There is no statistical evidence against linearity even with a large sample. **(B):** Spine plot by sex showing no significant deviation from linearity in either males or females.

NfL (C): Deviance test for comparing spline model to the null hypothesis of linear: p value = 0.000125. Evidence against perfect linearity with a large sample, with the graph suggesting that the rate of increase accerates somewhat with older age, but linear is still a reasonable approximation. **(D):** Spine plot by sex showing similar deviation from linearity in males and females.

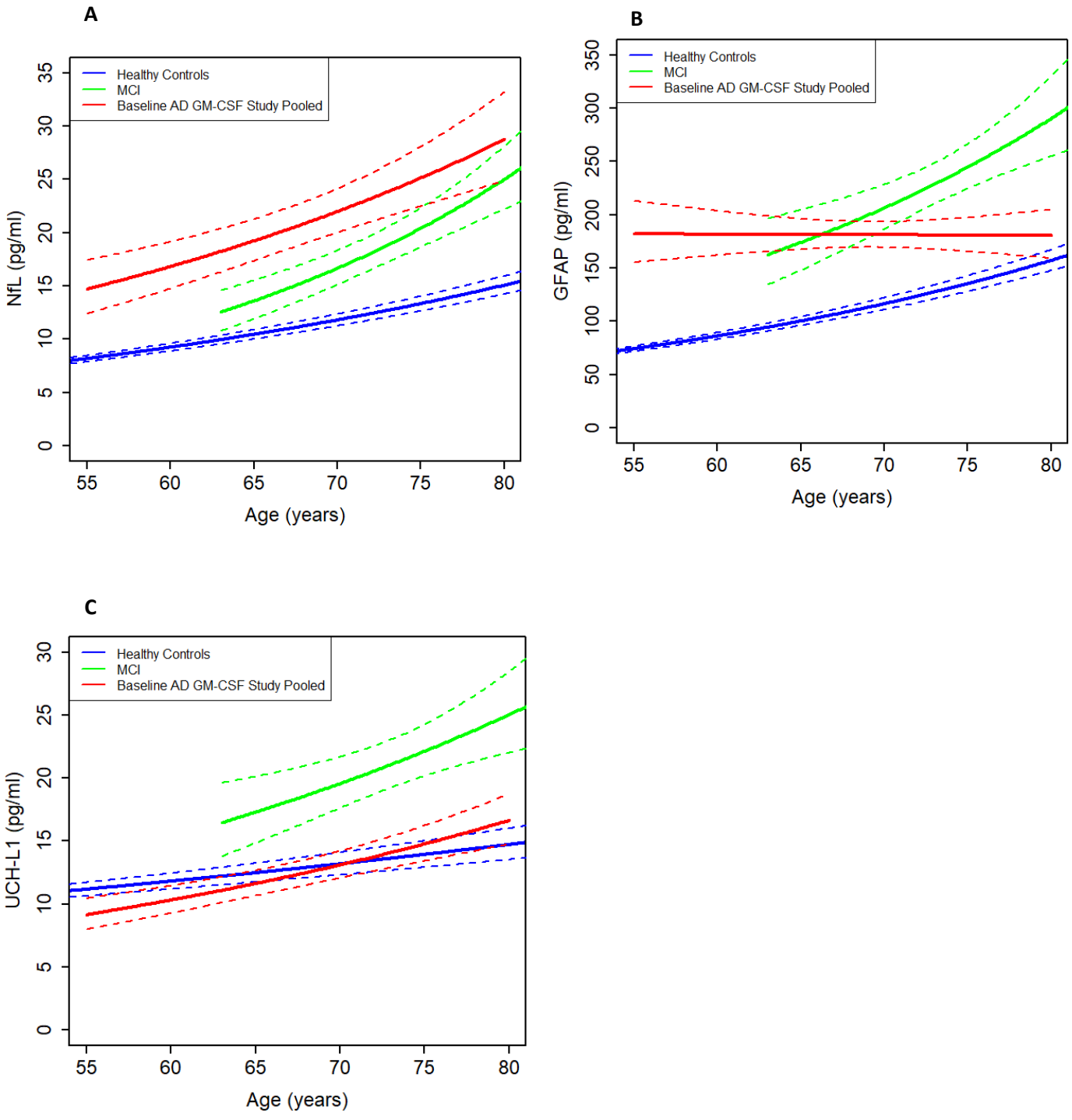

A
LN[UCH-L1 (pg/ml)]
LN[NfL (pg/ml)]
B
LN[UCH-L1 (pg/ml)]
LN[GFAP (pg/ml)]
C
LN[NfL (pg/ml)]
LN[GFAP (pg/ml)]

Supplementary Figure S2 (related to Figures 1-3: Correlations between plasma biomarkers of neurodegeneration and astrogliosis, UCH-L1, NfL, and GFAP).

Log transformed replicate measures of UCH-L1, NfL, and GFAP from all healthy control participants, age 2-85, were averaged over replicates and subjected to Pearson and Spearman correlation analyses and found to be highly correlated with each other. (**A**) UCH-L1:NfL, PC=0.37951, 95% CI: (0.280616, 0.470436), P = 3.157 * 10^(-12); (**B**) UCH-L1:GFAP, PC=0.42263, 95% CI: (0.327250, 0.509477), P = 4.441 * 10^(-15); and (**C**) NfL:GFAP PC=0.63743, 95% CI: (0.567089, 0.698536), P < 2.220 * 10^(-16).



Supplementary Figure S3 (related to Figures 1-3 and STAR Methods): Plot of Coefficients of Variance of UCH-L1 Measures for Normal Human Control Participants. UCH-L1 CVs from Table S5 are plotted as a histogram to indicate that the variance is an inherent feature of the measure determined by SIMOA and not due, for example, to unreliable outliers.


A
Healthy Controls
MCI
Baseline AD GM-CSF Study Pooled
NfL (pg/ml)
0
5
10
15
20
25
30
35
55
60
65
70
75
80
Age (years)
B
Healthy Controls
MCI
Baseline AD GM-CSF Study Pooled
GFAP (pg/ml)
0
50
100
150
200
250
300
350
55
60
65
70
75
80
Age (years)
C
Healthy Controls
MCI
Baseline AD GM-CSF Study Pooled
UCH-L1 (pg/ml)
0
5
10
15
20
25
30
55
60
65
70
75
80
Age (years)

Supplementary Figure S4; related to Figures 1-3. Comparisons between plasma NfL, GFAP, and UCH-L1 concentrations and age in participants with MCI due to AD, participants with mild-to-moderate AD, and healthy control participants.

We compared the concentrations of NfL, GFAP, and UCH-L1 in plasma samples from participants assessed as having MCI due to AD from the CUACC Bio-AD longitudinal observational study (n=32) (MCI), and 36 participants from the sargramostim/GM-CSF AD trial at baseline (before any placebo or GM-CSF intervention, shown as "Baseline AD GM-CSF Study Pooled"), and the corresponding segment of the Figure 1 age curve from the 317 healthy control participants. The mean age for the MCI participants was 73.7 years. (**A**) Plasma NfL concentrations in participants with MCI or mild-to-moderate AD are higher, correlating with disease progression, than in age-matched healthy control participants and also show an increase with age (MCI geometric mean estimate 19.33 pg/ml, 95% CI: (16.84, 22.19) at age 73.7; mild-to-moderate AD geometric mean estimate 24.25 pg/ml, 95% CI: (19.74, 29.80) at age 73.7 and baseline calibrated) compared to age-matched healthy control participants (geometric mean estimate 12.92 pg/ml, 95% CI: (11.81, 14.13); MCI/HC ratio estimate=1.497, 95% CI: (1.272, 1.762), $p=5.923x10^{-6}$; AD/HC ratio estimate=1.878, 95% CI: (1.501, 2.348), $p=6.718x10^{-7}$). NfL was marginally statistically non-significantly higher in AD than in MCI at 73.7 years (ratio estimate=1.254, 95% CI: (0.983, 1.601), p=0.0680). The estimated exponential change in plasma NfL for MCI was 4.138% per year (95% CI: (1.664%, 6.672%), p=0.0017) and for mild-to-moderate AD was 2.714% per year (95% CI: (0.625%, 4.847%), p=0.0122). The differences between the MCI or mild-to-moderate AD rate of change and healthy control rate of change were not statistically significant for NfL, but the power of the comparison test is limited because of the small sample size of the mild-to-moderate AD group (interaction test p value=0.3953). (**B**) Participants with MCI or mild-to-moderate AD exhibit a higher concentration of plasma GFAP (MCI: geometric mean estimate=233.86 pg/ml, 95% CI: (194.22, 281.60); AD: geometric mean estimate=180.95 pg/ml, 95% CI: (151.51, 216.12)) at the MCI mean age of 73,7 and baseline calibrated) compared to age-matched healthy control participants (HC: geometric mean estimate=130.08 pg/ml, 95% CI: (117.62, 143.86); MCI/HC ratio estimate=1.798, 95% CI: (1.458, 2.217), $p=7.816x10^{-7}$; AD/HC ratio estimate=1.391, 95% CI: (1.136, 1.704), p=0.0018)). GFAP levels for mild-to-moderate AD were statistically significantly lower than for MCI at 73.7 years (AD/MCI ratio estimate=0.774, 95% CI: (0.601, 0.996), p=0.0464)). The levels for the MCI participants increase with age (estimated change per year=3.466%, 95% CI: (0.170%, 6.871%), p=0.0398), but the levels do not change for the mild-to-moderate AD participants (estimated change per year=-0.030%, 95% CI: (-2.061%, 2.042%), p=0.9761). The test for a difference in the rate of change between healthy controls and mild-to-moderate AD was statistically significant (p=0.0056), as was the omnibus test for differences in the rate of change among the three groups for ages greater than or equal to 30 years (p=0.0200). (**C**) The mean of plasma UCH-L1 concentrations in participants with MCI due to AD is statistically significantly higher (MCI: geometric mean estimate=21.41 pg/ml, 95% CI: (18.11, 25.31); AD: geometric mean estimate=14.30 pg/ml, 95% CI: (12.20, 16.77); MCI/AD ratio estimate=1.497, 95% CI: (1.193, 1.878), p=0.0007), at mean age of 73.7 years than for healthy control participants, indicating the initiation of neurodegeneration beyond that caused by normal aging alone. Notably, the mean plasma concentrations of UCH-L1 at baseline for mild-to-moderate AD participants in the sargramostim/GM-CSF AD trial are similar to the concentrations in the healthy control participants at the corresponding age (HC: geometric mean estimate=13.73 pg/ml, 95% CI: (11.85, 15.91); AD/HC ratio estimate=1.042, 95%: (0.840, 1.292), p=0.7072; MCI/HC ratio estimate=1.559, 95% CI: (1.251, 1.944), p=0.0001), possibly because neuronal loss has reduced the numbers of neurons available to release UCH-L1, but the increase with age remains statistically significant (estimated change per year=2.420%, 95% CI: (0.783%, 4.083%), p=0.0054). The estimated slopes of the association between plasma UCH-L1 concentrations and age in the participants with MCI due to AD (estimated change per year=2.499%, 95% CI: (-0.446%, 5.531%), p=0.0941) or with mild-to-moderate AD are between 2 to 2.5, which is the slope of the correlation between plasma UCH-L1 concentrations and age in the healthy control participants in **Figure 1** (estimated change per year=1.110%, 95% CI: (0.716%, 1.505%), $p=5.504x10^{-8}$), but the differences are not statistically significant, most likely due to the small numbers of participants in the MCI due to AD group and in the mild-to-moderate AD group (interaction test p value=0.2040).

Principal Investigator: Huntington Potter, PhD
COMIRB No: 12-1273
Version Date: July 29, 2019

Study Title: *Pilot Phase 2 Trial of the Safety & Efficacy of GM-CSF (Leukine®) in the Treatment of Alzheimer's Disease*

Some people in this study may have a medical condition or a disability that does not allow them to make important decisions for themselves. If you have been asked to decide for someone else whether they should be in this study, please read this consent form carefully. In this form, we use the words "you" and "your." If you are reading this form and deciding for someone else, the words 'you' and 'your' refer to that other person, not to you.

You are being asked to be in a research study because you have been diagnosed with mild-to-moderate Alzheimer's disease. This form provides you with information about the study. A member of the research team will describe this study to you and answer all of your questions. Please read the information below and ask questions about anything you don't understand before deciding whether or not to take part.

Why is this study being done?

Alzheimer's disease is a major medical problem in the elderly, affecting 12% of those over age 65 and 40-50% of those over age 85. Current treatments offer minor benefits in slowing the development of memory problems but do not stop or reverse the damage from the disease. The purpose of this study is to determine if injections of a medicine called Leukine®, the study drug, in people with Alzheimer's disease is safe and effective in improving cognitive function and memory.

The study drug, Leukine®, is approved by the FDA (Food and Drug Administration) to help stimulate bone marrow in patients with low numbers of white blood cells or to help recipients or donors of bone marrow transplants. However, researchers think that the study drug potentially has other, "off label" uses, particularly in Alzheimer's disease patients. Studies in mice with Alzheimer's disease have shown that the study drug improves their memory and although we do not know whether this means that the study drug will improve memory in human Alzheimer's disease patients, an initial study in human cancer patients has also shown the study drug to improve memory.

The purpose of this study is to make sure that the study drug is safe for use with Alzheimer's patients and to see whether it improves their memory.

Other people in this study?

Up to *75* people from your area will be in the study.

What happens if I join this study?

If you join the study, you will help researchers determine if the study drug if safe to use in adults with mild-to-moderate Alzheimer's disease and if it has an effect on memory improvement. You will need to have someone accompany you to the study visits.

Initial Screening: at this visit, we will review with you the reasons for the study, the nature of the study treatment and the study procedures, the number of visits involved, the risks and the possible benefits to your participation. You will have an opportunity to read this consent form and have your questions answered after which you can either sign this form, or decide not to participate in the study.

If you have signed this form, we will administer a short test of memory called the mini mental state exam (MMSE).

Depending on your result, you may or may not qualify to be in this study. If you do qualify, we will proceed with the screening process. This includes recording your medical history, your medications, your blood pressure, pulse and weight and performing a heart tracing (ECG) and a physical exam. We will draw one tablespoon of your blood to determine your blood count and the health of your liver and kidneys.

We will then schedule you for an MRI of the brain (this is a scan of your brain).

Screening PET Scan

Once the results of the above are available and if you qualify, we will schedule you for a Positron Emission Tomography (PET) scan. The purpose of this scan is to measure the amount of amyloid in the brain. Amyloid is a protein found in the brain of patients with Alzheimer's disease.

The PET visit will last about 2 hours. You will be asked not to eat any food or drink anything (except for small sips of water) for 4 hours before the study visit. You will lie down on the scanner bed for the scan. Before the PET scan, an intravenous catheter (IV) will be placed in your arm or hand. This IV is placed so that the radiopharmaceutical (the imaging agent that binds to the amyloid protein) can be given for the scan. A skinny needle is used to guide a small skinny flexible plastic catheter into a vein. The needle is then removed and the catheter is temporarily left in the vein. The radiopharmaceutical will be injected into your vein using the IV. After the radiopharmaceutical has been injected, you will wait about 40 minutes before the scan begins The first part of the PET scan will be a quick (< 2 minute) computed tomography scan (CT scan, CAT scan) of your brain. This CT scan is done to help set up the PET scanner and to help with interpretation of the images of your brain obtained from the PET scan. It is important to not move your head position between the CT scan and the PET scan.

You will continue to lay on the scanner bed quietly without sleeping for the PET scan portion of the exam. The scan will last approximately 20 minutes. During the entire scan, you will be able to communicate with the scanner staff at all times. You can ask to end the scan at any time. The staff will try to make you as comfortable as possible by providing padding, pillows, and blankets. After

the PET scan, you are encouraged to drink water to urinate and help remove the imaging agent from your body.

When the results of the PET scan are available, a study doctor will contact you to discuss the results.

If you qualify for the study based on the PET scan and all other screening procedures, you will be eligible to return for the study visits.

The initial screening PET scan will take approximately 2 hours to complete.

Study visits: these visits can begin as soon as one week after the initial screen visit or as long as four weeks later. The total number of study visits is 15 with a total of 9 separate blood draws.

Study visit 1: at this visit we will:

- Record your vital signs (blood pressure, pulse and weight)
- Review your medications and changes in your health since your prior visit.
- Perform several paper and pencil tests of your cognition (brain function).
- We will draw a tablespoon of blood for a blood count and to measure proteins (biomarkers) that are associated with Alzheimer's disease.
- This study will have two different groups of research subjects like you. You will be assigned to receive either the study medication (Leukine), or a placebo. A placebo is a pill or a liquid that looks like medicine but is not real. It will have no medical effect on you. To decide which group you will be in, we will use a method of chance. This method is like flipping a coin or rolling dice. You will not know which treatment group you are in. Neither will your study doctor. This information needs to be kept secret so that the study is based on scientific results, not on peoples' opinions. However, we can give this information out if you have an emergency. If you are in an emergency, make sure you tell the emergency staff about this study. They can contact us, and we will give them all relevant information.
- The study nurse or clinician will administer the study drug or the placebo under the skin. Depending on your weight, this may be given as 1 or 2 injections

Study visits 2 to 14: at these visits, we will ask you about any new medications you may have taken or any new problems you may have experienced since your last injection. You will receive the study drug injection(s) five times a week and twice a week we will take a tablespoon of blood to measure your blood count. We will also take your vital signs during these visits.

Study visit 15*:* at this visit, we will ask you about any new medications or problems since your last injection. We will record your blood pressure and pulse and you will have a physical exam, and the paper and pencil tests of your brain function. We will obtain a tablespoon of your blood to determine your blood count, the function of your liver and kidneys, and protein (biomarker) levels. You will then receive your final injection of the study drug and then we will perform an MRI scan.

Follow up visits:

Follow up visit 1*:* is approximately 6-weeks/45 days after your last study injection. At this visit, we will review what medications you are on and record any problems that you may have had. We will record your blood pressure and pulse rate, perform a physical exam and you will have the paper and pencil tests of your brain function. We will obtain a tablespoon of your blood to determine your blood count, the function of your liver and kidneys, and protein (biomarker) levels.

You will also be scheduled for a final MRI scan and a PET scan within a week of this follow-up visit.

Follow up visit 2: this visit is approximately 6-weeks/45 days after the first follow up visit (e.g. 3 months after the end of the treatment phase). At this visit, we will review what medications you are on and record any problems that you may have had. We will record your blood pressure and pulse rate, perform a physical exam and you will have the paper and pencil tests of your brain function. We will obtain a tablespoon of your blood to determine your blood count, the function of your liver and kidneys, and protein (biomarker) levels.

*There are a total of 9 blood draws that will be done over the course of this study for safety and research purposes. If there is any blood left over at the First Study visit, Study visit 15 and Follow up visits 1 and 2, we would like to store it for possible future research related to Alzheimer's disease. At the end of this main study consent form you will be given the opportunity to agree or not agree to the storage of your left over blood for future research. You can still participate in the main study if you decide not to allow your left over blood to be stored and used for future research.

Total time

Although the majority of the time needed to participate in this study is in the first month during the treatment period, you will be asked to participate in the few follow up visits for a total study time of about 5 months. Please review the following study schedule.

STUDY SCHEDULE																					
VISITS	**SCREEN**	**TREATMENT PHASE**																		**Follow up 1**	**End of study**
DAYS	7-28	1	2	3	4	5			6	7	8	9	10			11	12	13	14	15	45 days	90 days
Informed consent	X																					
Pregnancy test	X																					
Kidney function	X																					
Mental Screening Assessment	X	X																		X	X	X
Medical history	X																					
Review of other medications	X	X	X	X	X	X			X	X	X	X	X			X	X	X	X	X	X	X
Side effects		X	X	X	X	X			X	X	X	X	X			X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X			X	X	X	X	X			X	X	X	X	X	X	X
Physical exam	X																			X	X	X
Injection site review		X	X	X	X	X			X	X	X	X	X			X	X	X	X	X		
ECG	X																					
MRI	X																			X	X	
PET	X																				X	
LABS																						
Blood draws	X	X			X				X			X				X				X	X	X
Detailed Mental Assessment		X																		X	X	X
Study drug injections		X	X	X	X	X			X	X	X	X	X			X	X	X	X	X		

What are the possible discomforts or risks?

Risk of Loss of Confidentiality

There is a risk that people outside of the research team will see your confidential research information. We will do all that we can to protect your information, but it cannot be guaranteed.

Side effects of the study drug: Leukine.

You may have problems because of the drug used in this study. These problems are called side effects. Some side effects are just a bother and others could harm you. There may be some side effects that we do not know about yet. The research might involve risks to you that are currently unforeseeable.

Here are the known side effects that could happen with Leukine®

There is always a chance that any medical treatment may cause you some discomfort or harm and the drugs or procedures in this study are no different. We will do everything possible to keep you from being harmed. There may be other risks or side effects that occur which we do not know about at this time. It is important for you to tell us when you experience such a side effect.

The following side effects occur more frequently in patients taking Leukine® as compared to those taking placebo.
Common but non-life threatening side effects include:

- Fatigue
- Lack of energy
- Rash
- Itching
- Vomiting
- Diarrhea
- Abdominal pain
- Sore throat
- Chills
- Bone pains
- Joint pains
- Weight loss or gain
- Elevations in kidney or liver enzymes have been reported
- Fluid retention
- Injection site irritation
- Insomnia
- Headaches

- Passing fevers

Less common but potentially serious side effects include:

- Bleeding into the eye
- Allergic reactions to the study drug (indicated by rashes or shortness of breath)
- Excessive increase in white blood cell count. This could lead to:
  - Extreme bone pain
  - Elevated blood pressure
  - Potential strokes
  - Heart failure
  - Lung problems
  - Fluid retention in the lung lining and in the heart lining
- Shortness of breath has been reported in those given Leukine®
- Occasional heart rhythm changes (supraventricular tachycardia)

Risks of Amyloid Related Imaging Abnormality (ARIA)

ARIA has been identified as an event that may occur following treatment with certain drugs that specifically target amyloid in the brain. There is no evidence that Leukine causes or increases risk of ARIA. However, there is a risk that an ARIA event may take place following removal of amyloid from the brain. ARIA events are identified and monitored using Magnetic Resonance Imaging (MRI).

ARIA events can be classified into two main categories:

o (ARIA-E) - Amyloid Related Imaging Abnormality due to Edema or swelling in the brain

o (ARIA-H) - Amyloid Related Imaging Abnormality due to Microhemorrhaging or small amount of bleeding in the brain

Risks of Having a Magnetic Resonance Imaging (MRI) procedure:

- In this study we will take Magnetic Resonance Images (MRI's) of your head. The MRI machine uses powerful magnetic waves to take pictures inside the body. The waves themselves are not harmful, but they can cause metal to heat up and electronics to stop working.
- **You should NOT have an MRI if you have <u>metal</u> or <u>electronic devices</u> inside your body. Heart pacemakers and insulin pumps are examples of electronic devices.**
- The MRI machine is a small round tube. It might make you uncomfortable if you do not like tight spaces.
- The most common side effect of having an MRI is flashing lights in the eyes. This is caused by the magnetic waves and is not harmful. Some people also experience warmth and reddening of the skin. This usually goes away after a few minutes.

- **If you are pregnant, be sure to tell the person giving you the MRI.**

Risks of PET Procedure:

Possible discomforts or risks of an IV

As mentioned above, an IV catheter will be temporarily placed in your arm or hand to inject the imaging agent. You will feel a brief sharp pain/discomfort when the needle used to place the IV catheter is placed through your skin. Sometimes the placement of an IV may cause redness, swelling or bruising at the site of the IV. This is temporary and should get better on its own. In very rare cases, placement of an IV could cause infection or a blood clot. During the injection of the imaging agent through the IV, you may feel a warm or tingling sensation at the site of the injection. The IV will be placed in the arm during the 20 minute scan and removed after the scan before you go home.

Possible discomforts or risks of a PET/CT scan

You will be asked to lie down on the scanner bed for 20 minutes to complete the scan. Pillows, padding and blankets can be provided for your comfort, but there may be some discomfort from lying down on the scanner bed. Some people may also feel claustrophobia (fear of being in tight places).

CT and PET scans use radiation to get pictures of your body. Exposure to radiation could increase the risk of developing cancer. Exposure to high levels of radiation have been linked with the increased risk of developing cancer. Low dose radiation, like that used in the medical imaging in this study, has not been shown to increase the risk of developing cancer. However it is good to keep exposure to radiation as low as reasonably possible. The Food and Drug Administration (FDA) has set guideline limits on radiation exposure for research participants (whole body limit of 30 mSv for a single imaging scan; 50 mSv for the entire year). The whole body estimated radiation from a single Amyloid PET/CT scan for this study of 3.5 mSv is much less than the established limit.

Although not everyone is exposed to radiation from medical imaging scans, everyone is exposed to natural background radiation. There is natural background radiation in the soil, water and vegetables that we eat, in the air from the sun, and within small amounts of radioactive atoms in our own bodies. The total amount of radiation you will receive from this single PET/CT scan (3.5 mSv). However, this scan is performed twice, once at screening and again at follow up 1 (+/- 7 days), with total radiation exposure of approximately 7 mSv. This is a little less radiation than the natural background radiation that you would expect to receive from living in the Denver area for 2 years (8 mSv). It is also below the established guideline limits for research participants by the FDA and the University of Colorado Radioactive Drug Research Committee (RDRC).

If you would like more information regarding radiation, please ask the study coordinator or investigator for a copy of "The University of Colorado Radiology Adult Dose-Risk Smartcard."

Please inform your doctor if you have had any radiation exposure in the past year, from research studies or standard clinical procedures. This is suggested to limit your radiation exposure and minimize risk. Radiation exposure includes x-rays, cardiac catheterization, fluoroscopy, as well as any scans that included the injection of radioactive materials.

Risks of the radiopharmaceutical (Pittsburgh Compound B)

Pittsburgh Compound B does not have any known side effects. As with the administration of any drug, however, there is the rare risk (less than 1 out of 100 people) of side effects, and you will be monitored for any reactions. The PET Facility provides a fully equipped medical cart, and a physician will always be available.

Risks of having blood taken:

- In this study we will need to get about a tablespoon of blood from you on a total of 9 occasions. We will get blood by putting a needle into one of your veins and letting the blood flow into a glass tube. You may feel some pain when the needle goes into your vein. A day or two later, you may have a small bruise where the needle went under the skin.

Risks from multiple study drug injections:

- Common side effects include bruising, swelling and pain at the site of the needle insertion.
- It is rare, but an infection can occur at the site of the needle insertion. A clinician will examine the injection sites weekly and more often if needed.

We will tell you as soon as we can, if we find out more information about the side effects that are caused by Leukine®.

During the course of this study, we may find more information that could be important to you. This includes information that, once learned, might cause you to change your mind about your willingness to be in this study. We will notify you as soon as possible if such information becomes available.

What are the possible benefits of the study?

This study is designed for the researcher to learn more about whether the study drug is safe to use for patients with Alzheimer's disease and to investigate whether the drug improves their memory.

However, there is no guarantee that your health will improve if you join this study. Also, there could be risks to being in this study. If there are risks, these are described in the section describing the discomforts or risks.

By volunteering, you are helping us learn more about Alzheimer's disease. We will learn more about what does or does not help. What we learn may help others with this disease.

Are there alternative treatments?

There may be other ways of treating your Alzheimer's disease. These other ways include using medications such as Donepezil, Rivastigmine, Galantamine or Memantine. You could also choose to get no treatment at all.

You should talk to your doctor about your choices. Make sure you understand all of your choices before you decide to take part in this study. You may leave this study and still have these other choices available to you.

Who is paying for this study?

This research is being paid for by The Dana Foundation.

Will I be paid for being in the study?

As help towards travel costs (including visits for the brain MRI), you will receive a $75 gift card at the end of the three week treatment period even if you cannot or choose not to complete the study, and you will receive a $25 gift card after each of the follow up visits for a total of $125.

Will I have to pay for anything?

All study related procedures such as physical exams, blood draws, tests of brain function and injections of the study drug are provided at no cost to you.

Conflict of interest disclosures

Dr. Huntington Potter and Dr. Timothy Boyd own intellectual property related to this study as inventors of the patent for the product being tested. Please ask the study staff if you have any questions/concerns about this.

Is my participation voluntary?

Taking part in this study is voluntary. You have the right to choose not to take part in this study. If you choose to take part, you have the right to stop at any time. If you refuse or decide to withdraw later, you will not lose any benefits or rights to which you are entitled.

If you leave this study, and are a patient in the Memory and Dementia Clinic, you will still receive your normal medical care. The only medical care that you will lose is the medical care you are getting as part of this study. You might be able to get that same kind of medical care outside of the study. Ask your study doctor.

Can I be removed from this study?

The study doctor may decide to stop your participation without your permission if the study doctor thinks that being in the study may cause you harm, or for any other reason. If the study drug increases your white blood cell count above an unusual level we will either decrease your dosage or discontinue the drug. We will monitor your blood count until it returns to normal.

In addition, the sponsor may stop the study at any time.

What happens if I am injured or hurt during the study?

If you have an injury while you are in this study, you should call Dr. Huntington Potter 303-724-7385 or Dr. Jonathan Woodcock 303-724-7863 immediately.

We will arrange to get you medical care if you have an injury that is caused by this research. However, you or your insurance company will have to pay for that care.

Who do I call if I have questions?

The researchers carrying out this study are Dr. Huntington Potter and Dr. Jonathan Woodcock. You may ask any questions you have now. If you have questions, concerns, or complaints later, you may call Dr. Huntington Potter at 303-724-7385 or Dr. Jonathan Woodcock at 303-724-7863. You will be given a copy of this form to keep.

You may have questions about your rights as someone in this study. You can call Dr. Huntington Potter 303-724-7385 with questions. You can also call the responsible Institutional Review Board (COMIRB). You can call them at 303-724-1055.

A description of this trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

A Subject Advocate is Also Available to Answer Questions

You may have questions about your rights as someone in this study. You can call Dr. Huntington Potter 303-724-7385 or Dr. Jonathan Woodcock 303-724-7863 with questions. You can **also** talk to a Subject Advocate at the Clinical Translation Research Center (CTRC). The phone number there is 720-848-6662.

Additional Optional Study Procedures

The following are additional optional study procedures that you are being asked to consider. You may choose to participate in any, or none, of the following procedures. Your decision to participate, or to not participate, in these additional procedures will not affect your ability to participate in the main study you agreed to above.

Optional Consent and Authorization for Data and Blood Banking for Future Research

Drs. Huntington Potter and Jonathan Woodcock would like to keep some of the data and blood that is taken during the study but is not used for other tests. If you agree, the data and blood samples will be kept and may be used in future research to learn more about Alzheimer's disease. The research that is done with your data and blood samples is not designed to specifically help you. It might help people who Alzheimer's disease and other diseases in the future. Reports about research done with your data and blood samples will not be given to you or your doctor. These reports will not be put in your health records. The research using your data and blood samples will not affect your care.

The choice to let Drs. Huntington Potter and Jonathan Woodcock keep the data and blood samples for future research is up to you. No matter what you decide to do, it will not

affect the care that you will receive as part of the study. If you decide now that your data and blood samples can be kept for research, you can change your mind at any time and contact your study doctor to let him or her know that you do not want Drs. Huntington Potter and Jonathan Woodcock to use your data and blood samples any longer, and they will no longer be used for research. Otherwise, they may be kept until they are used up, or until Drs. Huntington Potter and Jonathan Woodcock decides to destroy them.

When your data and blood samples are given to other researchers in the future, Drs. Huntington Potter and Jonathan Woodcock will not give them your name, address, phone number or any other information that will let the researchers know who you are.

Sometimes data and blood samples are used for genetic research (about diseases that are passed on in families). Even if your data and blood samples are used for this kind of research, the results will not be told to you and will not be put in your health records. Your data and blood samples will only be used for research and will not be sold. The research done with your data and blood samples may help to develop new products in the future, but there is no plan for you to be paid.

The possible benefits of research from your data and blood samples include learning more about what causes Alzheimer's disease and other diseases, how to prevent them and how to treat them. The greatest risk to you is the release of your private information. Drs. Huntington Potter and Jonathan Woodcock will protect your records so that your name, address and phone number will be kept private. The chance that this information will be given to someone else is very small. There will be no cost to you for any data or blood sample collection and storage by Drs. Huntington Potter and Jonathan Woodcock.

Please read each sentence below and think about your choice. After reading each sentence, circle "yes" or "no." If you have questions, please talk to your doctor or nurse. Remember, no matter what you decide to do about the storage and future use of your data and blood samples, you may still take part in the study.

I give my permission for my data and blood to be stored in a central tissue bank at the Linda Crinc Institute for Down Syndrome and the Colorado Intellectual & Developmental Disabilities Research Center for future use by the study investigators:

1. I give my permissions for my data and blood to be kept by Drs. Huntington Potter and Jonathan Woodcock for use in future research to learn more about how to prevent, detect, or treat Alzheimer's disease.

☐ Yes ☐ No _____Initials

2. I give my permissions for my data and blood to be used for research about other health problems (for example: causes of heart disease, osteoporosis, diabetes).

☐ Yes ☐ No _____Initials

3. I give my permission for my study doctor (or someone he or she chooses) to contact me in the future to ask me to take part in more research.

☐ Yes ☐ No _____Initials

Who will see my research information?

The University of Colorado Denver and the hospital(s) it works with have rules to protect information about you. Federal and state laws including the Health Insurance Portability and Accountability Act (HIPAA) also protect your privacy. This part of the consent form tells you what information about you may be collected in this study and who might see or use it.
The institutions involved in this study include:

- University of Colorado Denver
- University of Colorado Hospital
- The Linda Crnic Institute for Down Syndrome

We cannot do this study without your permission to see, use and give out your information. You do not have to give us this permission. If you do not, then you may not join this study.

We will see, use and disclose your information only as described in this form and in our Notice of Privacy Practices; however, people outside the University of Colorado Denver and its affiliate hospitals may not be covered by this promise.

We will do everything we can to keep your records a secret. It cannot be guaranteed.

The use and disclosure of your information has no time limit. You can cancel your permission to use and disclose your information at any time by writing to the study's Primary Investigators, at the name and address listed below. If you do cancel your permission to use and disclose your information, your part in this study will end and no further information about you will be collected. Your cancellation would not affect information already collected in this study.

Huntington Potter, Ph.D.
Director of Alzheimer's Disease Programs
Anschutz Medical Campus
University of Colorado, Denver MS 8608
12700 E. 19th Ave.
Aurora CO 80218

Jonathan H. Woodcock, M.D.
Clinical Director, Memory and Dementia Clinic
Anschutz Medical Campus
Academic Office Building 1, Room # 5209
12631 East 17th Avenue
Aurora, CO 80045

Both the research records that identify you and the consent form signed by you may be looked at by others who have a legal right to see that information.

- Federal offices such as the Food and Drug Administration (FDA) that protect research subjects like you.
- People at the Colorado Multiple Institutional Review Board (COMIRB)
- The study doctor and the rest of the study team.
- Officials at the institution where the research is being conducted and officials at other institutions involved in this study who are in charge of making sure that we follow all of the rules for research

We might talk about this research study at meetings. We might also print the results of this research study in relevant journals. But we will always keep the names of the research subjects, like you, private.

You have the right to request access to your personal health information from the Investigator. To ensure proper evaluation of test results, your access to these study results may not be allowed until after the study has been completed.

The investigator (or staff acting on behalf of the investigator) will use your information for the research outlined in this consent form. They will also make *all or some* of the following health information about you collected in this study available to:

- Quest Diagnostics

Information about you that will be seen, collected, used and disclosed in this study:

- Name and Demographic Information (age, sex, ethnicity, address, phone number, highest level of education obtained)
- Portions of my previous and current Medical Records that are relevant to this study, including but not limited to Diagnosis(es), History and Physical, laboratory or tissue studies, radiology studies, procedure results
- Research Visit and Research Test records
- Psychological tests
- Blood samples and the data with the blood samples.

What happens to Data and Blood Specimens that are collected in this study?

Scientists at the University of Colorado Denver and the hospitals involved in this study work to find the causes and cures of disease. The data and blood specimens collected from you during this study are important to this study and to future research. If you join this study:

- The data and blood specimens are given by you to the investigators for this research and so no longer belong to you.
- Both the investigators and any sponsor of this research may study your data and blood specimens collected from you.
- If data and blood specimens are in a form that identifies you, UCD or the hospitals involved in this study may use them for future research only with your consent or IRB approval.
- Any product or idea created by the researchers working on this study will not belong to you.

There is no plan for you to receive any financial benefit from the creation, use or sale of such a product or idea.

HIPAA Authorization for Optional Additional Study Procedures

In this form, you were given the option to agree to additional, optional research procedures. You must also give us your permission, under HIPAA rules, to use and disclose the information collected from these optional procedures, as described above.

If you decline to give us permission to use and disclose your information, you cannot take part in these optional procedures, but you can still participate in the main study. Please initial next to your choice:

_____ I give permission for my information, from the optional procedures I have agreed to above, to be used and disclosed as described in this section.

_____ I do not give permission for my information for any optional procedures to be used and disclosed; I understand that I will not participate in any optional procedures.

Agreement to be in this study

I have read this paper about the study or it was read to me. I understand the possible risks and benefits of this study. I know that being in this study is voluntary. I choose to be in this study: I will get a copy of this consent form.

Signature:_____ Date:_____

Time:_____

Print Name:_____

Consent form explained by:_____ Date:_____

_____Time:_____

Print Name: _____

Investigator: _____ Date: _____
Investigator must sign within ___[maximum 30] days

Time:_____

[If Applicable, Signature Line For witness. Required for consent of non-reading and non-English speaking subjects]

Witness Signature:_____ Date: _____
Time:_____

Print Name: _____

Witness of Signature ☐

Witness of consent process ☐

[If Applicable, Signature Line For Legally Authorized Representative]

_____ Date_____
Legally Authorized Representative

Print Name: _____

Principal Investigator: **Brianne Bettcher, Ph.D.**
COMIRB No: **15-1774**
Version Date: **July 8, 2020**
Alzheimer's Disease, Mild Cognitive Impairment Consent Form

Study Title: **University of Colorado Alzheimer's and Cognition Center (CUACC) Longitudinal Biomarker and Clinical Phenotyping Study**

You are being asked to be in a research study. This form provides you with information about the study. A member of the research team will describe this study to you and answer all of your questions. Please read the information below and ask questions about anything you don't understand before deciding whether or not to take part.

Some people in this study may have a medical condition or a disability that does not allow them to make important decisions for themselves. If you have been asked to decide for someone else whether they should be in this study, please read this consent form carefully.

In this form, we use the words "you" and "your." If you are reading this form and deciding for someone else, the words 'you' and 'your' refer to that other person, not to you.

Why is this study being done?

This study plans to establish a large bank of blood, cerebral spinal fluid (CSF),imaging, and clinical data. These data and samples will be used for future research into the causes of Alzheimer's disease (AD), Down Syndrome (DS) and other diseases that cause thinking and memory problems. This future research will also study how treatments for these diseases work. This research may help develop new treatments for some diseases of the nervous system and help us understand these diseases better.

You are being asked to be in this research study because you have AD, DS, or are being evaluated for thinking/memory issues, or

You must also have a study partner (a family member or close friend) who knows you well and is willing to accompany you to annual research visits. Certain parts of the annual research visits may be completed remotely, and the study partner may be asked to accompany you to the virtual visit.

Other people in this study

Up to 800 people from your area will participate in the study.

What happens if I join this study?

If you agree to join this study, we may ask you to come into the clinic for a screening visit. The screening visit may also be completed remotely (i.e., by telehealth/video). At this visit your medical history will be reviewed and you will have a neurological and physical exam to make sure that you meet the criteria to be enrolled.

If you qualify, you will undergo the following procedures once a year until the study ends. Each visit may last around 4-5 hours. We will ask you to continue to provide data and samples for the study for four annual visits (i.e., baseline visit and 3 yearly follow-up visits).

- Physical/neurological exam
- Complete set of tests that assess memory, attention, behavior, and other thinking skills. This set of tests will take about 2-3 hours to complete. Some of the tests will be done on paper and some may be done on a computer or ipad.
- Complete questionnaires about your health history.
- Have about 4.5 tablespoons of blood collected for research analysis. Sometimes data and samples are used for genetic research (about diseases that are passed on in families). Even if your data and samples are used for this kind of research, the results will not be told to you and will not be put in your health records
- Have a magnetic resonance imaging procedure (MRI). This procedure will involve lying still on your back for about 45 minutes in a machine that take images ("pictures") of your brain.
- Have your study partner (a family member or close friend) answer questions each year you participate in the study.
- Have your medical records reviewed and information about your general health and thinking/memory issues recorded

Telehealth/Remote Administration of Study Procedures: Some procedures may be completed remotely (i.e., while you are at home) using video or phone administration by the study staff, as detailed below.

Consent

- E-consent may be obtained. The purpose, procedures, risks, and benefits may be explained to you remotely using HIPAA-compliant programs (e.g., Zoom, Skype, Vidyo) by the site investigator or study staff. If interested, you may sign the e-consent form via out REDCap system.

Screening, Baseline, and Annual Follow-Up

- You may have your standard clinical information, medications and medical history reviewed, surveys/tests administered, and a neurological exam performed via video programs (e.g., Zoom, Skype, Vidyo). A brief interview with your study partner may also be conducted by phone or by video programs.

- Surveys and questionnaires may be sent securely and directly to you for completion before your in-person visit.
- All other study procedures will be performed at your next in-person visit and cannot be completed remotely.

The following procedures are optional and will be completed once a year if you agree. Please check YES or NO for each of the following. You can change your mind about participating in these procedures at any time:

- Lumbar Puncture: This involves insertion of a needle in the lower back and collection of about 2 tablespoons of cerebrospinal fluid. Please note that if you are taking a blood thinner or have a history of back surgery, you may not be eligible to participate in this procedure.

_____YES_____NO_____Initials

If you come to UCH for your standard (non-research) clinical care and have a blood draw or lumbar puncture we may collect any leftover samples from these procedures for the bank.

_____YES_____NO_____N/A_____Initials

- Contact for future research recruitment: If you agree, we would like to use the data collected in this study to assess if you may be eligible for future clinical research studies.

  I am interested in being contacted for participation in future research studies:

_____YES _____NO _____Initials

- Contact for University of Colorado Alzheimer's and Cognition Center newsletter and events: If you agree, we would like to send you a newsletter and schedule of the center's events.

  I am interested in being contacted for center news and events:
_____YES _____NO _____Initials

What are the possible discomforts or risks?

Risks of Having Blood Taken

We will need to get about 4.5 tablespoons of blood from you at each blood draw. We will get blood by putting a needle into one of your veins and letting the blood flow into a glass tube. You may feel some pain when the needle goes into your vein. A day or two later, you may have a small bruise where the needle went under the skin. The risks of having blood taken in the study is comparable with the risk of donating a unit of blood but the amount is only 1/6th as much.

Risks of Thinking/Memory Testing

The only risks associated with the thinking/memory testing is possible boredom or fatigue. You will be able to stop this testing at any time if you wish.

Risks of Having an MRI

In this study we will take Magnetic Resonance Images (MRI's) of your head. The MRI machine uses powerful magnetic waves to take pictures inside the body. The waves themselves are not harmful, but they can cause metal to heat up and electronics to stop working.

You should NOT have an MRI if you have metal or electronic devices inside your body. Heart pacemakers and insulin pumps are examples of electronic devices.

The MRI machine is a small round tube. It might make you uncomfortable if you do not like tight spaces.

The most common side effect of having an MRI is flashing lights in the eyes. This is caused by the magnetic waves and is not harmful. Some people also experience warmth and reddening of the skin. This usually goes away after a few minutes.

Risks of Having a Lumbar Puncture

Risks associated with lumbar puncture (LP) include:

- Pain
- Bleeding
- Infection
- headache

To limit discomfort, the skin, surrounding tissue and underlying muscle will be numbed with a drug called lidocaine. This is similar to the numbing medication delivered during a dental procedure, such as a tooth extraction. Lidocaine injection may cause a burning sensation. This sensation is brief and generally associated only with the administration of the drug.

During the LP you may experience:

- Brief pain lasting seconds in the lower back during insertion of the needle, especially if the needle contacts bone. This occurs about 1 in 5 times.
- Less than 1 in 10 patients experience a brief pain shooting down the leg if the LP needle touches a nerve root. Rarely, this sensation or some numbness in the leg may persist for days.
- Less than 1 in 100 patients are allergic to the iodine solution used to clean their back and will develop rash.
- After LP, 5 to 15 out of 100 individuals develop a headache that is worse when standing and disappears when lying down. It typically begins 6-48 hours after the LP, and may last 1 to 6 days. Lying flat and taking caffeine sodium benzoate will treat the headache.
- About 3 in 100 individuals with post-LP headache also develop double vision that can last a few days to a few weeks.
- About 3 in 100 individuals will develop temporary hearing problems or ringing in the ears lasting a few days.
- After a successful LP, less than 2 in 100 individuals will have a little bleeding at the LP site. This clears rapidly.
- Rarely, infection may occur at or near the LP site.

To reduce the likelihood of a temporary headache after the LP, you will be asked to lie on your back for a short period of time after the procedure. You will not receive any routine drugs for the purpose of this study. Post-LP headache may be treated with oral hydration, analgesics such as acetaminophen, or ibuprofen and caffeine. If the

headache persists for more than 7 days, an epidural blood patch may be advised for relief. If you require a blood patch, you or your insurance company will have to pay for that care. This procedure involves the collection of blood from your arm, which is then administered into the epidural space outside the membranes surrounding the spinal cord to seal the LP needle tract that is leaking fluid. The procedure is similar to that of a LP and carries similar risks for discomfort, bleeding and infection. It generally cures the headache in 10 to 15 minutes.

Because of the risk of temporary visual blurring or headache, you should arrange for transportation after the procedure in case you are temporarily unable to drive.

Risks of Genome Wide Association Studies (GWAS)

Should data be submitted to the GWAS database, it will be coded, meaning it will not include anything that might directly identify me. There is a slight risk there could be a breach in the security of this database system resulting in the unauthorized access to my information. Safeguards are in place to minimize this risk. Data is for broad sharing with approved investigators. It is possible that a study finding could one day help people of the same race, ethnicity or sex as you. However, it is also possible through these kinds of studies that genetic traits might come to be associated with your group. In some cases this could reinforce stereotypes.

Confidentiality

Some of the CSF, blood (including DNA and RNA samples) and certain medical information about you (for example, diagnosis and age) may be shared with other scientists or companies outside of UCH/UCD. However, your name, address, phone number, or any other information that would identify you will not be released. If these samples are sent to other researchers, only coded data, which does not include anything that might directly identify you, will be shared. Blood may also be used in the future to generate cell lines. Cell lines are made from white blood cells that are mixed with a solution that allows for permanent cell growth, immortalizing cells for future genetic research. The cell lines may be stored at The National Cell Repository for Alzheimer's disease (NCRAD) which is a National Institutes of Health funded facility that provides genetic materials to researchers throughout the country. The cell lines stored at NCRAD will be identified with a code number. Other researchers will not receive your name or other identifying information. Coded data linked to cell lines will be kept on a secure computer at NCRAD. This information can only be accessed by authorized investigators. A summary of coded data will be made available to researchers via a website that will be maintained by NCRAD. Centers may also be requested to contribute other biological samples such as serum and cerebrospinal fluid, using agreed upon protocols, for trans-center studies examining biomarkers that might relate to risk, diagnosis or progression of AD.

There is a risk that people outside of the research team will see your research information. Only HIPAA-compliant video platforms will be used during remote evaluations to protect your confidentiality. We will do all that we can to protect your information, but it cannot be guaranteed. There may be risks that are unknown at this time.

Genetic Information Nondiscrimination Act (GINA)

A Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. This law generally will protect you in the following ways:

- Health insurance companies and group health plans may not request your genetic information that we get from this research.
- Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility or premiums.
- Employers with 15 or more employees may not use your genetic information that we get from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment.

All health insurance companies and group health plans must follow this law by May 21, 2010. All employers with 15 or more employees must follow this law as of November 21, 2009.

Be aware that this new Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

What are the possible benefits?

This study is designed for the researcher to learn more about Alzheimer's disease (AD), Down Syndrome (DS) and other diseases that cause thinking and memory problems.

This study is not designed to treat any illness or to improve your health. Also, there may be risks, as discussed in the section describing the discomforts or risks.

Can these research results be used in my clinical care?

The assessments and tests done in this study are NOT the same as those used to diagnose medical problems and are done for research purposes. We will not tell you or your doctor the results. There will be no formal radiological read on your MRI scan.

However, when these tests are performed for research, there is a chance of finding something medically unexpected. Unexpected findings can be medically important or

medically unimportant. Findings that are medically important have clear clinical significance and are those for which treatment might be available. You will only be informed of findings with clear significance. You will not be informed of findings judged as medically unimportant by our clinicians.

If you have concerns about your risk of developing Alzheimer's disease, you should talk to your clinical doctor.

Will I be paid?

You will not be paid for participation in this study,

Will I have to pay for anything?

It will not cost you anything to be in the study.

Is my participation voluntary?

Taking part in this study is voluntary. You have the right to choose not to take part in this study. If you choose to take part, you have the right to stop at any time. If you refuse or decide to withdraw later, you will not lose any benefits or rights to which you are entitled.

Can I be removed from this study?

The study doctor may decide to stop your participation without your permission if the study doctor thinks that being in the study may cause you harm, or for any other reason.

What happens if I am injured or hurt during the study?

If you have an injury while you are in this study, you should call Dr. Bettcher immediately. Her phone number is303-724-3941.

We will arrange to get you medical care if you have an injury that is caused by this research. However, you or your insurance company will have to pay for that care.

Who do I call if I have questions?

The researcher carrying out this study is Dr. Brianne Bettcher. You may ask any questions you have now. If you have questions, concerns, or complaints later, you may call Dr. Bettcher at303-724-3941. You will be given a copy of this form to keep.

You may have questions about your rights as someone in this study. You can call Dr. Bettcher with questions. You can also call the responsible Institutional Review Board (COMIRB). You can call them at 303-724-1055.

You may also talk to a Subject Advocate at the Clinical and Translational Research

Center (CTRC) The number there is 720-848-6662.

Who will see my research information?

The University of Colorado Denver (UCD) and its affiliated hospital(s) have rules to protect information about you. Federal and state laws including the Health Insurance Portability and Accountability Act (HIPAA) also protect your privacy. This part of the consent form tells you what information about you may be collected in this study and who might see or use it.

The institutions involved in this study include

- University of Colorado Denver
- University of Colorado Hospital

We cannot do this study without your permission to see, use and give out your information. You do not have to give us this permission. If you do not, then you may not join this study.

We will see, use and disclose your information only as described in this form and in our Notice of Privacy Practices; however, people outside the UCD and its affiliate hospitals may not be covered by this obligation.

We will do everything we can to maintain the confidentiality of your personal information but confidentiality cannot be guaranteed.

The use and disclosure of your information has no time limit. You can cancel your permission to use and disclose your information at any time by writing to the study's Principal Investigator (PI), at the name and address listed below. If you do cancel your permission to use and disclose your information, your part in this study will end and no further information about you will be collected. Your cancellation would not affect information already collected in this study.

Brianne Bettcher, PhD
12469 E. 17th Place, Mail Stop F429
Aurora, CO 80045

Both the research records that identify you and the consent form signed by you may be looked at by others who have a legal right to see that information, such as:

- Federal offices such as the Food and Drug Administration (FDA) and the Office of Human Research Protections (OHRP) that protect research subjects like you.
- People at the Colorado Multiple Institutional Review Board (COMIRB)
- The study doctor and the rest of the study team.
- Officials at the institution where the research is conducted and officials at other institutions involved in this study who are in charge of making sure that we follow all of the rules for research

In addition, other researchers outside of UCD may also have access to your de-identified data. We might talk about this research study at meetings. We might also print the results of this research study in relevant journals. But we will always keep the

names of the research subjects, like you, private. Specifically, the results of some of the thinking tests done on an iPad will be shared with the University of California San Francisco Memory and Aging Center. Your name or any other information that identifies you will not be shared.

You have the right to request access to your personal health information from the Investigator.

Information about you that will be seen, collected, used and disclosed in this study:

- Portions of your previous and current Medical Records that are relevant to this study, including but not limited to Diagnosis(es), History and Physical, laboratory studies, radiology studies, procedure results
- Research Visit and Research Test records
- Imaging studies
- Psychological tests

What happens to Data, Blood, and Specimens that are collected in this study?

Scientists at the University of Colorado Denver and the hospitals involved in this study work to find the causes and cures of disease. The data, blood and specimens collected from you during this study are important to this study and to future research. If you join this study:

- The data, blood, or other specimens given by you to the investigators for this research no longer belong to you.
- Both the investigators and any sponsor of this research may study your data, blood, or other specimens collected from you. De-identified specimens may also be shared with other scientists or companies outside of UCH/UCD.
- If data, blood, or other specimens are in a form that identifies you, UCD or the hospitals involved in this study may use them for future research only with your consent or Institutional Review Board (IRB) approval.
- Any product or idea created by the researchers working on this study will not belong to you.
- There is no plan for you to receive any financial benefit from the creation, use or sale of such a product or idea.

HIPAA Authorization for Optional Additional Study Procedures

In this form, you were given the option to agree to optional study procedures and to having your data used to assess eligibility for future research. You must also give us your permission, under HIPAA rules, to use and disclose the information collected from these optional procedures, as described above.

If you decline to give us permission to use and disclose your information, you cannot take part in these optional procedures, but you can still participate in the main study. Please

initial next to your choice:

_____I give permission for my information, from the optional procedures I have agreed to above, to be used and disclosed as described in this section.

_____ I **do not** give permission for my information for any optional procedures to be used and disclosed; I understand that I will not participate in any optional procedures.

Agreement to be in this study and use my data

I have read this paper about the study or it was read to me. I understand the possible risks and benefits of this study. I understand and authorize the access, use and disclosure of my information as stated in this form. I know that being in this study is voluntary. I choose to be in this study: I will get a signed and dated copy of this consent form.

Signature:_____ Date:_____

Print Name:_____

Consent form explained by:_____ Date:_____

Print Name: _____

_____ Date:_____
Legally Authorized Representative (if applicable)

Print Name: _____

For use with non-English speaking and non-reading subjects:

Witness Signature:_____ Date:_____

Witness Print Name:_____ Witness of Signature ☐

Witness of consent process ☐

Study Partner Information and Consent

As the subject's study partner, you have important tasks that need to be carried out in order for the study to be conducted in the best manner possible. These responsibilities include:

1) You must attend the first research visit (aspects of which may be conducted remotely), and then must either attend or be available by phone for annual research visits.
2) You are an important source of information about the subject. You will be asked questions either in person, on the phone, by video call, and/or through online surveys to find out whether there are any changes in the subject.

If for some reason you become unable to carry out your responsibilities or do not wish to be the study partner, please tell the study coordinator immediately. You may be asked, if possible, to select a substitute who can take over your duties.

Agreement to be the study partner for this study

I have read all the preceding information which describes both the subject's participation in the study and my involvement as the subject's study partner. I understand the possible risks and benefits of this study. I understand and authorize the access, use and disclosure of information as stated in this form. I know that being in this study is voluntary. I choose to be in this study: I will get a signed and dated copy of this consent form.

Study Partner Signature:_____Date:_____

Print Name:_____

Person Obtaining Consent:_____ Date:_____

Print Name:_____

Principal Investigator: **Brianne Bettcher, Ph.D.**
COMIRB No: **15-1774**
Version Date: **July 8, 2020**
Healthy Control Consent Form

Study Title: **University of Colorado Alzheimer's and Cognition Center (CUACC) Longitudinal Biomarker and Clinical Phenotyping Study**

You are being asked to be in a research study. This form provides you with information about the study. A member of the research team will describe this study to you and answer all of your questions. Please read the information below and ask questions about anything you don't understand before deciding whether or not to take part.

Why is this study being done?

This study plans to establish a large bank of blood, cerebral spinal fluid (CSF), imaging, and clinical data. These data and samples will be used for research into the causes of Alzheimer's disease (AD), Down Syndrome (DS) and other diseases that cause thinking and memory problems. This future research will also study how treatments for these diseases work. This research may help develop new treatments for some diseases of the nervous system and help us understand these diseases better.

You are being asked to be in this research study because
you do not have AD, DS, or thinking/memory issues and are in good general health.

You must also have a study partner (a family member or close friend) who knows you well and is willing to complete a questionnaire once a year either over the phone or in person while you participate in this study. If you receive a diagnosis of Mild Cognitive Impairment (MCI) or AD during the course of this study, this partner must be willing to attend annual research visits with you in person.

Other people in this study

Up to 800 people from your area will participate in the study.

What happens if I join this study?

If you agree to join this study, we may ask you to come into the clinic for a screening visit. The screening visit may also be completed remotely (i.e., by telehealth/video). At this visit your medical history will be reviewed and you will have a neurological and physical exam to make sure that you meet the criteria to be enrolled.

Telehealth/Remote Administration of Study Procedures: Some procedures may be completed remotely (i.e., while you are at home) using video or phone administration by the study staff, as detailed below.

Consent

- E-consent may be obtained. The purpose, procedures, risks, and benefits may be explained to you remotely using HIPAA-compliant programs (e.g., Zoom, Skype, Vidyo) by the site investigator or study staff. If interested, you may sign the e-consent form via out REDCap system.

Screening, Baseline, and Annual Follow-Up

- You may have your standard clinical information, medications and medical history reviewed, surveys/tests administered, and a neurological exam performed via video programs (e.g., Zoom, Skype, Vidyo). A brief interview with your study partner may also be conducted by phone or by video programs.
- Surveys and questionnaires may be sent securely and directly to you for completion before your in-person visit.
- All other study procedures will be performed at your next in-person visit and cannot be completed remotely.

If you qualify, you will undergo the following procedures once a year until the study ends. Each visit may last around 4-5 hours. We will ask you to continue to provide data and samples for the study for four annual visits (i.e., baseline visit and 3 yearly follow-up visits).

- Physical/neurological exam
- Complete set of tests that assess memory, attention, behavior, and other thinking skills. This set of tests will take about 2-3 hours to complete. Some of the tests will be done on paper and some may be done on a computer or ipad.
- Complete questionnaires about your health history.
- Have about 4.5 tablespoons of blood collected for research analysis. Sometimes data and samples are used for genetic research (about diseases that are passed on in families). Even if your data and samples are used for this kind of research, the results will not be told to you and will not be put in your health records
- Have a magnetic resonance imaging procedure (MRI). This procedure will involve lying still on your back for about 45 minutes in a machine that take images (“pictures”) of your brain.
- Have your study partner (a family member or close friend) answer questions each year you participate in the study.
- Have your medical records reviewed and information about your general health and thinking/memory issues recorded

The following procedures are optional and will be completed once a year if you agree. Please check YES or NO for each of the following. You can change your mind about participating in these procedures at any time:

- Lumbar Puncture: This involves insertion of a needle in the lower back and collection of about 2 tablespoons of cerebrospinal fluid. Please note that if you are taking a blood thinner or have a history of back surgery, you may not be eligible to participate in this procedure.

_____YES _____NO _____Initials

- If you come to UCH for your standard (non-research) clinical care and have a blood draw or lumbar puncture we may collect any leftover samples from these procedures for the bank.

_____YES _____NO _____N/A_____Initials

- Contact for future research recruitment: If you agree, we would like to use the data collected in this study to assess if you may be eligible for future clinical research studies.

  I am interested in being contacted for participation in future research studies:

_____YES _____NO _____Initials

- Contact for University of Colorado Alzheimer's and Cognition Center newsletter and events: If you agree, we would like to send you a newsletter and schedule of the center's events.

  I am interested in being contacted for center news and events:
_____YES _____NO _____Initials

What are the possible discomforts or risks?

Risks of Having Blood Taken

We will need to get about 4.5 tablespoons of blood from you at each blood draw. We will get blood by putting a needle into one of your veins and letting the blood flow into a glass tube. You may feel some pain when the needle goes into your vein. A day or two later, you may have a small bruise where the needle went under the skin. The risks of having blood taken in the study is comparable with the risk of donating a unit of blood but the amount is only 1/6th as much.

Risks of Thinking/Memory Testing

The only risks associated with the thinking/memory testing is possible boredom or fatigue. You will be able to stop this testing at any time if you wish.

Risks of Having an MRI

In this study we will take Magnetic Resonance Images (MRI's) of your head. The MRI machine uses powerful magnetic waves to take pictures inside the body. The waves themselves are not harmful, but they can cause metal to heat up and electronics to stop working.

You should NOT have an MRI if you have metal or electronic devices inside your body. Heart pacemakers and insulin pumps are examples of electronic devices.

The MRI machine is a small round tube. It might make you uncomfortable if you do not like tight spaces.

The most common side effect of having an MRI is flashing lights in the eyes. This is caused by the magnetic waves and is not harmful. Some people also experience warmth and reddening of the skin. This usually goes away after a few minutes.

Risks of Having a Lumbar Puncture

Risks associated with lumbar puncture (LP) include:

- Pain
- Bleeding
- Infection
- headache

To limit discomfort, the skin, surrounding tissue and underlying muscle will be numbed with a drug called lidocaine. This is similar to the numbing medication delivered during a dental procedure, such as a tooth extraction. Lidocaine injection may cause a burning sensation. This sensation is brief and generally associated only with the administration of the drug.

During the LP you may experience:

- Brief pain lasting seconds in the lower back during insertion of the needle, especially if the needle contacts bone. This occurs about 1 in 5 times.
- Less than 1 in 10 patients experience a brief pain shooting down the leg if the LP needle touches a nerve root. Rarely, this sensation or some numbness in the leg may persist for days.

- Less than 1 in 100 patients are allergic to the iodine solution used to clean their back and will develop rash.
- After LP, 5 to 15 out of 100 individuals develop a headache that is worse when standing and disappears when lying down. It typically begins 6-48 hours after the LP, and may last 1 to 6 days. Lying flat and taking caffeine sodium benzoate will treat the headache.
- About 3 in 100 individuals with post-LP headache also develop double vision that can last a few days to a few weeks.
- About 3 in 100 individuals will develop temporary hearing problems or ringing in the ears lasting a few days.
- After a successful LP, less than 2 in 100 individuals will have a little bleeding at the LP site. This clears rapidly.
- Rarely, infection may occur at or near the LP site.

To reduce the likelihood of a temporary headache after the LP, you will be asked to lie on your back for a short period of time after the procedure. You will not receive any routine drugs for the purpose of this study. Post-LP headache may be treated with oral hydration, analgesics such as acetaminophen, or ibuprofen and caffeine. If the headache persists for more than 7 days, an epidural blood patch may be advised for relief. If you require a blood patch, you or your insurance company will have to pay for that care. This procedure involves the collection of blood from your arm, which is then administered into the epidural space outside the membranes surrounding the spinal cord to seal the LP needle tract that is leaking fluid. The procedure is similar to that of a LP and carries similar risks for discomfort, bleeding and infection. It generally cures the headache in 10 to 15 minutes.

Because of the risk of temporary visual blurring or headache, you should arrange for transportation after the procedure in case you are temporarily unable to drive.

Risks of Genome Wide Association Studies (GWAS)

Should data be submitted to the GWAS database, it will be coded, meaning it will not include anything that might directly identify me. There is a slight risk there could be a breach in the security of this database system resulting in the unauthorized access to my information. Safeguards are in place to minimize this risk. Data is for broad sharing with approved investigators. It is possible that a study finding could one day help people of the same race, ethnicity or sex as you. However, it is also possible through these kinds of studies that genetic traits might come to be associated with your group. In some cases this could reinforce stereotypes.

Confidentiality

Some of the CSF, blood (including DNA and RNA samples) and certain medical information about you (for example, diagnosis and age) may be shared with other

scientists or companies outside of UCH/UCD. However, your name, address, phone number, or any other information that would identify you will not be released. If these samples are sent to other researchers, only coded data, which does not include anything that might directly identify you, will be shared. Blood may also be used in the future to generate cell lines. Cell lines are made from white blood cells that are mixed with a solution that allows for permanent cell growth, immortalizing cells for future genetic research. The cell lines may be stored at The National Cell Repository for Alzheimer's disease (NCRAD) which is a National Institutes of Health funded facility that provides genetic materials to researchers throughout the country. The cell lines stored at NCRAD will be identified with a code number. Other researchers will not receive your name or other identifying information. Coded data linked to cell lines will be kept on a secure computer at NCRAD. This information can only be accessed by authorized investigators. A summary of coded data will be made available to researchers via a website that will be maintained by NCRAD. Centers may also be requested to contribute other biological samples such as serum and cerebrospinal fluid, using agreed upon protocols, for trans-center studies examining biomarkers that might relate to risk, diagnosis or progression of AD.

There is a risk that people outside of the research team will see your research information. Only HIPAA-compliant video platforms will be used during remote evaluations to protect your confidentiality. We will do all that we can to protect your information, but it cannot be guaranteed. There may be risks that are unknown at this time.

Genetic Information Nondiscrimination Act (GINA)

A Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. This law generally will protect you in the following ways:

- Health insurance companies and group health plans may not request your genetic information that we get from this research.
- Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility or premiums.
- Employers with 15 or more employees may not use your genetic information that we get from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment.

All health insurance companies and group health plans must follow this law by May 21, 2010. All employers with 15 or more employees must follow this law as of November 21, 2009.

Be aware that this new Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

What are the possible benefits?

This study is designed for the researcher to learn more about Alzheimer's disease (AD), Down Syndrome (DS) and other diseases that cause thinking and memory problems.

This study is not designed to treat any illness or to improve your health. Also, there may be risks, as discussed in the section describing the discomforts or risks.

Will I be paid?

You will not be paid for participation in this study.

Can these research results be used in my clinical care?

The assessments and tests done in this study are NOT the same as those used to diagnose medical problems and are done for research purposes. We will not tell you or your doctor the results. There will be no formal radiological read on your MRI scan.

However, when these tests are performed for research, there is a chance of finding something medically unexpected. Unexpected findings can be medically important or medically unimportant. Findings that are medically important have clear clinical significance and are those for which treatment might be available. You will only be informed of findings with clear significance. You will not be informed of findings judged as medically unimportant by our clinicians.
If you have concerns about your risk of developing Alzheimer's disease, you should talk to your clinical doctor.

Will I have to pay for anything?

It will not cost you anything to be in the study.

Is my participation voluntary?

Taking part in this study is voluntary. You have the right to choose not to take part in this study. If you choose to take part, you have the right to stop at any time. If you refuse or decide to withdraw later, you will not lose any benefits or rights to which you are entitled.

Can I be removed from this study?

The study doctor may decide to stop your participation without your permission if the study doctor thinks that being in the study may cause you harm, or for any other reason.

What happens if I am injured or hurt during the study?

If you have an injury while you are in this study, you should call Dr. Bettcher immediately. Her phone number is303-724-3941.

We will arrange to get you medical care if you have an injury that is caused by this research. However, you or your insurance company will have to pay for that care.

Who do I call if I have questions?

The researcher carrying out this study is Dr. Brianne Bettcher. You may ask any questions you have now. If you have questions, concerns, or complaints later, you may call Dr. Bettcher at303-724-3941. You will be given a copy of this form to keep.

You may have questions about your rights as someone in this study. You can call Dr. Bettcher with questions. You can also call the responsible Institutional Review Board (COMIRB). You can call them at 303-724-1055.

You may also talk to a Subject Advocate at the Clinical and Translational Research Center (CTRC) The number there is 720-848-6662.

Who will see my research information?

The University of Colorado Denver (UCD) and its affiliated hospital(s) have rules to protect information about you. Federal and state laws including the Health Insurance Portability and Accountability Act (HIPAA) also protect your privacy. This part of the consent form tells you what information about you may be collected in this study and who might see or use it.

The institutions involved in this study include

- University of Colorado Denver
- University of Colorado Hospital

We cannot do this study without your permission to see, use and give out your information. You do not have to give us this permission. If you do not, then you may not join this study.

We will see, use and disclose your information only as described in this form and in our Notice of Privacy Practices; however, people outside the UCD and its affiliate hospitals may not be covered by this obligation.

We will do everything we can to maintain the confidentiality of your personal information but confidentiality cannot be guaranteed.

The use and disclosure of your information has no time limit. You can cancel your permission to use and disclose your information at any time by writing to the study's Principal Investigator (PI), at the name and address listed below. If you do cancel your

permission to use and disclose your information, your part in this study will end and no further information about you will be collected. Your cancellation would not affect information already collected in this study.

Brianne Bettcher, PhD
12469 E. 17th Place, Mail stop F429
Aurora, CO 80045

Both the research records that identify you and the consent form signed by you may be looked at by others who have a legal right to see that information, such as:

- Federal offices such as the Food and Drug Administration (FDA) and the Office of Human Research Protections (OHRP) that protect research subjects like you.
- People at the Colorado Multiple Institutional Review Board (COMIRB)
- The study doctor and the rest of the study team.
- Officials at the institution where the research is conducted and officials at other institutions involved in this study who are in charge of making sure that we follow all of the rules for research

In addition, other researchers outside of UCD may also have access to your de-identified data. Specifically, the results of some of the thinking tests done on an ipad will be shared with the University of California San Francisco Memory and Aging Center. Your name or any other information that identifies you will not be shared.

We might talk about this research study at meetings. We might also print the results of this research study in relevant journals. But we will always keep the names of the research subjects, like you, private.

You have the right to request access to your personal health information from the Investigator.

Information about you that will be seen, collected, used and disclosed in this study:

- Portions of your previous and current Medical Records that are relevant to this study, including but not limited to Diagnosis(es), History and Physical, laboratory studies, radiology studies, procedure results
- Research Visit and Research Test records
- Imaging studies
- Psychological tests

What happens to Data, Blood, and Specimens that are collected in this study?

Scientists at the University of Colorado Denver and the hospitals involved in this study work to find the causes and cures of disease. The data, blood and specimens collected

from you during this study are important to this study and to future research. If you join this study:

- The data, blood, or other specimens given by you to the investigators for this research no longer belong to you.
- Both the investigators and any sponsor of this research may study your data, blood, or other specimens collected from you. De-identified specimens may also be shared with other scientists or companies outside of UCH/UCD.
- If data, blood, or other specimens are in a form that identifies you, UCD or the hospitals involved in this study may use them for future research only with your consent or Institutional Review Board (IRB) approval.
- Any product or idea created by the researchers working on this study will not belong to you.
- There is no plan for you to receive any financial benefit from the creation, use or sale of such a product or idea.

HIPAA Authorization for Optional Additional Study Procedures

In this form, you were given the option to agree to optional study procedures and to having your data used to assess eligibility for future research. You must also give us your permission, under HIPAA rules, to use and disclose the information collected from these optional procedures, as described above.

If you decline to give us permission to use and disclose your information, you cannot take part in these optional procedures, but you can still participate in the main study. Please initial next to your choice:

_____ I give permission for my information, from the optional procedures I have agreed to above, to be used and disclosed as described in this section.

_____ I **do not** give permission for my information for any optional procedures to be used and disclosed; I understand that I will not participate in any optional procedures.

Agreement to be in this study and use my data

I have read this paper about the study or it was read to me. I understand the possible risks and benefits of this study. I understand and authorize the access, use and disclosure of my information as stated in this form. I know that being in this study is voluntary. I choose to be in this study: I will get a signed and dated copy of this consent form.

Signature:_____ Date:_____

Print Name:_____

Consent form explained by:_____ Date:_____

Print Name: _____

_____ Date_____
Legally Authorized Representative (if applicable)

Print Name: _____

For use with non-English speaking and non-reading subjects:

Witness Signature: _____ Date_____

Witness Print Name:_____

Witness of Signature ☐

Witness of consent process ☐

Principal Investigator: **Kavita Nair, PhD**
COMIRB No: **21-3703**
Version Date: **15Dec2021**

Study Title: Establishing Demographic and Comorbidity Specific Reference Ranges in Healthy Controls to Assess the Value of CNS Biomarkers in Multiple Sclerosis and Others Neurodegenerative Disorders

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Some people in this study may be under the age of 18. If you are a parent/guardian and have been asked to decide for a child under 18 whether they should be in this study, please read this consent form carefully. In this form, we use the words "you" and "your." If you are reading this form and deciding for a child, the words 'you' and 'your' refer to the child, not to you.

You are being asked to be in a research study. This form provides you with information about the study. A member of the research team will describe this study to you and answer all of your questions. Please read the information below and ask questions about anything you don't understand before deciding whether or not to take part.

Why is this study being done?

This study plans to learn more about the amounts of certain substances in the blood of healthy individuals of different ages, weights, and genders. This information will be compared to data from patients with multiple sclerosis in the future.

You are being asked to be in this research study because you are a healthy individual between the ages of 13 and 85.

Other people in this study

Up to 400 people from your area will participate in the study.

Up to 600 people around the country will be in the study.

What happens if I join this study?

If you join the study, you will:

- Complete a questionnaire about your medical history
- Have your blood pressure taken
- Have about 4 teaspoons of blood drawn

These procedures should take about 30 minutes.

If the study doctors need clarification ion any of your medical history information, they may call you at a later date to obtain more information.

Optional: height and weight
If you are willing, we would also like to record your height and weight. This is optional. Please indicate you decision by checking one of the options below and writing your initials:

_____ YES, I agree to have the study team measure my height and weight

_____ NO, I do not want the study team to measure my height and weight

_____ Initials

Optional: contact for future research

I give my permission for my study doctor (or someone he or she chooses) to keep my contact and basic health information for purposes of contacting me in the future to ask me to take part in more research.

_____Yes _____ No _____Initials

Optional Consent for Data and Specimen Banking for Future Research

The study doctors would like to keep some of the blood that is taken during the study but is not used for other tests. If you agree, the data and samples will be kept and may be used in future research to learn more about autoimmune diseases. The research that is done with your data and samples is not designed to specifically help you.

The choice to let the study doctors keep the data and samples for future research is up to you. No matter what you decide to do, you can still take part in this study. If you decide now that your data and samples can be kept for research, you can change your mind at any time and contact your study doctor, and they will no longer be used for research. Otherwise, they may be kept until they are used up, or until the study doctors decide to destroy them.

When your data and samples are given to other researchers in the future, the study doctors will not give them your name, address, phone number or any other information that will let the researchers know who you are.

Sometimes data and samples are used for genetic research (about diseases that are passed on in families). Even if your data and samples are used for this kind of research, the results will not be told to you and will not be put in your health records. Your data and samples will only be used for research and will not be sold. The research done with your data and samples may help to develop new products in the future, but there is no plan for you to be paid.

We may share data from our research with other researchers or data banks. One such data bank is called dbGAP, which collects genetic and other data and is sponsored by the National Institutes of Health. By broadly sharing data in data banks like this, we can make our discoveries more accessible to other researchers. Information which directly identifies you will not be sent to these data banks.

Because your genetic information is unique to you, there is a small risk that someone could connect the information back to you. Also, genetic research and broadly sharing data may involve risks to you or people like yourself that are unknown at this time.

The possible benefits of research from your data and samples include learning more about diseases, how to prevent them and how to treat them. The greatest risk to you is the release of your private information. The research team will protect your records so that your name, address and phone number will be kept private. The chance that this information will be given to

someone else is very small. There will be no cost to you for any data or sample collection and storage.

Please read the sentence below and think about your choice. After reading each sentence, check “yes” or “no.” If you have questions, please talk to your doctor or nurse. Remember, no matter what you decide to do about the storage and future use of your data and samples, you may still take part in the study.
I give my permission for my data and blood to be stored in a central tissue bank at University of Colorado Anschutz Medical Campus for use in future research:

_____ YES _____ NO _____ Initials

What are the possible discomforts or risks?

In this study, we will need to get about 4 teaspoons of blood from you. We will get blood by putting a needle into one of your veins and letting the blood flow into a glass tube. You may feel some pain when the needle goes into your vein. A day or two later, you may have a small bruise under the skin.

Other possible risks include boredom/discomfort while filling out the medical history information.

There is a risk that people outside of the research team will see your research information. We will do all that we can to protect your information, but it can not be guaranteed.

What are the possible benefits of the study?

This study is designed for the researcher to learn more about the amounts of certain substances in the blood of healthy individuals.

This study is not designed to treat any illness or to improve your health.

Who is paying for this study?

This research is being funded by Bristol Myers Squibb.

Will I be paid for being in the study?

You will be paid $20 gift card for having your blood drawn for this study.

It is important to know that payments for participation in a study is taxable income.

Will I have to pay for anything?

It will not cost you anything to be in the study.

Is my participation voluntary?

Taking part in this study is voluntary. You have the right to choose not to take part in this study. If you choose to take part, you have the right to stop at any time. If you refuse or decide to withdraw later, you will not lose any benefits or rights to which you are entitled.

Can I be removed from this study?

The study doctor may decide to stop your participation without your permission if the study doctor thinks that being in the study may cause you harm, or for any other reason.

Who do I call if I have questions?

The researcher carrying out this study is Dr. Kavita Nair. You may ask any questions you have now. If you have questions, concerns, or complaints later, you may call Dr. Nair at 303-724-2635. You will be given a copy of this form to keep.

You may have questions about your rights as someone in this study. You can call Dr. Nair with questions. You can also call the responsible Institutional Review Board (COMIRB). You can call them at 303-724-1055.

Who will see my research information?

The University of Colorado Denver | Anschutz Medical Campus (the University) and its affiliated health systems have rules to protect information about you. Federal and state laws including the Health Insurance Portability and Accountability Act (HIPAA) also protect your privacy. This part of the consent form tells you what information about you may be collected in this study and who might see or use it.

The institutions involved in this study include:

- University of Colorado Denver | Anschutz Medical Campus
- University of Colorado Health

We cannot do this study without your permission to see, use and give out your information. You do not have to give us this permission. If you do not, then you may not join this study.

We will see, use and disclose your information only as described in this form and in our Notice of Privacy Practices; however, people outside the University and its affiliate hospitals may not be covered by this obligation.

We will do everything we can to maintain the confidentiality of your personal information but confidentiality cannot be guaranteed.

The use and disclosure of your information has no time limit. You can cancel your permission to use and disclose your information at any time by writing to the study's Principal Investigator (PI), at the name and address listed below. If you do cancel your permission to use and disclose your information, your part in this study will end and no further information about you will be collected. Your cancellation would not affect information already collected in this study.

Kavita Nair, PhD
12850 East Montview Blvd, C-238
Aurora, Colorado 80045

Both the research records that identify you and the consent form signed by you may be looked at by others who have a legal right to see that information, such as:

- Federal offices such as the Food and Drug Administration (FDA) and the Office of Human Research Protections (OHRP) that protect research subjects like you.
- The Institutional Review Board that is responsible for overseeing this research

- The study doctor and the rest of the study team.
- Bristol Myers Squibb, who is the company paying for this research study.
- Officials at the institution where the research is conducted and officials at other institutions involved in this study who are in charge of making sure that we follow all of the rules for research

We might talk about this research study at meetings. We might also print the results of this research study in relevant journals. But we will always keep the names of the research subjects, like you, private.

You have the right to request access to your personal health information from the Investigator.

Information about you that will be seen, collected, used and disclosed in this study:

- Name and Demographic Information (age, sex, ethnicity, address, phone number, etc.)
- Information about your medical history that is relevant to this study, including but not limited to Diagnosis(es), History and Physical, laboratory or tissue studies, radiology studies, procedure results

What happens to Data and Blood that are collected in this study?

Scientists at the University and the health systems involved in this study work to find the causes and cures of disease. The data, tissue, blood and specimens collected from you during this study are important to this study and to future research. If you join this study:

- The data and blood given by you to the investigators for this research no longer belong to you.
- Both the investigators and any sponsor of this research may study your data and blood, collected from you.
- If data or blood are in a form that identifies you, the University or the health systems involved in this study may use them for future research only with your consent or Institutional Review Board (IRB) approval.
- Any product or idea created by the researchers working on this study will not belong to you.
- There is no plan for you to receive any financial benefit from the creation, use or sale of such a product or idea.

HIPAA Authorization for Optional Additional Study Procedures

In this form, you were given the option to agree to additional, optional research procedures. You must also give us your permission, under HIPAA rules, to use and disclose the information collected from these optional procedures, as described above.

If you decline to give us permission to use and disclose your information, you cannot take part in these optional procedures, but you can still participate in the main study. Please initial next to your choice:

_____ I give permission for my information, from the optional procedures I have agreed to above, to be used and disclosed as described in this section.

_____ I do not give permission for my information for any optional procedures to be used and disclosed; I understand that I will not participate in any optional procedures.

Agreement to be in this study and use my data

I have read this paper about the study or it was read to me. I understand the possible risks and benefits of this study. I understand and authorize the access, use and disclosure of my information as stated in this form. I know that being in this study is voluntary. I choose to be in this study: I will get a signed and dated copy of this consent form.

Subject or Parent/Guardian Signature:_____Date:_____

Print Name:_____

Consent form explained by:_____ Date:_____

Print Name:_____

----------Use the following only if applicable----------

Signature: _____ Date:_____
(Child Subject 13-17 years old; ***In addition*** to Parent Signature)

Print Name:_____

[**If Applicable**, add Signature Line for witness; required for consent of non-reading subjects and consent using a short form]

----------Use the following only if applicable----------

A signature of a witness is required for consent of non-reading subjects and consent using a short form.

Witness Signature: _____ Date:_____

Print Name:_____

Witness of Signature ☐

Witness of consent process ☐

Study Title: Research to Develop the Human Trisome Project Biobank

In this consent form the word 'you' refers to you or your dependents.

You are being asked to be in a research study. This form provides you with information about the study. A member of the research team will describe this study to you and answer all of your questions. Please read the information below and ask questions about anything you don't understand before deciding whether or not to take part.

In this form, we use the words "you" and "your." If you are reading this form and deciding for someone else, the words "you" and "your" refer to that other person, not to you.

Why is this study being done?

This study plans to learn more about Down syndrome (DS) and other medical conditions that are more likely to affect people with DS. Family members of people with DS and unrelated people may also join the study for comparison.

The investigators want to make it easier to learn about the symptoms, causes, and long-term changes in DS and other co-occurring conditions. We will do this by collecting your health data and biological samples and storing all this information in one place. We will then share de-identified data and samples with other approved researchers. These researchers may also be studying other conditions in addition to DS, and so samples and data may be used for future, unspecified research. This centralized system helps increase the speed of DS research and research overall.

The investigators and other approved researchers will use both clinical information and biological samples from the same people to look at relationships between physical symptoms, behavior, genes, and other parts of human biology, such as proteins and metabolites.

By looking at all of these different pieces together, we can begin to unravel why some people with Down syndrome are pre-disposed to some co-occurring conditions but protected from others.

Other people in this study

Up to 2500 people will participate in the study divided between those with and without DS.

What happens if I join this study?

1) You will have a blood draw.
2) We will rub a sponge or swab in your mouth.
3) We may ask you to spit into a tube or rinse your mouth and spit into a tube.
4) *Optional* - You may provide a skin tape sample, urine sample, and/or stool sample (we may send you home with (or mail) a stool sample kit).
5) *Optional* – We may bank your tissue samples that are discarded from medically necessary procedures.
6) You will answer questions about your health history, which takes 15-30 minutes.
7) We will review your medical records. You may be asked to complete a Medical Records Request Form if you see a doctor outside of the University of Colorado or Children's Hospital Colorado system. The type of information we are collecting is listed later in this consent form. If you live in Colorado, we may request your immunization records from the Colorado Immunization Information Systems (CIIS).

8) We may contact you annually for the next 4 years after your initial visit to update your health information and give additional research samples. We may also access your medical record.
9) *Optional Procedures (see Optional Procedures section for details)*
    a. You may provide a skin tape sample, urine sample, and/or stool sample (we may send you home with (or mail) a stool sample kit).
    b. We may bank your tissue samples that are discarded from medically necessary procedures.
    c. We may contact you more often than once a year for an additional blood sample.

The blood and other samples and data we collect will be de-identified, assigned a Study ID, and stored for future, unspecified use for approved research projects. The Linda Crnic Institute keeps a linker file of your Study ID and name to track your clinical information and samples and to match them with results from research analyses. The study team at the Linda Crnic Institute acts as an 'honest broker' and uses only the Study ID to identify samples and data and does not release your personal information. When we share your samples or data with collaborators for research projects, the information will not be identifiable to them, but the code will still allow the study team to link the information back to your other information. If you sign a separate consent form to be in other studies that tell you that they will ask to use samples collected through the HTP Biobank, we will work with those investigators to link your information so we can answer the study questions. Both study teams will work to ensure your privacy at all times.

We will also perform tests on the samples to get information, such as the DNA. We might also change some of the cells in the blood sample so that they live for a very long time. This allows for more research to be done with a single sample of your blood than if we did not change these cells.

Your health information from your medical records and a health information questionnaire that you fill out will be entered into a password-protected and encrypted database.

As part of this research, you are giving your permission to the primary investigator to use, transfer and share your de-identified information with other researchers and companies acting on their behalf and partners of the research program here or abroad who are approved by the Human Trisome Project Steering Committee to do research with the samples in this biobank. By signing this consent form, you agree for use of your coded data in future ethically approved research and for non-identifying information to be stored on an online portal that is accessed by researchers to look at the combined information from all participants in the study.

Optional Procedures

The below are optional procedures. You do not have to agree in order to participate in the research today and participation does not affect your compensation. The risks of participating in this additional procedure(s) are minimal, including the risk of loss of privacy. Your participation is always optional, and you can end your participation at any time.

Optional Consent – Skin Swab/Tape Sample
You may provide a skin swab sample so investigators can look at the molecules (including DNA), cells and microbes on your skin. We collect this by rubbing a swab on your skin or pressing a piece of tape onto your skin. Do you agree to provide a skin sample?

☐ Yes ☐ No _____Initials

Optional Consent - Urine Sample
You may provide a urine sample so investigators can look at molecules, cells and microbes in it, and take any cells in the urine and change them to become a more immature type of cell to study many aspects of biology. Do you agree to provide a urine sample?

☐ Yes ☐ No _____Initials

Optional Consent - Stool Sample
You may provide a stool sample so investigators can look at molecules (including DNA), cells and microbes that live inside humans and help our bodies run well. Do you agree to provide a stool sample?

Participants that provide an optional stool sample will be compensated $50.

☐ Yes ☐ No _____Initials

Optional Consent – Tissue Collection
Sometimes you may have medical procedures that result in waste tissue, for example when tonsils are removed. Do you agree that we can bank your tissue samples for research purposes?

☐ Yes ☐ No _____Initials

Optional Consent – FibroScan
Some participants may be invited to undergo a non-invasive ultrasound FibroScan of the liver. The procedure will take about 30 minutes, and the results of this research procedure will be returned to you.

☐ Yes ☐ No _____Initials

Optional Consent – Additional Sample Collection
We may ask you to return for a blood draw or other sample collection sooner than once a year. This sample may be used to test our instruments, to check levels of a medication that you have reported taking, or for other research purposes. This is an additional, voluntary sample(s). Not all participants who agree will be asked for additional sample(s). **You can change your mind at any time, and it will not affect your participation in other aspects of this study**. You will be compensated $100 for each additional blood draw and updated health information. Would you like to provide an additional sample(s), including a blood draw?

☐ Yes ☐ No _____Initials

Optional Consent – Behaviors and Thinking Surveys
We may ask you or your study partner to complete surveys with questions about your behaviors, feelings, and the way you think. You may choose to take part in online only surveys or in-person assessments, or both. In-person assessments are video-recorded for scoring purposes. This study is designed to learn more about Down syndrome and how thinking is related to the molecules that are in your blood. The surveys do not all have to be completed at the same time.

Participants will be compensated $100/total to complete all surveys, with $50 for the Vineland-3 and $50 for completion of all additional surveys via cash or gift card (physical or electronic). In-person participants may receive an additional $50 in compensation if further additional assessments are performed (up to $150/total to complete all surveys).

☐ Yes ☐ No _____Initials

Optional Consent – Return of Research Results
In very rare instances, we may find results about your health that we feel are important for you and your doctor to know. We may require that these results are returned to you through a medical doctor or genetic counselor whom you choose. It is important to note that these results will then become part of your medical record, and you will be responsible for any costs associated with the return of these results and any treatment your doctor thinks is necessary. You should not expect to receive results from this study and should not rely on this study for diagnosis of any disease.

A Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate

against you based on your genetic information. This law generally will protect you in the following ways:

- Health insurance companies and group health plans may not request your genetic information that we get from this research.
- Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility or premiums.
- Employers with 15 or more employees may not use your genetic information that we get from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment.

All health insurance companies and group health plans must follow this law by May 21, 2010. All employers with 15 or more employees must follow this law as of November 21, 2009. Be aware that this new Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance. If we incidentally identify evidence of non-paternity or non-maternity, this information will never be released to you.

Would you like to be contacted with results which the study doctor feels are important for me to know?

☐ Yes ☐ No _____Initials

Optional Consent - Additional Research
Would you like to be contacted for participation in future research studies involving Down syndrome?

☐ Yes ☐ No _____Initials

What are the possible discomforts or risks?

In this study we will need to collect blood from you. You may feel some pain when the needle goes into your skin, and you may develop a small bruise. The tongue swab or saliva collection may feel slightly uncomfortable. There is a small risk that people outside of the research team will see your research information. We will do all that we can to protect your information, but it cannot be guaranteed.

What are the possible benefits of the study?

This study is designed to learn more about Down syndrome and related conditions. Although there are no direct benefits to you, it may help future generations understand the unique issues associated with Down syndrome.

This study is not designed to treat any illness or to improve your health. The information collected will not be put in your medical record.

Who is paying for this study?

This research is being sponsored by the Linda Crnic Institute for Down Syndrome and National Institutes of Health (NIH), National Institute of Neurological Disorders and Stroke (NINDS), National Institute of Child Health and Human Development (NICHD), National Institute on Deafness and Other Communication Disorders (NIDCD), National Institute on Aging (NIA) and the GLOBAL Down Syndrome Foundation.

Will I be paid for being in the study?

You will be paid to participate in this study. You will be paid $100 for participating in the first blood draw and completing the first survey. You will be paid $100 for each additional blood draw and survey completion up to five annual visits. The total amount you could be paid is $500 per year. Additional optional visits or procedures may increase the amount you are paid in total.

It is important to know that payments for participation in a study are taxable income.

Will I have to pay for anything?

Linda Crnic Institute for Down Syndrome will provide the tests and procedures at no cost during this study. Tests and procedures that are done only for the study will not be billed to you or your insurance company. You or your insurance company may be billed for any standard medical care given during this research study.

Is my participation voluntary?

Taking part in this study is voluntary. You have the right to choose not to take part in this study. If you choose to take part, you have the right to stop at any time. If you refuse or decide to withdraw later, you will not lose any benefits or rights to which you are entitled.

Can I be removed from this study?

The study doctor may decide to stop your participation without your permission if the study doctor thinks that being in the study may cause you harm, or for any other reason.

Who do I call if I have questions?

The researcher carrying out this study is Joaquín M. Espinosa, PhD. You may ask any questions you have now. If you have questions, concerns, or complaints later, you may call Dr. Espinosa at (303) 724-7389. You will be given a copy of this form to keep.

You may have questions about your rights as someone in this study. You can call Dr. Espinosa with questions at (303) 724-7389. You can also call the Colorado Multiple Institutional Review Board (COMIRB) at (303) 724-1055.

Who will see my research information?

The University of Colorado Denver (UCD) and its affiliated hospital(s) have rules to protect information about you. Federal and state laws including the Health Insurance Portability and Accountability Act (HIPAA) also protect your privacy. This part of the consent form tells you what information about you may be collected in this study and who might see or use it.

The institutions involved in this study include:

- University of Colorado Denver
- University of Colorado Hospital
- Children's Hospital Colorado (Children's Colorado)

Children's Colorado shares a medical record system with the Barbara Davis Center and PedsConnect; therefore, it is also possible that your information could be viewed by healthcare professionals at these organizations.

It is possible that in the future de-identified genetic information could be sent to a national database (e.g. dbGAP).

We cannot do this study without your permission to see, use and give out your information. You do not have to give us this permission. If you do not, then you may not join this study.

We will see, use and disclose your information only as described in this form and in our Notice of Privacy Practices; however, people outside the UCD and its affiliate hospitals may not be covered by this obligation. We will do everything we can to maintain the confidentiality of your personal information, but confidentiality cannot be guaranteed.

The use and disclosure of your information has no time limit. You can cancel your permission to use and disclose your information at any time by writing to the study Principal Investigator (PI), at the name and address listed below. If you do cancel your permission to use and disclose your information, your

part in this study will end and no further information about you will be collected. Your cancellation would not affect information already collected in this study.

Joaquín Espinosa, PhD

Linda Crnic Institute for Down Syndrome, Mail Stop 8608

12700 E 19th Ave. Aurora, CO 80045

Both the research records that identify you and the consent form signed by you may be looked at by others who have a legal right to see that information, such as:

- Federal offices such as the Food and Drug Administration (FDA) and the Office of Human Research Protections (OHRP) that protect research participants like you.
- People at the Colorado Multiple Institutional Review Board (COMIRB).
- The study doctor and the rest of the study team.
- Officials at the institution where the research is conducted and officials at other institutions involved in this study who are in charge of making sure that we follow all of the rules for research.
- Research collaborators or external companies that perform analyses related to the study goals who may receive samples with a Study ID and/or limited data sets.

We might talk about this research study at meetings. We might also print the results of this research study in relevant journals. But we will always keep the names of the research participants, like you, private.

You have the right to request access to your personal health information from the Investigator.

The Investigator (or staff acting on behalf of the Investigator) will use your information for the research outlined in this consent form. They will also make *all or some* of the following health information about you collected in this study available to people who will look at the DNA, protein and other aspects of your blood, and your provider if you wish.

Information about you that will be collected in this study (information that does not identify you will be shared with investigators accessing your samples):

- Name and Demographic Information (age, sex, ethnicity, address, phone number, etc.
- Portions of your previous and current Medical Records that are relevant to this study, including but not limited to diagnosis(es), history and physical, laboratory or tissue studies, radiology studies, procedure results, immunizations.
- Research visit and research test records.
- Psychological and mental health tests.
- Tissue samples and the data with the samples.

A Certificate of Confidentiality has been obtained from the Federal Government for this study to help insure your privacy. This Certificate means that the researchers cannot be forced to disclose information that may identify you, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative or other proceedings. But, if you request disclosure, we can release the information.

The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally funded projects or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA).

What will happen to my recorded information?

In this study, we will be recording the administration of the optional in-person cognitive assessments and photographs of your skin, if you have agreed to these procedures. We will use digital video and audio recordings and digital photographs, which will be kept on a secure and access-restricted computer drive. We will keep this information secure and private. We will store it for up to 50 years. At the end of that time, we will destroy it.

What happens to data, tissue, blood and specimens that are collected in this study?

Scientists at the University of Colorado Denver and the hospitals involved in this study work to find the causes and cures of disease. The data, tissue, blood and specimens collected from you during this study are important to this study and to future research. If you join this study:

- The data, tissue, blood, or other specimens given by you to the investigators for this research no longer belong to you.
- Both the investigators and any sponsor of this research may study your data, tissue, blood, or other specimens collected from you.
- If data, tissue, blood, or other specimens are in a form that identifies you, UCD or the hospitals involved in this study may use them for future research only with your consent or Institutional Review Board (IRB) approval.
- Any product or idea created by the researchers working on this study will not belong to you.
- There is no plan for you to receive any financial benefit from the creation, use or sale of such a product or idea.

HIPAA Authorization for Optional Additional Study Procedures

In this form, you were given the option to agree to additional, optional research procedures. You must also give us your permission, under HIPAA rules, to use and disclose the information collected from these optional procedures, as described above.

Some of these optional procedures may involve genetic testing or the use of your genetic information. Your genetic information will not be released to others.

If you decline to give us permission to use and disclose your information, you cannot take part in these optional procedures, but you can still participate in the main study. Please initial next to your choice:

_____ I give permission for my information, from the optional procedures I have agreed to above, to be used and disclosed as described in this section.

_____ I **do not** give permission for my information for any optional procedures to be used and disclosed; I understand that I will not participate in any optional procedures.

Consent and Authorization Form

Agreement to be in this study and use my data

I have read this paper about the study or it was read to me. I understand the possible risks and benefits of this study. I understand and authorize the access, use and disclosure of my information as stated in this form. I know that being in this study is voluntary. I choose to be in this study. I will get a signed and dated copy of this consent form.

Permission to Enroll

I have been told about the study. I know what is expected of me. I was allowed to ask questions. I had all my questions answered. I give permission to enroll in this study.

Self-Consent - *(over 18 without guardian/representative)*

_____ Date: _____
Signature of Participant

_____
Printed Name of Participant

Legal Guardian or Legally Authorized Representative (LAR) - *(as applicable: Parent, Legal Guardian, MDPOA for Healthcare, LAR)*

_____
Name of Participant

_____ Date: _____
Signature of Parent, Legal Guardian, MDPOA for Healthcare or LAR

_____
Printed Name of Parent, Legal Guardian, MDPOA for Healthcare or LAR

Witness - *required for consent of non-English speaking or non-reading participants*

_____ Date: _____
Signature of Witness (non-study team member)

_____
Printed Name of Witness (non-study team member)

Self-Assent - *for children aged 13-17 who can read and understand this form*

_____ Date: _____
Signature of Child (consent must also be signed by Parent, Guardian or LAR)

FOR STUDY STAFF USE ONLY

Consent form explained by -

_____ Date: _____
Signature of Study Team

_____
Printed Name of Study Team

The FREQ Procedure

Frequency
Row Pct

Table 1 of Treatment by gender

Controlling for data_set=Leukine Study

Treatment	gender		
	Female	Male	Total
External AD	0 .	0 .	0
External Controls	0 .	0 .	0
External MCI	0 .	0 .	0
External T21	0 .	0 .	0
Leukine	12 60.00	8 40.00	20
Placebo	11 55.00	9 45.00	20
Total	23	17	40

Frequency
Row Pct

Table 2 of Treatment by gender

Controlling for data_set=Bio AD

Treatment	gender		
	Female	Male	Total
External AD	6 46.15	7 53.85	13
External Controls	48 69.57	21 30.43	69
External MCI	16 50.00	16 50.00	32
External T21	0 .	0 .	0
Leukine	0 .	0 .	0
Placebo	0 .	0 .	0
Total	70	44	114

Frequency
Row Pct

Table 3 of Treatment by gender

Controlling for data_set=HTP

Treatment	gender		
	Female	Male	Total
External AD	0 .	0 .	0
External Controls	56 54.37	47 45.63	103
External MCI	0 .	0 .	0
External T21	150 47.47	166 52.53	316

The FREQ Procedure

Frequency
Row Pct

Table 3 of Treatment by gender

Controlling for data_set=HTP

Treatment	gender		
	Female	Male	Total
Leukine	0 .	0 .	0
Placebo	0 .	0 .	0
Total	206	213	419

Frequency
Row Pct

Table 4 of Treatment by gender

Controlling for data_set=Nair

Treatment	gender		
	Female	Male	Total
External AD	0 .	0 .	0
External Controls	93 64.14	52 35.86	145
External MCI	0 .	0 .	0
External T21	0 .	0 .	0
Leukine	0 .	0 .	0
Placebo	0 .	0 .	0
Total	93	52	145

The MEANS Procedure

Analysis Variable : Age										
data_set	Treatment	N Obs	N	Mean	Std Dev	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Leukine Study	Leukine	20	20	66.9500000	6.4845971	56.0000000	62.0000000	67.5000000	71.0000000	80.0000000
	Placebo	20	20	70.1500000	6.4176976	55.0000000	66.0000000	72.5000000	74.5000000	78.0000000
Bio AD	External AD	13	13	66.9230769	9.0596315	54.0000000	57.0000000	68.0000000	73.0000000	81.0000000
	External Controls	69	69	69.5072464	6.3630902	53.0000000	65.0000000	70.0000000	73.0000000	83.0000000
	External MCI	32	32	73.6562500	5.8287905	63.0000000	70.0000000	73.5000000	78.5000000	87.0000000
HTP	External Controls	103	103	28.1242984	15.2107619	1.7998047	14.5996094	27.6992188	38.6953125	61.2968750
	External T21	316	316	23.1516932	12.2932819	0.9772949	13.9492188	22.7968750	31.7656250	57.5937500
Nair	External Controls	145	145	40.8688578	18.9390208	16.3984375	24.5000000	35.7968750	54.8984375	85.8906250

Supplemental Table S5 related to Figures 1-3: ICFs and Demographics of all human data sets.

Supplementary Table S6: Inclusion-Exclusion

Criteria Bio-AD Longitudinal Observational Study

Healthy Control Bio-AD Participants (n=69):

Community dwelling older adults age 53+ with the following exclusions:

1. No diagnosis of MCI or Dementia and no evidence of a neurodegenerative phenotype based on a neurological exam.
2. No major psychiatric disorder, current non-AD neurological condition known to affect cognition (e.g., Parkinson's disease; large vessel infarct; multiple sclerosis),
3. No current evidence or history in the past 2 years of a focal brain lesion, current substance abuse, significant systemic medical illness or active neoplastic disease (e.g., active cancer), significant sensory or motor deficits that would interfere with cognitive testing, or traumatic brain injury with loss of consciousness greater than 5 minutes.

Of the 69 Bio-AD healthy control participants, 4 (5.80%) affirmatively had diabetes, all of them Type 2, 19 (27.54%) affirmatively had recent/active depression, 2 (2.90%) affirmatively had remote/inactive hypertension, 37 (53.62%) affirmatively had recent/active hypercholesterolemia, 3 (4.35%) affirmatively had remote/inactive hypercholesterolemia, 13 (18.84%) affirmatively had a depression episode within the last two years, and 18 (26.09%) affirmatively had a depression episode more than two years ago. Missing or unknowns were counted in the denominator.

Bio-AD Participants with MCI (n=32):

1. All participants were reviewed at a case consensus conference with a board-certified neuropsychologist, board-certified behavioral neurologist, and clinical research coordinator. Adjudication of MCI due to possible AD and AD dementia was based on NIA-AA clinical criteria, with additional categorization of atypical AD phenotypes based on published diagnostic criteria (e.g., Posterior Cortical Atrophy [PCA].

Of the 32 Bio-AD MCI participants, 3 (9.38%) affirmatively had diabetes, all of them Type 2, 18 (56.25%) affirmatively had recent/active depression, 2 (6.25%) affirmatively had remote/inactive hypertension, 15 (46.88%) affirmatively had recent/active hypercholesterolemia, 1 (3.13%) affirmatively had remote/inactive hypercholesterolemia, 6 (18.75%) affirmatively had a depression episode within the last two years, and 6 (18.75%) affirmatively had a depression episode more than two years ago. Missing or unknowns were counted in the denominator.

GM-CSF/sargramostim Clinical Trial Participants (AD) at Baseline (n=36):

Inclusion/exclusion criteria:

Subjects must be:
1. Age 55 to 85 years;
2. Mild-to-moderate AD diagnosis (MMSE 10-26 inclusive);
3. Evidence of elevated cortical amyloid by PET using florbetapir F18 (Amyvid)
[i.e. a positive scan], assessed qualitatively according to the Amyvid product label.
4. If on anti-dementia treatment should be on stable treatment for at least 2 months (i.e.
cholinesterase inhibitor and/or Memantine or Axona);
5. Stable on all other medications for at least 30 days prior to screen;
6. Fluent in English;
7. Physically able to participate by medical history, clinical exam and tests;
8. Have a study partner to accompany them to scheduled visits.

Exclusion criteria are:
1. Clinically relevant arrhythmias or uncontrolled hypertension;
2. Resting pulse less than 50;
3. Active cancer other than non-melanoma skin cancers;

4. Use of another investigatory drug within 2 months of screening;
5. Significant stroke or head trauma by history or MRI;
6. Contraindication for having a MRI;
7. DSM-IV criteria for a current major psychiatric disorder;
8. Sensitivity to yeast or yeast products;
9. Impaired kidney function as measured by a Glomerular Filtration Rate less than 60Ml/min;
10. Preexisting fluid retention, pulmonary infiltrates, or congestive heart failure;
11. History of moderate-to-severe lung disease;
12. History of moderate-to-severe liver disease;
13. Pregnant women, any women who feel they are likely to become pregnant during the study;
No participants enrolled had diabetes.

MS Cohort Healthy Control Participants:
We included data from individuals, ages 13-85, collected at various community events to obtain a representative sample of the Colorado population based on the US 2020 census. Participants were enrolled at community events including at local college campuses, a 5K race, outside a community of independent eateries, the Rocky Mountain Multiple Sclerosis Center Education Summits and kickoff events, the Anschutz Medical Campus Block Party, and at the University's recreational center. Individuals in the older age range were collected from the Senior's Clinic at the Anschutz Medical Campus.

The inclusion criteria were as follows:

1. Male and female participants from the ages of 13 to 85.
2. Willing and able to complete the medical history screening questionnaire.
3. Ability to get blood drawn.
4. Able to provide consent or have a parent or guardian who can provide consent.

The exclusion criteria were as follows:

- Pregnant or nursing at the time of sample collection.
- First degree relative (parent, sibling, or child) of a person diagnosed with MS
- Prior or concurrent diagnosis of Type 1 and Type 2 diabetes mellitus.
- Prior or concurrent diagnosis of a malignant neoplasm in the last 5 years (skin tumors other than melanoma were not exclusionary (e.g., basal cell or squamous cell).
- Prior or concurrent diagnosis of epilepsy or a seizure disorder.
- Prior or concurrent chemotherapy in the last 2 years.
- Brain or spinal surgery or trauma in the last 2 years.
- History of stroke at any time
- History of severe concussion or concussion with loss of consciousness in the last 2 years.
- History of having undergone cranial radiation.
- Prior known or diagnosed COVID-19 infection requiring hospitalization.

We created a screening tool that allowed self-reporting for the absence or presence of comorbidities. Specific comorbidities were reviewed by a treating neurologist specializing in MS to determine inclusion (prior or concurrent migraines) or exclusion (previous diagnosis of autoimmune disease) prior to biomarker testing.

Human Trisome Project normal controls

1. Healthy Community-dwelling volunteers
2. No diagnosis of Down syndrome
3. No serious illnesses, including cancer.

Specifically:

Control cohort from the Crnic Institute Human Trisome Project employed for analyses	
Karyotype	D21 (euploid controls), n=103
Median Age-- years (Range)	27.7 (4.2 – 61.3)
gender, no. (%)	
Female	56 (54.4%)
Male	47 (45.6%)
Official diagnosis for Down syndrome-- no. (%)	0
Institutionalized	0 (0.0%)
Medical history-- no. (% with condition) [unknown]	
Obese (BMI >30)	4/54 (7.41%) [49]
Any congenital Heart Defect (CHD)	2/103 (1.94%) [0]
Any autoimmune skin conditions (alopecia areata, atopic dermatitis/eczema, hidradenitis suppurativa, vitiligo, boils, folliculitis, and psoriasis)	3/53 (5.66%) [50]
Autism spectrum disorder	1/103 (1.00%) [0]
Anxiety	19/102 (18.63%) [1]
Depression	11/103 (10.68%) [0]
Any seizure history (infantile spasms, neonatal seizure, febrile seizure, general seizure)	2/93 (2.15%) [10]
Celiac disease	2/103 (0.97%) [0]
Any hearing loss condition (conductive hearing loss, mixed hearing loss, sensorineural hearing loss)	2/101 (1.98%) [2]
Any sleep apnea	2/40 (5.00%)[63]
Thyroid dysfunction (Grave's hyperthyroidism, Hashimoto's hypothyroidism, hypo- and hyper-thyroidism, positive antimicrosomal antibodies, positive TPO or Tg antibodies, and subclinical hypothyroidism)	6/55 (10.91%) [48]
Reactive airway disease	2/54 (3.70%) [49]
Frequency/recurrent pneumonia infection	0/73 (0%) [30]
Pulmonary hypertension	0/73 (0%) [30]
Cataracts	1/52 (1.92%) [51]
Recurrent otitis media	1/53 (1.89%) [50]
Regression	0/103 (0%) [0]

Supplemental Table S6 related to Figures 1-3: Inclusion and exclusion criteria are provided for all human data sets.

The FREQ Procedure

Frequency

Table of time_cat by Treatment

time_cat	Treatment				
	External Controls	External MCI	Leukine	Placebo	Total
External	660	64	0	0	724
Baseline	0	0	52	52	104
15 days	0	0	42	51	93
45 days	0	0	49	50	99
90 days	0	0	48	51	99
Total	660	64	191	204	1119

The MEANS Procedure

Analysis Variable : Age									
study_ind	N Obs	N	Mean	Std Dev	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
0	346	346	45.8985335	22.6908432	1.7998047	26.7968750	43.4960938	67.5000000	87.0000000
1	36	36	69.0833333	6.2990929	55.0000000	64.5000000	70.5000000	73.5000000	80.0000000

The MEANS Procedure

Analysis Variable : Age									
Treatment	N Obs	N	Mean	Std Dev	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
External Controls	314	314	43.0697216	21.8483041	1.7998047	24.6992188	39.0468750	63.0000000	85.8906250
External MCI	32	32	73.6562500	5.8287905	63.0000000	70.0000000	73.5000000	78.5000000	87.0000000
Leukine	18	18	67.8333333	6.1381162	56.0000000	64.0000000	68.5000000	71.0000000	80.0000000
Placebo	18	18	70.3333333	6.3801162	55.0000000	68.0000000	72.5000000	74.0000000	78.0000000

The Mixed Procedure

Estimates								
Label	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper
External Controls, Age = 67.8333333	2.5553	0.06570	318	38.89	<.0001	0.05	2.4260	2.6845
External MCI, Age = 67.8333333	2.9202	0.1167	30	25.02	<.0001	0.05	2.6818	3.1585
Leukine, Baseline, Age = 67.8333333	2.5667	0.1055	16.9	24.33	<.0001	0.05	2.3440	2.7894
Leukine, Day 18, Age = 67.8333333	1.8669	0.1700	11.2	10.98	<.0001	0.05	1.4937	2.2400
Leukine, Follow Up 1, Age = 67.8333333	2.4451	0.1087	14.3	22.49	<.0001	0.05	2.2123	2.6778
Leukine, Follow Up 2, Age = 67.8333333	2.4857	0.1124	16.8	22.11	<.0001	0.05	2.2482	2.7231
Placebo, Baseline, Age = 67.8333333	2.4758	0.09538	18.5	25.96	<.0001	0.05	2.2757	2.6758
Placebo, Day 18, Age = 67.8333333	2.5154	0.07371	17.1	34.13	<.0001	0.05	2.3600	2.6708
Placebo, Follow Up 1, Age = 67.8333333	2.5736	0.1099	14.3	23.41	<.0001	0.05	2.3382	2.8090
Placebo, Follow Up 2, Age = 67.8333333	2.5875	0.07071	12.4	36.60	<.0001	0.05	2.4340	2.7410
Pooled Baseline, Age = 67.8333333	2.5212	0.07111	34.7	35.46	<.0001	0.05	2.3768	2.6656
Leukine, Day 18, Age = 67.8333333, Baseline Calibrated	1.8214	0.1645	14	11.07	<.0001	0.05	1.4687	2.1741
Leukine, Follow Up 1, Age = 67.8333333, Baseline Calibrated	2.3996	0.1028	22.7	23.35	<.0001	0.05	2.1869	2.6123
Leukine, Follow Up 2, Age = 67.8333333, Baseline Calibrated	2.4402	0.09415	27.5	25.92	<.0001	0.05	2.2472	2.6332
Placebo, Day 18, Age = 67.8333333, Baseline Calibrated	2.5609	0.08397	30.2	30.50	<.0001	0.05	2.3895	2.7323
Placebo, Follow Up 1, Age = 67.8333333, Baseline Calibrated	2.6190	0.1197	21.9	21.89	<.0001	0.05	2.3708	2.8673
Placebo, Follow Up 2, Age = 67.8333333, Baseline Calibrated	2.6330	0.08588	32.6	30.66	<.0001	0.05	2.4582	2.8078
External Controls, Age = 73.6562500	2.6195	0.07477	317	35.04	<.0001	0.05	2.4724	2.7666
External MCI, Age = 73.6562500	3.0639	0.08190	30	37.41	<.0001	0.05	2.8966	3.2312
Leukine, Baseline, Age = 73.6562500	2.7059	0.1148	21.6	23.57	<.0001	0.05	2.4676	2.9442
Placebo, Baseline, Age = 73.6562500	2.6150	0.09680	18.8	27.02	<.0001	0.05	2.4123	2.8177
Pooled Baseline, Age = 73.6562500	2.6605	0.07886	39.7	33.74	<.0001	0.05	2.5010	2.8199
Leukine vs. Placebo, Baseline	0.09093	0.1422	34.6	0.64	0.5267	0.05	-0.1979	0.3797
Day 18 vs. Baseline, Placebo	0.03966	0.08749	16.1	0.45	0.6563	0.05	-0.1457	0.2250
Day 18 vs. Baseline, Leukine	-0.6998	0.1618	12.9	-4.33	0.0008	0.05	-1.0497	-0.3500
Follow Up 1 vs. Baseline, Placebo	0.09781	0.1247	16	0.78	0.4441	0.05	-0.1665	0.3621
Follow Up 1 vs. Baseline, Leukine	-0.1216	0.1017	14.4	-1.20	0.2511	0.05	-0.3392	0.09596
Follow Up 2 vs. Baseline, Placebo	0.1118	0.09347	16.6	1.20	0.2486	0.05	-0.08581	0.3093
Follow Up 2 vs. Baseline, Leukine	-0.08102	0.07809	13.7	-1.04	0.3175	0.05	-0.2489	0.08686
Leukine vs. Placebo, Day 18 vs. Baseline	-0.7395	0.1839	20.2	-4.02	0.0007	0.05	-1.1230	-0.3560
Leukine vs. Placebo, Follow Up 1 vs. Baseline	-0.2194	0.1609	29.7	-1.36	0.1828	0.05	-0.5481	0.1093
Leukine vs. Placebo, Follow Up 2 vs. Baseline	-0.1928	0.1218	30	-1.58	0.1239	0.05	-0.4415	0.05594
Leukine Baseline vs. External Controls, Age = 67.8333333	0.01142	0.1243	32.2	0.09	0.9274	0.05	-0.2416	0.2645
Placebo Baseline vs. External Controls, Age = 67.8333333	-0.07952	0.1158	39.6	-0.69	0.4964	0.05	-0.3137	0.1546
Pooled Baseline vs. External Controls, Age = 67.8333333	-0.03405	0.09681	110	-0.35	0.7257	0.05	-0.2259	0.1578
Leukine Baseline vs. External MCI, Age = 67.8333333	-0.3535	0.1573	45.3	-2.25	0.0296	0.05	-0.6702	-0.03669
Placebo Baseline vs. External MCI, Age = 67.8333333	-0.4444	0.1507	48.4	-2.95	0.0049	0.05	-0.7474	-0.1414
Pooled Baseline vs. External MCI, Age = 67.8333333	-0.3989	0.1367	50.4	-2.92	0.0052	0.05	-0.6734	-0.1245

The Mixed Procedure

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper
External MCI vs. External Controls, Age = 67.8333333	0.3649	0.1339	51.5	2.72	0.0088	0.05	0.09609	0.6337
Leukine Day 18 vs. External Controls, Age = 67.8333333	-0.6884	0.1822	14.8	-3.78	0.0019	0.05	-1.0772	-0.2996
Leukine Follow Up 1 vs. External Controls, Age = 67.8333333	-0.1102	0.1270	26.5	-0.87	0.3935	0.05	-0.3711	0.1507
Leukine Follow Up 2 vs. External Controls, Age = 67.8333333	-0.06961	0.1302	30	-0.53	0.5969	0.05	-0.3355	0.1963
Placebo Day 18 vs. External Controls, Age = 67.8333333	-0.03985	0.09874	53.4	-0.40	0.6881	0.05	-0.2379	0.1582
Placebo Follow Up 1 vs. External Controls, Age = 67.8333333	0.01830	0.1281	26.1	0.14	0.8875	0.05	-0.2449	0.2815
Placebo Follow Up 2 vs. External Controls, Age = 67.8333333	0.03224	0.09652	41.8	0.33	0.7400	0.05	-0.1626	0.2271
Leukine Day 18 vs. External Controls, Age = 67.8333333, Baseline Calibrated	-0.7339	0.1771	18.9	-4.14	0.0006	0.05	-1.1048	-0.3630
Leukine Follow Up 1 vs. External Controls, Age = 67.8333333, Baseline Calibrated	-0.1557	0.1220	44.6	-1.28	0.2085	0.05	-0.4014	0.09005
Leukine Follow Up 2 vs. External Controls, Age = 67.8333333, Baseline Calibrated	-0.1151	0.1148	59.5	-1.00	0.3202	0.05	-0.3448	0.1146
Placebo Day 18 vs. External Controls, Age = 67.8333333, Baseline Calibrated	0.005614	0.1066	75.9	0.05	0.9581	0.05	-0.2067	0.2180
Placebo Follow Up 1 vs. External Controls, Age = 67.8333333, Baseline Calibrated	0.06376	0.1365	36.8	0.47	0.6432	0.05	-0.2129	0.3404
Placebo Follow Up 2 vs. External Controls, Age = 67.8333333, Baseline Calibrated	0.07771	0.1081	79.2	0.72	0.4744	0.05	-0.1375	0.2929
Leukine Baseline vs. External Controls, Age = 73.6562500	0.08637	0.1370	43.3	0.63	0.5317	0.05	-0.1899	0.3626
Placebo Baseline vs. External Controls, Age = 73.6562500	-0.00456	0.1223	47	-0.04	0.9704	0.05	-0.2506	0.2415
Pooled Baseline vs. External Controls, Age = 73.6562500	0.04091	0.1087	130	0.38	0.7072	0.05	-0.1741	0.2559
Leukine Baseline vs. External MCI, Age = 73.6562500	-0.3580	0.1410	41.5	-2.54	0.0150	0.05	-0.6427	-0.07327
Placebo Baseline vs. External MCI, Age = 73.6562500	-0.4489	0.1268	42	-3.54	0.0010	0.05	-0.7048	-0.1930
Pooled Baseline vs. External MCI, Age = 73.6562500	-0.4034	0.1137	67.5	-3.55	0.0007	0.05	-0.6303	-0.1765
External MCI vs. External Controls, Age = 73.6562500	0.4443	0.1109	94.6	4.01	0.0001	0.05	0.2242	0.6645
Study AD Age (per year)	0.02391	0.007786	22.9	3.07	0.0054	0.05	0.007801	0.04002
External MCI Age (per year)	0.02468	0.01428	30	1.73	0.0941	0.05	-0.00447	0.05384
External Controls Age (per year)	0.01104	0.001982	312	5.57	<.0001	0.05	0.007137	0.01494
Age Slopes (per year): Study AD vs External Controls	0.01287	0.008034	26	1.60	0.1212	0.05	-0.00364	0.02939
Age Slopes (per year): External MCI vs External Controls	0.01365	0.01441	31.2	0.95	0.3511	0.05	-0.01574	0.04304
Age Slopes (per year): Study AD vs External MCI	-0.00077	0.01626	45.3	-0.05	0.9623	0.05	-0.03352	0.03197

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
Leukine vs. Placebo, Change from Baseline, All	3	25.7	5.51	0.0046
Leukine vs. Placebo, Change from Baseline, Follow Ups 1 and 2	2	25.3	1.38	0.2704
Leukine vs External Controls, Age = 67.8333333, Post Baseline	3	14.6	5.28	0.0113
Placebo vs External Controls, Age = 67.8333333, Post Baseline	3	17.5	0.32	0.8075
Leukine vs External Controls, Age = 67.8333333, Post Baseline	3	14.6	5.28	0.0113

The Mixed Procedure

Contrasts				
Label	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Placebo vs External Controls, Age = 67.8333333, Post Baseline	3	17.5	0.32	0.8075
Leukine vs External Controls, Same Age, Post Baseline	4	16.5	4.70	0.0101
Placebo vs External Controls, Same Age, Post Baseline	4	22.7	0.89	0.4857
Leukine vs External Controls, Post Baseline, Overall	5	22.5	11.02	<.0001
Placebo vs External Controls, Post Baseline, Overall	5	37.1	12.61	<.0001
Leukine vs External Controls, Age = 67.8333333, Baseline Calibrated, Post Baseline	3	17.1	5.87	0.0060
Placebo vs External Controls, Age = 67.8333333, Baseline Calibrated, Post Baseline	3	17.9	0.38	0.7718
Leukine vs External Controls, Same Age, Baseline Calibrated, Post Baseline	4	19.1	5.05	0.0060
Placebo vs External Controls, Same Age, Baseline Calibrated, Post Baseline	4	22.7	0.92	0.4716
Leukine vs External Controls, Baseline Calibrated, Post Baseline, Overall	5	25.6	11.04	<.0001
Placebo vs External Controls, Baseline Calibrated, Post Baseline, Overall	5	39.2	11.46	<.0001
Age Slopes Overall	3	36.6	14.48	<.0001
Treatment x Age Slopes Interaction Overall	2	28.3	1.68	0.2040

Obs	Label	Geo_Mean_Est	Geo_Mean_CI_L	Geo_Mean_CI_U
1	External Controls, Age = 67.8333333	12.8749	11.3138	14.6515
2	External MCI, Age = 67.8333333	18.5444	14.6118	23.5353
3	Leukine, Baseline, Age = 67.8333333	13.0227	10.4230	16.2709
4	Leukine, Day 18, Age = 67.8333333	6.4679	4.4536	9.3932
5	Leukine, Follow Up 1, Age = 67.8333333	11.5315	9.1370	14.5536
6	Leukine, Follow Up 2, Age = 67.8333333	12.0092	9.4711	15.2275
7	Placebo, Baseline, Age = 67.8333333	11.8908	9.7350	14.5240
8	Placebo, Day 18, Age = 67.8333333	12.3719	10.5910	14.4522
9	Placebo, Follow Up 1, Age = 67.8333333	13.1126	10.3625	16.5927
10	Placebo, Follow Up 2, Age = 67.8333333	13.2968	11.4044	15.5032

Obs	Label	Geo_Mean_Est	Geo_Mean_CI_L	Geo_Mean_CI_U
1	External Controls, Age = 67.8333333	12.8749	11.3138	14.6515
2	External MCI, Age = 67.8333333	18.5444	14.6118	23.5353
3	Pooled Baseline, Age = 67.8333333	12.4439	10.7706	14.3771
4	Leukine, Day 18, Age = 67.8333333, Baseline Calibrated	6.1804	4.3434	8.7943
5	Leukine, Follow Up 1, Age = 67.8333333, Baseline Calibrated	11.0190	8.9076	13.6307
6	Leukine, Follow Up 2, Age = 67.8333333, Baseline Calibrated	11.4754	9.4611	13.9186
7	Placebo, Day 18, Age = 67.8333333, Baseline Calibrated	12.9474	10.9076	15.3687
8	Placebo, Follow Up 1, Age = 67.8333333, Baseline Calibrated	13.7226	10.7058	17.5895
9	Placebo, Follow Up 2, Age = 67.8333333, Baseline Calibrated	13.9153	11.6837	16.5732

Obs	Label	Geo_Mean_Est	Geo_Mean_CI_L	Geo_Mean_CI_U
1	External Controls, Age = 73.6562500	13.7295	11.8514	15.9052
2	External MCI, Age = 73.6562500	21.4106	18.1128	25.3090
3	Leukine, Baseline, Age = 73.6562500	14.9681	11.7940	18.9964
4	Placebo, Baseline, Age = 73.6562500	13.6670	11.1592	16.7384
5	Pooled Baseline, Age = 73.6562500	14.3028	12.1951	16.7747

Obs	Label	Ratio_Est	Ratio_CI_L	Ratio_CI_U	T_Statistic	p_value
1	Leukine vs. Placebo, Baseline	1.09520	0.82048	1.46191	0.64	0.5267
2	Day 18 vs. Baseline, Placebo	1.04046	0.86443	1.25234	0.45	0.6563
3	Day 18 vs. Baseline, Leukine	0.49666	0.35006	0.70466	-4.33	0.0008
4	Follow Up 1 vs. Baseline, Placebo	1.10276	0.84666	1.43631	0.78	0.4441
5	Follow Up 1 vs. Baseline, Leukine	0.88549	0.71235	1.10072	-1.20	0.2511
6	Follow Up 2 vs. Baseline, Placebo	1.11825	0.91777	1.36252	1.20	0.2486
7	Follow Up 2 vs. Baseline, Leukine	0.92217	0.77965	1.09075	-1.04	0.3175
8	Leukine vs. Placebo, Day 18 vs. Baseline	0.47735	0.32530	0.70046	-4.02	0.0007
9	Leukine vs. Placebo, Follow Up 1 vs. Baseline	0.80298	0.57804	1.11544	-1.36	0.1828
10	Leukine vs. Placebo, Follow Up 2 vs. Baseline	0.82466	0.64306	1.05753	-1.58	0.1239

Obs	Label	Ratio_Est	Ratio_CI_L	Ratio_CI_U	T_Statistic	p_value
1	Leukine Baseline vs. External Controls, Age = 67.8333333	1.01148	0.78534	1.30274	0.09	0.9274
2	Placebo Baseline vs. External Controls, Age = 67.8333333	0.92356	0.73076	1.16723	-0.69	0.4964
3	Pooled Baseline vs. External Controls, Age = 67.8333333	0.96652	0.79779	1.17094	-0.35	0.7257
4	Leukine Baseline vs. External MCI, Age = 67.8333333	0.70225	0.51158	0.96397	-2.25	0.0296
5	Placebo Baseline vs. External MCI, Age = 67.8333333	0.64121	0.47360	0.86813	-2.95	0.0049
6	Pooled Baseline vs. External MCI, Age = 67.8333333	0.67103	0.50999	0.88294	-2.92	0.0052
7	External MCI vs. External Controls, Age = 67.8333333	1.44035	1.10086	1.88453	2.72	0.0088

Obs	Label	Ratio_Est	Ratio_CI_L	Ratio_CI_U	T_Statistic	p_value
1	Leukine Day 18 vs. External Controls, Age = 67.8333333	0.50236	0.34054	0.74108	-3.78	0.0019
2	Leukine Follow Up 1 vs. External Controls, Age = 67.8333333	0.89566	0.68998	1.16265	-0.87	0.3935
3	Leukine Follow Up 2 vs. External Controls, Age = 67.8333333	0.93276	0.71495	1.21692	-0.53	0.5969
4	Placebo Day 18 vs. External Controls, Age = 67.8333333	0.96093	0.78831	1.17136	-0.40	0.6881
5	Placebo Follow Up 1 vs. External Controls, Age = 67.8333333	1.01846	0.78278	1.32511	0.14	0.8875
6	Placebo Follow Up 2 vs. External Controls, Age = 67.8333333	1.03277	0.84996	1.25490	0.33	0.7400

Obs	Label	Ratio_Est	Ratio_CI_L	Ratio_CI_U	T_Statistic	p_value
1	Leukine Day 18 vs. External Controls, Age = 67.8333333, Baseline Calibrated	0.48004	0.33127	0.69562	-4.14	0.0006
2	Leukine Follow Up 1 vs. External Controls, Age = 67.8333333, Baseline Calibrated	0.85585	0.66940	1.09423	-1.28	0.2085
3	Leukine Follow Up 2 vs. External Controls, Age = 67.8333333, Baseline Calibrated	0.89130	0.70839	1.12143	-1.00	0.3202
4	Placebo Day 18 vs. External Controls, Age = 67.8333333, Baseline Calibrated	1.00563	0.81323	1.24355	0.05	0.9581
5	Placebo Follow Up 1 vs. External Controls, Age = 67.8333333, Baseline Calibrated	1.06584	0.80824	1.40554	0.47	0.6432
6	Placebo Follow Up 2 vs. External Controls, Age = 67.8333333, Baseline Calibrated	1.08081	0.87154	1.34034	0.72	0.4744

Obs	Label	Ratio_Est	Ratio_CI_L	Ratio_CI_U	T_Statistic	p_value
1	Leukine Baseline vs. External Controls, Age = 73.6562500	1.09021	0.82708	1.43706	0.63	0.5317
2	Placebo Baseline vs. External Controls, Age = 73.6562500	0.99545	0.77832	1.27315	-0.04	0.9704
3	Pooled Baseline vs. External Controls, Age = 73.6562500	1.04175	0.84023	1.29161	0.38	0.7072
4	Leukine Baseline vs. External MCI, Age = 73.6562500	0.69910	0.52589	0.92935	-2.54	0.0150
5	Placebo Baseline vs. External MCI, Age = 73.6562500	0.63833	0.49421	0.82448	-3.54	0.0010
6	Pooled Baseline vs. External MCI, Age = 73.6562500	0.66802	0.53241	0.83817	-3.55	0.0007
7	External MCI vs. External Controls, Age = 73.6562500	1.55946	1.25128	1.94354	4.01	0.0001

Obs	Label	Ratio_Est	Ratio_CI_L	Ratio_CI_U	T_Statistic	p_value
1	Study AD Age (per year)	1.02420	1.00783	1.04083	3.07	0.0054
2	External MCI Age (per year)	1.02499	0.99554	1.05531	1.73	0.0941
3	External Controls Age (per year)	1.01110	1.00716	1.01505	5.57	<.0001
4	Age Slopes (per year): Study AD vs External Controls	1.01296	0.99636	1.02982	1.60	0.1212
5	Age Slopes (per year): External MCI vs External Controls	1.01374	0.98438	1.04397	0.95	0.3511
6	Age Slopes (per year): Study AD vs External MCI	0.99923	0.96704	1.03249	-0.05	0.9623

Obs	Label	Percent_Est	Percent_CI_L	Percent_CI_U	T_Statistic	p_value
1	Study AD Age (per year)	2.41977	0.78311	4.08302	3.07	0.0054
2	External MCI Age (per year)	2.49893	-0.44644	5.53145	1.73	0.0941
3	External Controls Age (per year)	1.10980	0.71628	1.50486	5.57	<.0001

Table S8 LN[UCH-L1]; related to Figure 1 Data and statistical calculations were analyzed for UCH-L1 levels in the plasma of the human participants.

The FREQ Procedure

Frequency

Table of time_cat by Treatment

time_cat	Treatment				
	External Controls	External MCI	Leukine	Placebo	Total
External	692	64	0	0	756
Baseline	0	0	52	52	104
15 days	0	0	44	51	95
45 days	0	0	49	50	99
90 days	0	0	48	51	99
Total	692	64	193	204	1153

The MEANS Procedure

Analysis Variable : Age									
study_ind	N Obs	N	Mean	Std Dev	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
0	349	349	45.7758943	22.6399945	1.7998047	26.7968750	43.2968750	67.3906250	87.0000000
1	36	36	69.0833333	6.2990929	55.0000000	64.5000000	70.5000000	73.5000000	80.0000000

The MEANS Procedure

Analysis Variable : Age									
Treatment	N Obs	N	Mean	Std Dev	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
External Controls	317	317	42.9614735	21.7825789	1.7998047	24.6992188	38.5000000	62.5937500	85.8906250
External MCI	32	32	73.6562500	5.8287905	63.0000000	70.0000000	73.5000000	78.5000000	87.0000000
Leukine	18	18	67.8333333	6.1381162	56.0000000	64.0000000	68.5000000	71.0000000	80.0000000
Placebo	18	18	70.3333333	6.3801162	55.0000000	68.0000000	72.5000000	74.0000000	78.0000000

The Mixed Procedure

Estimates								
Label	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper
External Controls, Age = 67.8333333	2.4165	0.04012	318	60.24	<.0001	0.05	2.3376	2.4955
External MCI, Age = 67.8333333	2.7258	0.09622	30	28.33	<.0001	0.05	2.5293	2.9223
Leukine, Baseline, Age = 67.8333333	2.9407	0.09965	16.6	29.51	<.0001	0.05	2.7301	3.1513
Leukine, Day 18, Age = 67.8333333	2.9404	0.1032	16.1	28.50	<.0001	0.05	2.7219	3.1590
Leukine, Follow Up 1, Age = 67.8333333	3.0196	0.09059	16.8	33.33	<.0001	0.05	2.8283	3.2108
Leukine, Follow Up 2, Age = 67.8333333	3.0157	0.1039	15.8	29.01	<.0001	0.05	2.7951	3.2363
Placebo, Baseline, Age = 67.8333333	3.1246	0.1532	17.8	20.40	<.0001	0.05	2.8025	3.4467
Placebo, Day 18, Age = 67.8333333	3.1023	0.1209	18	25.67	<.0001	0.05	2.8484	3.3562
Placebo, Follow Up 1, Age = 67.8333333	3.0671	0.1131	17.2	27.12	<.0001	0.05	2.8286	3.3055
Placebo, Follow Up 2, Age = 67.8333333	3.0726	0.1108	16.6	27.73	<.0001	0.05	2.8383	3.3068
Pooled Baseline, Age = 67.8333333	3.0326	0.09137	29.8	33.19	<.0001	0.05	2.8460	3.2193
Leukine, Day 18, Age = 67.8333333, Baseline Calibrated	3.0324	0.09914	32.8	30.59	<.0001	0.05	2.8306	3.2341
Leukine, Follow Up 1, Age = 67.8333333, Baseline Calibrated	3.1115	0.09749	33.1	31.92	<.0001	0.05	2.9132	3.3098
Leukine, Follow Up 2, Age = 67.8333333, Baseline Calibrated	3.1076	0.1008	33.2	30.83	<.0001	0.05	2.9026	3.3127
Placebo, Day 18, Age = 67.8333333, Baseline Calibrated	3.0104	0.07089	33.6	42.46	<.0001	0.05	2.8662	3.1545
Placebo, Follow Up 1, Age = 67.8333333, Baseline Calibrated	2.9751	0.08781	26.2	33.88	<.0001	0.05	2.7947	3.1556
Placebo, Follow Up 2, Age = 67.8333333, Baseline Calibrated	2.9806	0.08855	29.9	33.66	<.0001	0.05	2.7998	3.1615
External Controls, Age = 73.6562500	2.5586	0.04567	318	56.02	<.0001	0.05	2.4687	2.6484
External MCI, Age = 73.6562500	2.9619	0.06753	30	43.86	<.0001	0.05	2.8240	3.0998
Leukine, Baseline, Age = 73.6562500	3.0966	0.1157	22.9	26.77	<.0001	0.05	2.8573	3.3360
Placebo, Baseline, Age = 73.6562500	3.2805	0.1548	18.4	21.19	<.0001	0.05	2.9557	3.6053
Pooled Baseline, Age = 73.6562500	3.1886	0.1016	36.4	31.37	<.0001	0.05	2.9825	3.3946
Leukine vs. Placebo, Baseline	-0.1839	0.1827	29.8	-1.01	0.3223	0.05	-0.5572	0.1894
Day 18 vs. Baseline, Placebo	-0.02227	0.04701	17.4	-0.47	0.6415	0.05	-0.1213	0.07675
Day 18 vs. Baseline, Leukine	-0.00025	0.04795	17	-0.01	0.9959	0.05	-0.1014	0.1009
Follow Up 1 vs. Baseline, Placebo	-0.05751	0.09692	16.6	-0.59	0.5609	0.05	-0.2623	0.1473
Follow Up 1 vs. Baseline, Leukine	0.07889	0.06377	17.3	1.24	0.2325	0.05	-0.05545	0.2132
Follow Up 2 vs. Baseline, Placebo	-0.05202	0.1009	16.3	-0.52	0.6129	0.05	-0.2655	0.1614
Follow Up 2 vs. Baseline, Leukine	0.07501	0.05245	16.5	1.43	0.1713	0.05	-0.03589	0.1859
Leukine vs. Placebo, Day 18 vs. Baseline	0.02202	0.06715	34.3	0.33	0.7449	0.05	-0.1144	0.1584
Leukine vs. Placebo, Follow Up 1 vs. Baseline	0.1364	0.1160	28.9	1.18	0.2493	0.05	-0.1009	0.3737
Leukine vs. Placebo, Follow Up 2 vs. Baseline	0.1270	0.1137	24.6	1.12	0.2746	0.05	-0.1073	0.3614
Leukine Baseline vs. External Controls, Age = 67.8333333	0.5242	0.1074	22.4	4.88	<.0001	0.05	0.3016	0.7467
Placebo Baseline vs. External Controls, Age = 67.8333333	0.7081	0.1583	20.3	4.47	0.0002	0.05	0.3781	1.0381
Pooled Baseline vs. External Controls, Age = 67.8333333	0.6161	0.09979	42.3	6.17	<.0001	0.05	0.4148	0.8175
Leukine Baseline vs. External MCI, Age = 67.8333333	0.2149	0.1385	41.8	1.55	0.1284	0.05	-0.06470	0.4944
Placebo Baseline vs. External MCI, Age = 67.8333333	0.3988	0.1809	31.6	2.20	0.0349	0.05	0.03014	0.7674
Pooled Baseline vs. External MCI, Age = 67.8333333	0.3068	0.1327	59.7	2.31	0.0242	0.05	0.04137	0.5723

The Mixed Procedure

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper
External MCI vs. External Controls, Age = 67.8333333	0.3093	0.1042	41.2	2.97	0.0050	0.05	0.09880	0.5198
Leukine Day 18 vs. External Controls, Age = 67.8333333	0.5239	0.1107	21.3	4.73	0.0001	0.05	0.2939	0.7539
Leukine Follow Up 1 vs. External Controls, Age = 67.8333333	0.6030	0.09907	24	6.09	<.0001	0.05	0.3986	0.8075
Leukine Follow Up 2 vs. External Controls, Age = 67.8333333	0.5992	0.1114	20.8	5.38	<.0001	0.05	0.3674	0.8310
Placebo Day 18 vs. External Controls, Age = 67.8333333	0.6858	0.1273	22.2	5.39	<.0001	0.05	0.4218	0.9498
Placebo Follow Up 1 vs. External Controls, Age = 67.8333333	0.6505	0.1200	21.8	5.42	<.0001	0.05	0.4015	0.8996
Placebo Follow Up 2 vs. External Controls, Age = 67.8333333	0.6560	0.1178	21.2	5.57	<.0001	0.05	0.4111	0.9010
Leukine Day 18 vs. External Controls, Age = 67.8333333, Baseline Calibrated	0.6159	0.1070	44.3	5.76	<.0001	0.05	0.4004	0.8314
Leukine Follow Up 1 vs. External Controls, Age = 67.8333333, Baseline Calibrated	0.6950	0.1054	45.1	6.59	<.0001	0.05	0.4827	0.9073
Leukine Follow Up 2 vs. External Controls, Age = 67.8333333, Baseline Calibrated	0.6911	0.1085	44.5	6.37	<.0001	0.05	0.4726	0.9097
Placebo Day 18 vs. External Controls, Age = 67.8333333, Baseline Calibrated	0.5938	0.08146	58	7.29	<.0001	0.05	0.4308	0.7569
Placebo Follow Up 1 vs. External Controls, Age = 67.8333333, Baseline Calibrated	0.5586	0.09654	38.1	5.79	<.0001	0.05	0.3632	0.7540
Placebo Follow Up 2 vs. External Controls, Age = 67.8333333, Baseline Calibrated	0.5641	0.09722	43.3	5.80	<.0001	0.05	0.3681	0.7601
Leukine Baseline vs. External Controls, Age = 73.6562500	0.5381	0.1244	30.5	4.33	0.0002	0.05	0.2843	0.7919
Placebo Baseline vs. External Controls, Age = 73.6562500	0.7220	0.1614	21.7	4.47	0.0002	0.05	0.3869	1.0570
Pooled Baseline vs. External Controls, Age = 73.6562500	0.6300	0.1114	52.3	5.65	<.0001	0.05	0.4065	0.8536
Leukine Baseline vs. External MCI, Age = 73.6562500	0.1347	0.1339	37.8	1.01	0.3209	0.05	-0.1365	0.4059
Placebo Baseline vs. External MCI, Age = 73.6562500	0.3186	0.1689	25.5	1.89	0.0707	0.05	-0.02895	0.6662
Pooled Baseline vs. External MCI, Age = 73.6562500	0.2267	0.1220	61.1	1.86	0.0680	0.05	-0.01732	0.4707
External MCI vs. External Controls, Age = 73.6562500	0.4033	0.08152	62.5	4.95	<.0001	0.05	0.2404	0.5663
Study AD Age (per year)	0.02678	0.01010	32.7	2.65	0.0122	0.05	0.006234	0.04733
External MCI Age (per year)	0.04055	0.01177	30	3.44	0.0017	0.05	0.01651	0.06458
External Controls Age (per year)	0.02439	0.001212	316	20.12	<.0001	0.05	0.02201	0.02678
Age Slopes (per year): Study AD vs External Controls	0.002387	0.01017	33.7	0.23	0.8158	0.05	-0.01828	0.02306
Age Slopes (per year): External MCI vs External Controls	0.01615	0.01183	30.6	1.36	0.1822	0.05	-0.00799	0.04030
Age Slopes (per year): Study AD vs External MCI	-0.01376	0.01551	60.4	-0.89	0.3783	0.05	-0.04478	0.01725

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
Leukine vs. Placebo, Change from Baseline, All	3	30.3	0.80	0.5062
Leukine vs. Placebo, Change from Baseline, Follow Ups 1 and 2	2	28.4	0.73	0.4919
Leukine vs External Controls, Age = 67.8333333, Post Baseline	3	18.3	16.35	<.0001
Placebo vs External Controls, Age = 67.8333333, Post Baseline	3	17.7	10.94	0.0003
Leukine vs External Controls, Age = 67.8333333, Post Baseline	3	18.3	16.35	<.0001

The Mixed Procedure

Contrasts				
Label	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Placebo vs External Controls, Age = 67.8333333, Post Baseline	3	17.7	10.94	0.0003
Leukine vs External Controls, Same Age, Post Baseline	4	24.4	12.26	<.0001
Placebo vs External Controls, Same Age, Post Baseline	4	28	8.64	0.0001
Leukine vs External Controls, Post Baseline, Overall	5	29.9	119.66	<.0001
Placebo vs External Controls, Post Baseline, Overall	5	33.5	112.54	<.0001
Leukine vs External Controls, Age = 67.8333333, Baseline Calibrated, Post Baseline	3	20.9	16.48	<.0001
Placebo vs External Controls, Age = 67.8333333, Baseline Calibrated, Post Baseline	3	21.9	18.04	<.0001
Leukine vs External Controls, Same Age, Baseline Calibrated, Post Baseline	4	28	12.53	<.0001
Placebo vs External Controls, Same Age, Baseline Calibrated, Post Baseline	4	35.2	13.81	<.0001
Leukine vs External Controls, Baseline Calibrated, Post Baseline, Overall	5	33.8	119.84	<.0001
Placebo vs External Controls, Baseline Calibrated, Post Baseline, Overall	5	42.1	139.19	<.0001
Age Slopes Overall	3	44	141.27	<.0001
Treatment x Age Slopes Interaction Overall	2	32.2	0.96	0.3953

Obs	Label	Geo_Mean_Est	Geo_Mean_CI_L	Geo_Mean_CI_U
1	External Controls, Age = 67.8333333	11.2068	10.3563	12.1272
2	External MCI, Age = 67.8333333	15.2689	12.5449	18.5843
3	Leukine, Baseline, Age = 67.8333333	18.9289	15.3339	23.3667
4	Leukine, Day 18, Age = 67.8333333	18.9241	15.2088	23.5470
5	Leukine, Follow Up 1, Age = 67.8333333	20.4826	16.9167	24.8001
6	Leukine, Follow Up 2, Age = 67.8333333	20.4034	16.3645	25.4390
7	Placebo, Baseline, Age = 67.8333333	22.7504	16.4852	31.3966
8	Placebo, Day 18, Age = 67.8333333	22.2492	17.2599	28.6808
9	Placebo, Follow Up 1, Age = 67.8333333	21.4788	16.9222	27.2624
10	Placebo, Follow Up 2, Age = 67.8333333	21.5972	17.0875	27.2972

Obs	Label	Geo_Mean_Est	Geo_Mean_CI_L	Geo_Mean_CI_U
1	External Controls, Age = 67.8333333	11.2068	10.3563	12.1272
2	External MCI, Age = 67.8333333	15.2689	12.5449	18.5843
3	Pooled Baseline, Age = 67.8333333	20.7518	17.2185	25.0102
4	Leukine, Day 18, Age = 67.8333333, Baseline Calibrated	20.7467	16.9563	25.3843
5	Leukine, Follow Up 1, Age = 67.8333333, Baseline Calibrated	22.4552	18.4156	27.3809
6	Leukine, Follow Up 2, Age = 67.8333333, Baseline Calibrated	22.3684	18.2221	27.4581
7	Placebo, Day 18, Age = 67.8333333, Baseline Calibrated	20.2947	17.5707	23.4411
8	Placebo, Follow Up 1, Age = 67.8333333, Baseline Calibrated	19.5920	16.3576	23.4659
9	Placebo, Follow Up 2, Age = 67.8333333, Baseline Calibrated	19.7000	16.4405	23.6056

Obs	Label	Geo_Mean_Est	Geo_Mean_CI_L	Geo_Mean_CI_U
1	External Controls, Age = 73.6562500	12.9173	11.8072	14.1318
2	External MCI, Age = 73.6562500	19.3349	16.8441	22.1940
3	Leukine, Baseline, Age = 73.6562500	22.1233	17.4139	28.1062
4	Placebo, Baseline, Age = 73.6562500	26.5897	19.2154	36.7942
5	Pooled Baseline, Age = 73.6562500	24.2539	19.7377	29.8035

Obs	Label	Ratio_Est	Ratio_CI_L	Ratio_CI_U	T_Statistic	p_value
1	Leukine vs. Placebo, Baseline	0.83202	0.57282	1.20851	-1.01	0.3223
2	Day 18 vs. Baseline, Placebo	0.97797	0.88577	1.07977	-0.47	0.6415
3	Day 18 vs. Baseline, Leukine	0.99975	0.90355	1.10619	-0.01	0.9959
4	Follow Up 1 vs. Baseline, Placebo	0.94411	0.76924	1.15872	-0.59	0.5609
5	Follow Up 1 vs. Baseline, Leukine	1.08208	0.94606	1.23766	1.24	0.2325
6	Follow Up 2 vs. Baseline, Placebo	0.94931	0.76685	1.17519	-0.52	0.6129
7	Follow Up 2 vs. Baseline, Leukine	1.07790	0.96474	1.20432	1.43	0.1713
8	Leukine vs. Placebo, Day 18 vs. Baseline	1.02227	0.89191	1.17168	0.33	0.7449
9	Leukine vs. Placebo, Follow Up 1 vs. Baseline	1.14614	0.90402	1.45310	1.18	0.2493
10	Leukine vs. Placebo, Follow Up 2 vs. Baseline	1.13545	0.89826	1.43528	1.12	0.2746

Obs	Label	Ratio_Est	Ratio_CI_L	Ratio_CI_U	T_Statistic	p_value
1	Leukine Baseline vs. External Controls, Age = 67.8333333	1.68904	1.35204	2.11006	4.88	<.0001
2	Placebo Baseline vs. External Controls, Age = 67.8333333	2.03004	1.45944	2.82374	4.47	0.0002
3	Pooled Baseline vs. External Controls, Age = 67.8333333	1.85171	1.51402	2.26472	6.17	<.0001
4	Leukine Baseline vs. External MCI, Age = 67.8333333	1.23970	0.93735	1.63958	1.55	0.1284
5	Placebo Baseline vs. External MCI, Age = 67.8333333	1.48998	1.03060	2.15414	2.20	0.0349
6	Pooled Baseline vs. External MCI, Age = 67.8333333	1.35909	1.04224	1.77227	2.31	0.0242
7	External MCI vs. External Controls, Age = 67.8333333	1.36246	1.10385	1.68166	2.97	0.0050

Obs	Label	Ratio_Est	Ratio_CI_L	Ratio_CI_U	T_Statistic	p_value
1	Leukine Day 18 vs. External Controls, Age = 67.8333333	1.68862	1.34171	2.12523	4.73	0.0001
2	Leukine Follow Up 1 vs. External Controls, Age = 67.8333333	1.82768	1.48972	2.24232	6.09	<.0001
3	Leukine Follow Up 2 vs. External Controls, Age = 67.8333333	1.82062	1.44391	2.29560	5.38	<.0001
4	Placebo Day 18 vs. External Controls, Age = 67.8333333	1.98533	1.52471	2.58510	5.39	<.0001
5	Placebo Follow Up 1 vs. External Controls, Age = 67.8333333	1.91658	1.49406	2.45859	5.42	<.0001
6	Placebo Follow Up 2 vs. External Controls, Age = 67.8333333	1.92715	1.50850	2.46197	5.57	<.0001

Obs	Label	Ratio_Est	Ratio_CI_L	Ratio_CI_U	T_Statistic	p_value
1	Leukine Day 18 vs. External Controls, Age = 67.8333333, Baseline Calibrated	1.85125	1.49237	2.29644	5.76	<.0001
2	Leukine Follow Up 1 vs. External Controls, Age = 67.8333333, Baseline Calibrated	2.00370	1.62042	2.47765	6.59	<.0001
3	Leukine Follow Up 2 vs. External Controls, Age = 67.8333333, Baseline Calibrated	1.99595	1.60408	2.48356	6.37	<.0001
4	Placebo Day 18 vs. External Controls, Age = 67.8333333, Baseline Calibrated	1.81092	1.53846	2.13164	7.29	<.0001
5	Placebo Follow Up 1 vs. External Controls, Age = 67.8333333, Baseline Calibrated	1.74822	1.43790	2.12550	5.79	<.0001
6	Placebo Follow Up 2 vs. External Controls, Age = 67.8333333, Baseline Calibrated	1.75785	1.44496	2.13850	5.80	<.0001

Obs	Label	Ratio_Est	Ratio_CI_L	Ratio_CI_U	T_Statistic	p_value
1	Leukine Baseline vs. External Controls, Age = 73.6562500	1.71268	1.32877	2.20752	4.33	0.0002
2	Placebo Baseline vs. External Controls, Age = 73.6562500	2.05845	1.47242	2.87773	4.47	0.0002
3	Pooled Baseline vs. External Controls, Age = 73.6562500	1.87763	1.50148	2.34800	5.65	<.0001
4	Leukine Baseline vs. External MCI, Age = 73.6562500	1.14422	0.87242	1.50069	1.01	0.3209
5	Placebo Baseline vs. External MCI, Age = 73.6562500	1.37522	0.97146	1.94679	1.89	0.0707
6	Pooled Baseline vs. External MCI, Age = 73.6562500	1.25441	0.98283	1.60104	1.86	0.0680
7	External MCI vs. External Controls, Age = 73.6562500	1.49682	1.27176	1.76170	4.95	<.0001

Obs	Label	Ratio_Est	Ratio_CI_L	Ratio_CI_U	T_Statistic	p_value
1	Study AD Age (per year)	1.02714	1.00625	1.04847	2.65	0.0122
2	External MCI Age (per year)	1.04138	1.01664	1.06672	3.44	0.0017
3	External Controls Age (per year)	1.02469	1.02225	1.02714	20.12	<.0001
4	Age Slopes (per year): Study AD vs External Controls	1.00239	0.98188	1.02333	0.23	0.8158
5	Age Slopes (per year): External MCI vs External Controls	1.01628	0.99204	1.04112	1.36	0.1822
6	Age Slopes (per year): Study AD vs External MCI	0.98633	0.95621	1.01740	-0.89	0.3783

Obs	Label	Percent_Est	Percent_CI_L	Percent_CI_U	T_Statistic	p_value
1	Study AD Age (per year)	2.71426	0.62539	4.84650	2.65	0.0122
2	External MCI Age (per year)	4.13788	1.66438	6.67156	3.44	0.0017
3	External Controls Age (per year)	2.46940	2.22529	2.71410	20.12	<.0001

Supplemental Table S9 (related to Figure 2): Data and statistical calculations were analyzed for NfL levels in the plasma of the human participants.

The FREQ Procedure

Frequency	Table of time_cat by Treatment				
	Treatment				
time_cat	External Controls	External MCI	Leukine	Placebo	Total
External	689	64	0	0	753
Baseline	0	0	52	52	104
15 days	0	0	44	51	95
45 days	0	0	49	50	99
90 days	0	0	48	51	99
Total	689	64	193	204	1150

The MEANS Procedure

Analysis Variable : Age									
study_ind	N Obs	N	Mean	Std Dev	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
0	349	349	45.7758943	22.6399945	1.7998047	26.7968750	43.2968750	67.3906250	87.0000000
1	36	36	69.0833333	6.2990929	55.0000000	64.5000000	70.5000000	73.5000000	80.0000000

The MEANS Procedure

Analysis Variable : Age									
Treatment	N Obs	N	Mean	Std Dev	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
External Controls	317	317	42.9614735	21.7825789	1.7998047	24.6992188	38.5000000	62.5937500	85.8906250
External MCI	32	32	73.6562500	5.8287905	63.0000000	70.0000000	73.5000000	78.5000000	87.0000000
Leukine	18	18	67.8333333	6.1381162	56.0000000	64.0000000	68.5000000	71.0000000	80.0000000
Placebo	18	18	70.3333333	6.3801162	55.0000000	68.0000000	72.5000000	74.0000000	78.0000000

The Mixed Procedure

Estimates								
Label	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper
External Controls, Age = 67.8333333	4.6936	0.04334	317	108.29	<.0001	0.05	4.6083	4.7789
External MCI, Age = 67.8333333	5.2563	0.1296	30	40.56	<.0001	0.05	4.9917	5.5210
Leukine, Baseline, Age = 67.8333333	5.1416	0.09730	16.7	52.84	<.0001	0.05	4.9360	5.3471
Leukine, Day 18, Age = 67.8333333	5.1789	0.1070	16.6	48.39	<.0001	0.05	4.9527	5.4051
Leukine, Follow Up 1, Age = 67.8333333	5.2389	0.09274	15.9	56.49	<.0001	0.05	5.0422	5.4356
Leukine, Follow Up 2, Age = 67.8333333	5.2554	0.1223	15.5	42.96	<.0001	0.05	4.9955	5.5154
Placebo, Baseline, Age = 67.8333333	5.2585	0.1160	17.9	45.34	<.0001	0.05	5.0147	5.5022
Placebo, Day 18, Age = 67.8333333	5.3383	0.1167	17.7	45.76	<.0001	0.05	5.0928	5.5837
Placebo, Follow Up 1, Age = 67.8333333	5.2937	0.1084	17.1	48.84	<.0001	0.05	5.0652	5.5223
Placebo, Follow Up 2, Age = 67.8333333	5.3299	0.1025	17.7	52.01	<.0001	0.05	5.1143	5.5455
Pooled Baseline, Age = 67.8333333	5.2000	0.07569	32.9	68.70	<.0001	0.05	5.0460	5.3540
Leukine, Day 18, Age = 67.8333333, Baseline Calibrated	5.2373	0.09688	30.5	54.06	<.0001	0.05	5.0396	5.4351
Leukine, Follow Up 1, Age = 67.8333333, Baseline Calibrated	5.2973	0.08940	32.5	59.25	<.0001	0.05	5.1153	5.4793
Leukine, Follow Up 2, Age = 67.8333333, Baseline Calibrated	5.3139	0.1076	28.3	49.39	<.0001	0.05	5.0936	5.5342
Placebo, Day 18, Age = 67.8333333, Baseline Calibrated	5.2798	0.08619	29.8	61.26	<.0001	0.05	5.1038	5.4559
Placebo, Follow Up 1, Age = 67.8333333, Baseline Calibrated	5.2353	0.08809	28.5	59.43	<.0001	0.05	5.0550	5.4156
Placebo, Follow Up 2, Age = 67.8333333, Baseline Calibrated	5.2715	0.08270	31.1	63.74	<.0001	0.05	5.1028	5.4401
External Controls, Age = 73.6562500	4.8681	0.05118	317	95.12	<.0001	0.05	4.7675	4.9688
External MCI, Age = 73.6562500	5.4547	0.09095	30	59.97	<.0001	0.05	5.2690	5.6405
Leukine, Baseline, Age = 73.6562500	5.1398	0.1136	23	45.26	<.0001	0.05	4.9049	5.3747
Placebo, Baseline, Age = 73.6562500	5.2567	0.1180	18.7	44.54	<.0001	0.05	5.0094	5.5040
Pooled Baseline, Age = 73.6562500	5.1982	0.08767	37.3	59.30	<.0001	0.05	5.0207	5.3758
Leukine vs. Placebo, Baseline	-0.1169	0.1514	32.9	-0.77	0.4456	0.05	-0.4249	0.1911
Day 18 vs. Baseline, Placebo	0.07980	0.05690	17	1.40	0.1787	0.05	-0.04025	0.1999
Day 18 vs. Baseline, Leukine	0.03731	0.07336	15.9	0.51	0.6180	0.05	-0.1183	0.1929
Follow Up 1 vs. Baseline, Placebo	0.03527	0.07589	17.1	0.46	0.6479	0.05	-0.1248	0.1953
Follow Up 1 vs. Baseline, Leukine	0.09729	0.07363	16.8	1.32	0.2041	0.05	-0.05818	0.2528
Follow Up 2 vs. Baseline, Placebo	0.07145	0.07186	16.8	0.99	0.3342	0.05	-0.08028	0.2232
Follow Up 2 vs. Baseline, Leukine	0.1139	0.07880	16	1.45	0.1677	0.05	-0.05314	0.2809
Leukine vs. Placebo, Day 18 vs. Baseline	-0.04249	0.09284	30.5	-0.46	0.6504	0.05	-0.2320	0.1470
Leukine vs. Placebo, Follow Up 1 vs. Baseline	0.06202	0.1057	33.9	0.59	0.5614	0.05	-0.1529	0.2769
Leukine vs. Placebo, Follow Up 2 vs. Baseline	0.04242	0.1066	32.4	0.40	0.6934	0.05	-0.1747	0.2595
Leukine Baseline vs. External Controls, Age = 67.8333333	0.4480	0.1065	24	4.21	0.0003	0.05	0.2281	0.6678
Placebo Baseline vs. External Controls, Age = 67.8333333	0.5649	0.1238	23.2	4.56	0.0001	0.05	0.3089	0.8208
Pooled Baseline vs. External Controls, Age = 67.8333333	0.5064	0.08722	57.3	5.81	<.0001	0.05	0.3318	0.6811
Leukine Baseline vs. External MCI, Age = 67.8333333	-0.1148	0.1621	46.7	-0.71	0.4824	0.05	-0.4408	0.2113
Placebo Baseline vs. External MCI, Age = 67.8333333	0.002121	0.1739	46.9	0.01	0.9903	0.05	-0.3478	0.3520
Pooled Baseline vs. External MCI, Age = 67.8333333	-0.05632	0.1501	48.8	-0.38	0.7091	0.05	-0.3580	0.2453

The Mixed Procedure

Estimates								
Label	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper
External MCI vs. External Controls, Age = 67.8333333	0.5627	0.1366	37	4.12	0.0002	0.05	0.2859	0.8396
Leukine Day 18 vs. External Controls, Age = 67.8333333	0.4853	0.1155	22.4	4.20	0.0004	0.05	0.2461	0.7245
Leukine Follow Up 1 vs. External Controls, Age = 67.8333333	0.5453	0.1024	23.5	5.33	<.0001	0.05	0.3338	0.7568
Leukine Follow Up 2 vs. External Controls, Age = 67.8333333	0.5618	0.1298	19.7	4.33	0.0003	0.05	0.2909	0.8328
Placebo Day 18 vs. External Controls, Age = 67.8333333	0.6447	0.1245	22.9	5.18	<.0001	0.05	0.3871	0.9022
Placebo Follow Up 1 vs. External Controls, Age = 67.8333333	0.6001	0.1167	23	5.14	<.0001	0.05	0.3587	0.8416
Placebo Follow Up 2 vs. External Controls, Age = 67.8333333	0.6363	0.1113	24.5	5.72	<.0001	0.05	0.4069	0.8657
Leukine Day 18 vs. External Controls, Age = 67.8333333, Baseline Calibrated	0.5437	0.1061	43.7	5.12	<.0001	0.05	0.3298	0.7577
Leukine Follow Up 1 vs. External Controls, Age = 67.8333333, Baseline Calibrated	0.6037	0.09935	49.3	6.08	<.0001	0.05	0.4041	0.8033
Leukine Follow Up 2 vs. External Controls, Age = 67.8333333, Baseline Calibrated	0.6203	0.1160	38.1	5.35	<.0001	0.05	0.3855	0.8551
Placebo Day 18 vs. External Controls, Age = 67.8333333, Baseline Calibrated	0.5862	0.09647	46.6	6.08	<.0001	0.05	0.3921	0.7804
Placebo Follow Up 1 vs. External Controls, Age = 67.8333333, Baseline Calibrated	0.5417	0.09818	43.8	5.52	<.0001	0.05	0.3438	0.7396
Placebo Follow Up 2 vs. External Controls, Age = 67.8333333, Baseline Calibrated	0.5779	0.09337	50.2	6.19	<.0001	0.05	0.3904	0.7654
Leukine Baseline vs. External Controls, Age = 73.6562500	0.2717	0.1246	33.2	2.18	0.0364	0.05	0.01829	0.5250
Placebo Baseline vs. External Controls, Age = 73.6562500	0.3885	0.1286	26.4	3.02	0.0056	0.05	0.1243	0.6528
Pooled Baseline vs. External Controls, Age = 73.6562500	0.3301	0.1015	66.1	3.25	0.0018	0.05	0.1274	0.5328
Leukine Baseline vs. External MCI, Age = 73.6562500	-0.3149	0.1455	47.2	-2.16	0.0355	0.05	-0.6076	-0.02225
Placebo Baseline vs. External MCI, Age = 73.6562500	-0.1981	0.1490	39	-1.33	0.1915	0.05	-0.4995	0.1033
Pooled Baseline vs. External MCI, Age = 73.6562500	-0.2565	0.1263	65.9	-2.03	0.0464	0.05	-0.5087	-0.00427
External MCI vs. External Controls, Age = 73.6562500	0.5866	0.1044	51.5	5.62	<.0001	0.05	0.3771	0.7961
Study AD Age (per year)	-0.00030	0.01006	31.1	-0.03	0.9761	0.05	-0.02082	0.02022
External MCI Age (per year)	0.03407	0.01585	30	2.15	0.0398	0.05	0.001695	0.06645
External Controls Age (per year) (< 30)	-0.03286	0.005097	312	-6.45	<.0001	0.05	-0.04288	-0.02283
External Controls Age (per year) (>= 30)	0.02998	0.001825	316	16.43	<.0001	0.05	0.02639	0.03357
Age Slopes (per year): External Controls, >= 30 vs < 30	0.06283	0.006244	313	10.06	<.0001	0.05	0.05055	0.07512
Age Slopes (per year): Study AD vs External Controls (< 30)	0.03255	0.01128	48.8	2.89	0.0058	0.05	0.009882	0.05522
Age Slopes (per year): External MCI vs External Controls (< 30)	0.06693	0.01665	36.5	4.02	0.0003	0.05	0.03317	0.1007
Age Slopes (per year): Study AD vs External Controls (>= 30)	-0.03028	0.01023	33.2	-2.96	0.0056	0.05	-0.05108	-0.00948
Age Slopes (per year): External MCI vs External Controls (>= 30)	0.004097	0.01596	30.8	0.26	0.7991	0.05	-0.02846	0.03665
Age Slopes (per year): Study AD vs External MCI	-0.03438	0.01878	51	-1.83	0.0730	0.05	-0.07207	0.003320

The Mixed Procedure

Contrasts				
Label	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Leukine vs. Placebo, Change from Baseline, All	3	26.9	0.72	0.5475
Leukine vs. Placebo, Change from Baseline, Follow Ups 1 and 2	2	31.1	0.18	0.8397
Leukine vs External Controls, Age = 67.8333333, Post Baseline	3	17.1	12.43	0.0001
Placebo vs External Controls, Age = 67.8333333, Post Baseline	3	18.6	11.15	0.0002
Leukine vs External Controls, Age = 67.8333333, Post Baseline	3	17.1	12.43	0.0001
Placebo vs External Controls, Age = 67.8333333, Post Baseline	3	18.6	11.15	0.0002
Leukine vs External Controls, Same Age, Post Baseline	4	25.3	11.96	<.0001
Placebo vs External Controls, Same Age, Post Baseline	4	24.5	9.34	<.0001
Leukine vs External Controls, Post Baseline, Overall	6	36.7	69.47	<.0001
Placebo vs External Controls, Post Baseline, Overall	6	40.1	68.07	<.0001
Leukine vs External Controls, Age = 67.8333333, Baseline Calibrated, Post Baseline	3	20	14.18	<.0001
Placebo vs External Controls, Age = 67.8333333, Baseline Calibrated, Post Baseline	3	21.6	14.06	<.0001
Leukine vs External Controls, Same Age, Baseline Calibrated, Post Baseline	4	31.1	12.21	<.0001
Placebo vs External Controls, Same Age, Baseline Calibrated, Post Baseline	4	27.8	12.06	<.0001
Leukine vs External Controls, Baseline Calibrated, Post Baseline, Overall	6	49.2	72.14	<.0001
Placebo vs External Controls, Baseline Calibrated, Post Baseline, Overall	6	49	76.74	<.0001
Age Slopes Overall	4	54.3	70.14	<.0001
Treatment x Age Slopes Interaction Overall	2	32	4.43	0.0200
Age Slopes Differences Overall	3	46.1	35.11	<.0001

Obs	Label	Geo_Mean_Est	Geo_Mean_CI_L	Geo_Mean_CI_U
1	External Controls, Age = 67.8333333	109.246	100.316	118.971
2	External MCI, Age = 67.8333333	191.778	147.182	249.885
3	Leukine, Baseline, Age = 67.8333333	170.985	139.218	209.999
4	Leukine, Day 18, Age = 67.8333333	177.485	141.552	222.540
5	Leukine, Follow Up 1, Age = 67.8333333	188.456	154.805	229.423
6	Leukine, Follow Up 2, Age = 67.8333333	191.607	147.749	248.483
7	Placebo, Baseline, Age = 67.8333333	192.185	150.615	245.228
8	Placebo, Day 18, Age = 67.8333333	208.150	162.853	266.047
9	Placebo, Follow Up 1, Age = 67.8333333	199.085	158.409	250.205
10	Placebo, Follow Up 2, Age = 67.8333333	206.419	166.387	256.084

Obs	Label	Geo_Mean_Est	Geo_Mean_CI_L	Geo_Mean_CI_U
1	External Controls, Age = 67.8333333	109.246	100.316	118.971
2	External MCI, Age = 67.8333333	191.778	147.182	249.885
3	Pooled Baseline, Age = 67.8333333	181.275	155.399	211.460
4	Leukine, Day 18, Age = 67.8333333, Baseline Calibrated	188.167	154.408	229.307
5	Leukine, Follow Up 1, Age = 67.8333333, Baseline Calibrated	199.798	166.554	239.678
6	Leukine, Follow Up 2, Age = 67.8333333, Baseline Calibrated	203.139	162.976	253.199
7	Placebo, Day 18, Age = 67.8333333, Baseline Calibrated	196.334	164.639	234.132
8	Placebo, Follow Up 1, Age = 67.8333333, Baseline Calibrated	187.784	156.804	224.883
9	Placebo, Follow Up 2, Age = 67.8333333, Baseline Calibrated	194.702	164.487	230.466

Obs	Label	Geo_Mean_Est	Geo_Mean_CI_L	Geo_Mean_CI_U
1	External Controls, Age = 73.6562500	130.079	117.619	143.860
2	External MCI, Age = 73.6562500	233.864	194.220	281.601
3	Leukine, Baseline, Age = 73.6562500	170.682	134.948	215.879
4	Placebo, Baseline, Age = 73.6562500	191.845	149.815	245.666
5	Pooled Baseline, Age = 73.6562500	180.954	151.512	216.118

Obs	Label	Ratio_Est	Ratio_CI_L	Ratio_CI_U	T_Statistic	p_value
1	Leukine vs. Placebo, Baseline	0.88969	0.65383	1.21063	-0.77	0.4456
2	Day 18 vs. Baseline, Placebo	1.08307	0.96055	1.22122	1.40	0.1787
3	Day 18 vs. Baseline, Leukine	1.03802	0.88845	1.21276	0.51	0.6180
4	Follow Up 1 vs. Baseline, Placebo	1.03590	0.88271	1.21569	0.46	0.6479
5	Follow Up 1 vs. Baseline, Leukine	1.10218	0.94348	1.28757	1.32	0.2041
6	Follow Up 2 vs. Baseline, Placebo	1.07407	0.92286	1.25005	0.99	0.3342
7	Follow Up 2 vs. Baseline, Leukine	1.12061	0.94825	1.32431	1.45	0.1677
8	Leukine vs. Placebo, Day 18 vs. Baseline	0.95840	0.79297	1.15834	-0.46	0.6504
9	Leukine vs. Placebo, Follow Up 1 vs. Baseline	1.06398	0.85823	1.31906	0.59	0.5614
10	Leukine vs. Placebo, Follow Up 2 vs. Baseline	1.04333	0.83971	1.29634	0.40	0.6934

Obs	Label	Ratio_Est	Ratio_CI_L	Ratio_CI_U	T_Statistic	p_value
1	Leukine Baseline vs. External Controls, Age = 67.8333333	1.56514	1.25624	1.94999	4.21	0.0003
2	Placebo Baseline vs. External Controls, Age = 67.8333333	1.75920	1.36192	2.27237	4.56	0.0001
3	Pooled Baseline vs. External Controls, Age = 67.8333333	1.65933	1.39344	1.97597	5.81	<.0001
4	Leukine Baseline vs. External MCI, Age = 67.8333333	0.89158	0.64350	1.23528	-0.71	0.4824
5	Placebo Baseline vs. External MCI, Age = 67.8333333	1.00212	0.70627	1.42190	0.01	0.9903
6	Pooled Baseline vs. External MCI, Age = 67.8333333	0.94524	0.69911	1.27802	-0.38	0.7091
7	External MCI vs. External Controls, Age = 67.8333333	1.75547	1.33092	2.31545	4.12	0.0002

Obs	Label	Ratio_Est	Ratio_CI_L	Ratio_CI_U	T_Statistic	p_value
1	Leukine Day 18 vs. External Controls, Age = 67.8333333	1.62464	1.27903	2.06364	4.20	0.0004
2	Leukine Follow Up 1 vs. External Controls, Age = 67.8333333	1.72507	1.39622	2.13136	5.33	<.0001
3	Leukine Follow Up 2 vs. External Controls, Age = 67.8333333	1.75391	1.33758	2.29983	4.33	0.0003
4	Placebo Day 18 vs. External Controls, Age = 67.8333333	1.90534	1.47275	2.46499	5.18	<.0001
5	Placebo Follow Up 1 vs. External Controls, Age = 67.8333333	1.82236	1.43140	2.32010	5.14	<.0001
6	Placebo Follow Up 2 vs. External Controls, Age = 67.8333333	1.88950	1.50217	2.37670	5.72	<.0001

Obs	Label	Ratio_Est	Ratio_CI_L	Ratio_CI_U	T_Statistic	p_value
1	Leukine Day 18 vs. External Controls, Age = 67.8333333, Baseline Calibrated	1.72242	1.39067	2.13330	5.12	<.0001
2	Leukine Follow Up 1 vs. External Controls, Age = 67.8333333, Baseline Calibrated	1.82889	1.49793	2.23297	6.08	<.0001
3	Leukine Follow Up 2 vs. External Controls, Age = 67.8333333, Baseline Calibrated	1.85946	1.47035	2.35156	5.35	<.0001
4	Placebo Day 18 vs. External Controls, Age = 67.8333333, Baseline Calibrated	1.79718	1.48007	2.18224	6.08	<.0001
5	Placebo Follow Up 1 vs. External Controls, Age = 67.8333333, Baseline Calibrated	1.71891	1.41030	2.09505	5.52	<.0001
6	Placebo Follow Up 2 vs. External Controls, Age = 67.8333333, Baseline Calibrated	1.78224	1.47750	2.14982	6.19	<.0001

Obs	Label	Ratio_Est	Ratio_CI_L	Ratio_CI_U	T_Statistic	p_value
1	Leukine Baseline vs. External Controls, Age = 73.6562500	1.31214	1.01846	1.69050	2.18	0.0364
2	Placebo Baseline vs. External Controls, Age = 73.6562500	1.47483	1.13234	1.92091	3.02	0.0056
3	Pooled Baseline vs. External Controls, Age = 73.6562500	1.39111	1.13591	1.70364	3.25	0.0018
4	Leukine Baseline vs. External MCI, Age = 73.6562500	0.72984	0.54465	0.97799	-2.16	0.0355
5	Placebo Baseline vs. External MCI, Age = 73.6562500	0.82033	0.60686	1.10888	-1.33	0.1915
6	Pooled Baseline vs. External MCI, Age = 73.6562500	0.77376	0.60127	0.99574	-2.03	0.0464
7	External MCI vs. External Controls, Age = 73.6562500	1.79786	1.45809	2.21679	5.62	<.0001

Obs	Label	Ratio_Est	Ratio_CI_L	Ratio_CI_U	T_Statistic	p_value
1	Study AD Age (per year)	0.99970	0.97939	1.02042	-0.03	0.9761
2	External MCI Age (per year)	1.03466	1.00170	1.06871	2.15	0.0398
3	External Controls Age (per year) (< 30)	0.96768	0.95802	0.97743	-6.45	<.0001
4	External Controls Age (per year) (>= 30)	1.03043	1.02674	1.03414	16.43	<.0001
5	Age Slopes (per year): External Controls, >= 30 vs < 30	1.06485	1.05185	1.07801	10.06	<.0001
6	Age Slopes (per year): Study AD vs External Controls (< 30)	1.03309	1.00993	1.05677	2.89	0.0058
7	Age Slopes (per year): External MCI vs External Controls (< 30)	1.06922	1.03373	1.10593	4.02	0.0003
8	Age Slopes (per year): Study AD vs External Controls (>= 30)	0.97017	0.95020	0.99057	-2.96	0.0056
9	Age Slopes (per year): External MCI vs External Controls (>= 30)	1.00411	0.97194	1.03733	0.26	0.7991
10	Age Slopes (per year): Study AD vs External MCI	0.96621	0.93046	1.00333	-1.83	0.0730

Obs	Label	Percent_Est	Percent_CI_L	Percent_CI_U	T_Statistic	p_value
1	Study AD Age (per year)	-0.03040	-2.06088	2.04217	-0.03	0.9761
2	External MCI Age (per year)	3.46599	0.16966	6.87079	2.15	0.0398
3	External Controls Age (per year) (< 30)	-3.23221	-4.19776	-2.25692	-6.45	<.0001
4	External Controls Age (per year) (>= 30)	3.04291	2.67364	3.41350	16.43	<.0001

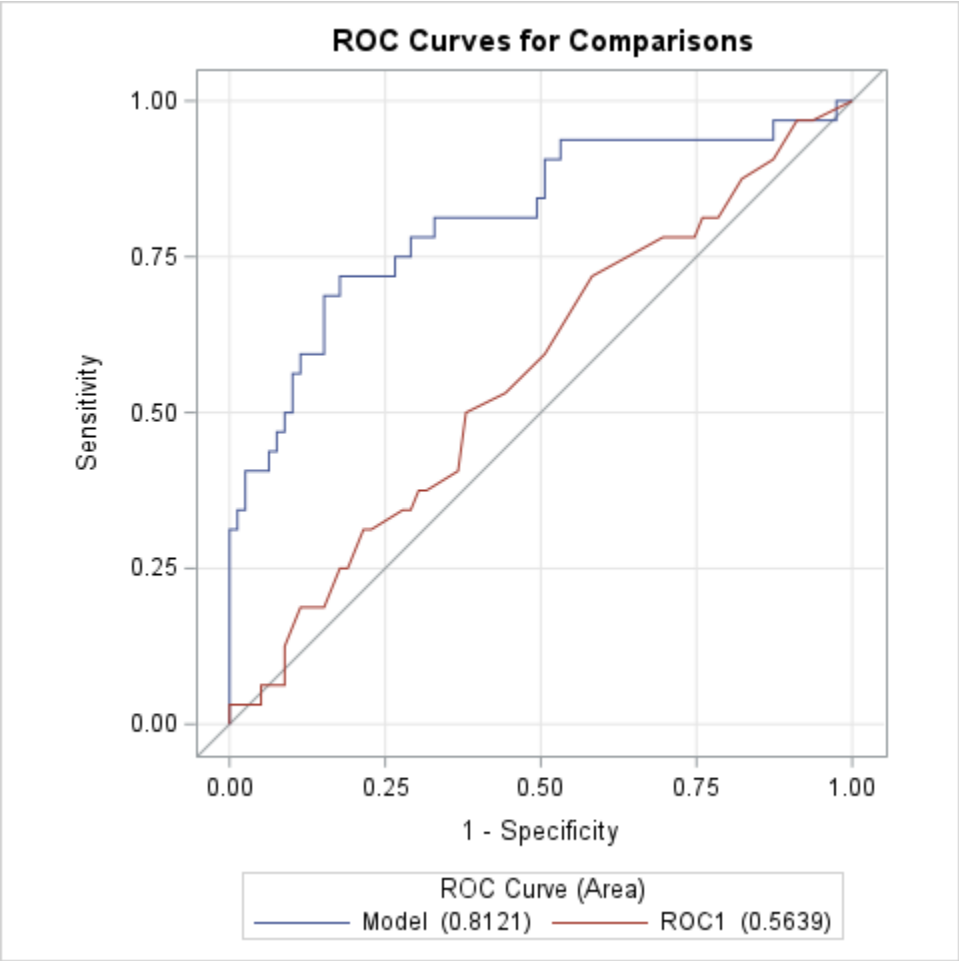
Supplemental Table S10 (related to Figure 3): Data and statistical calculations were analyzed for GFAP levels in the plasma of the human participants.

Table S11 ROC results; related to Figure S4

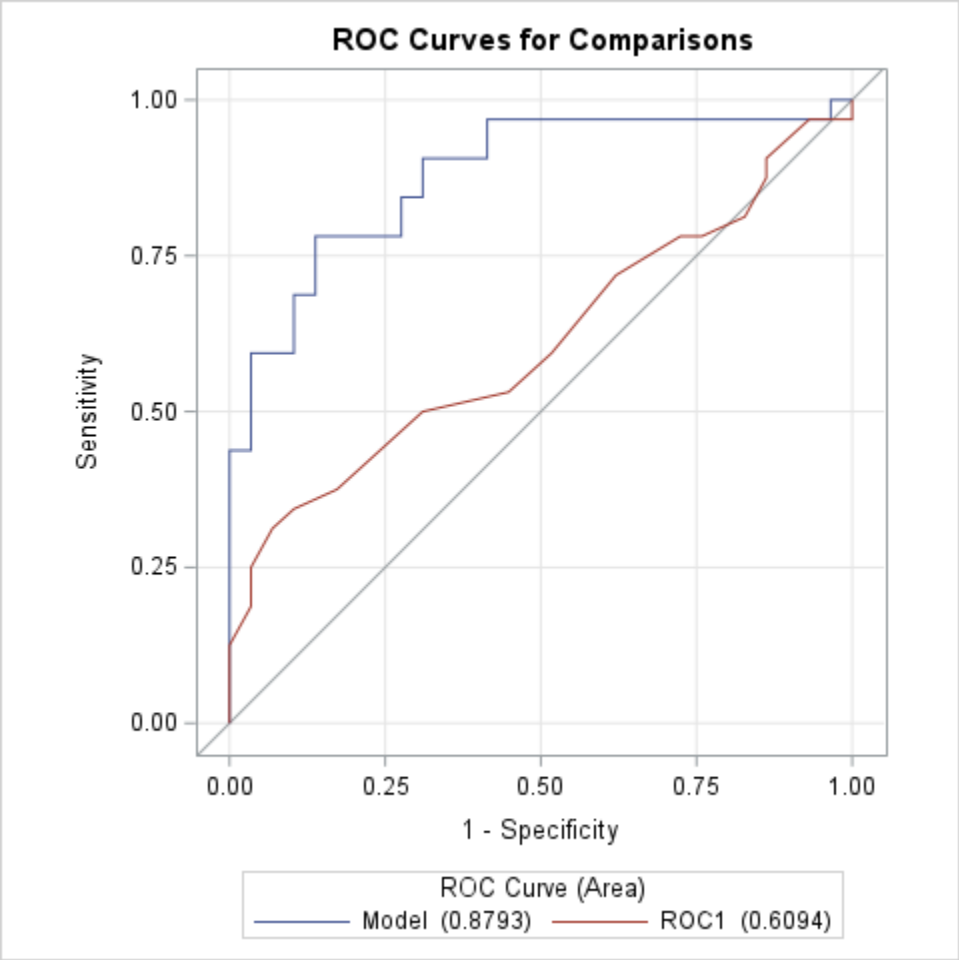
Contrast	Group	Number of Patients	Minimum Age (years)	Maximum Age (years)
MCI vs Healthy Controls	Healthy Controls	79	63	86
	MCI	32	63	87
MCI vs AD	AD	29	64	80
	MCI	32	63	87
AD vs Healthy Controls	Healthy Controls	103	55	80
	AD	36	55	80

Contrast	Model	ROC Area	Standard Error	95% Confidence Interval		p value
MCI vs Healthy Controls	age, LN[NfL], LN[GFAP], LN[UCH-L1]	0.8121	0.0493	0.7154	0.9088	
	age	0.5639	0.0602	0.4460	0.6818	
	difference	0.2482	0.0681	0.1147	0.3817	0.0003
					`	
MCI vs AD	age, LN[NfL], LN[GFAP], LN[UCH-L1]	0.8793	0.0444	0.7923	0.9664	
	age	0.6094	0.0730	0.4664	0.7524	
	difference	0.2699	0.0728	0.1272	0.4127	0.0002
AD vs Healthy Controls	age, LN[NfL], LN[GFAP], LN[UCH-L1]	0.8622	0.0373	0.7890	0.9353	
	age	0.5614	0.0536	0.4563	0.6664	
	difference	0.3008	0.0673	0.1690	0.4327	<0.0001

MCI vs control, full model vs age only model:



MCI vs AD, full model vs age only model:



AD vs control, full model vs age only model:

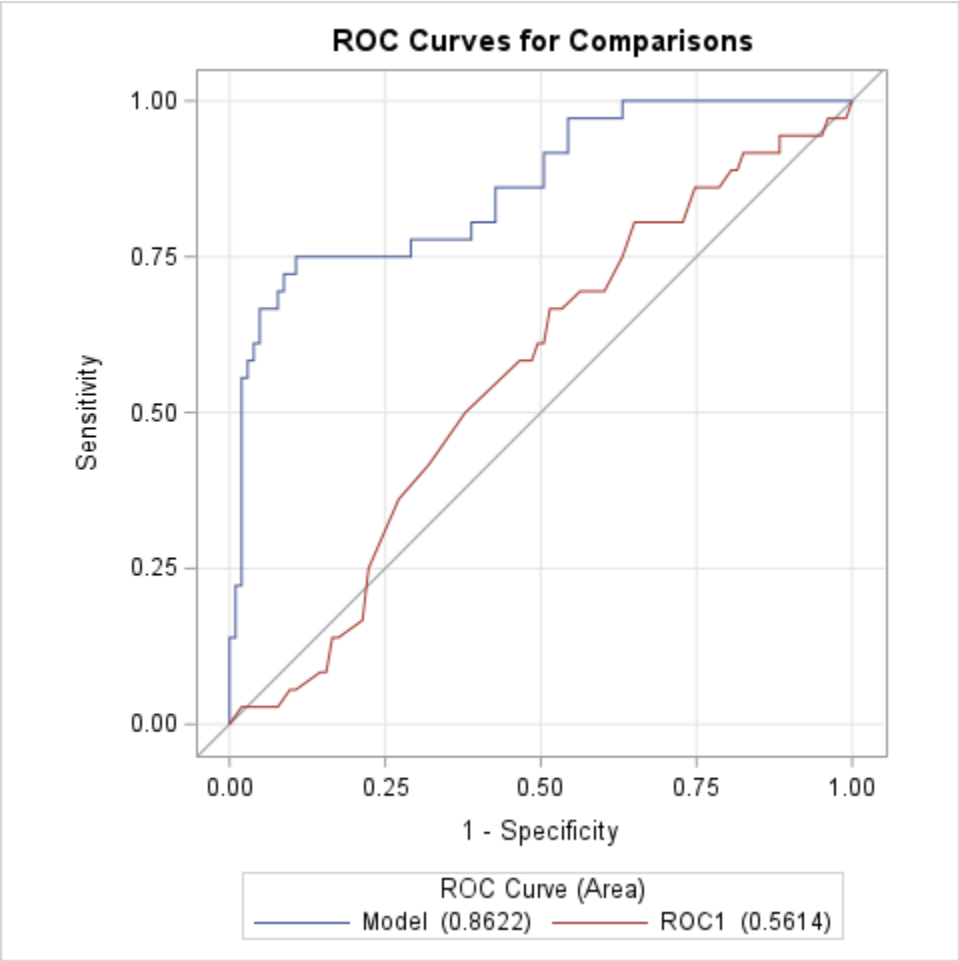


Table S11. Receiver Operating Characteristic (ROC) results; related to Figure S4. ROC curves were calculated to compare MCI vs Control, MCI vs AD and AD vs Control. The full model includes age, LN(UCH-L1), LN(NfL), and LN(GFAP), which is plotted for each comparison and compared to the model using only age as discriminator. The results indicate that the full model can well distinguish MCI from NC, MCI from AD, and AD from NC.