CLINICAL OUTCOMES AND BIOMARKER FINDINGS FROM A RANDOMIZED, PLACEBO-CONTROLLED TRIAL OF BEZISTERIM IN SUBJECTS WITH MILD TO MODERATE PROBABLE ALZHEIMER'S DISEASE

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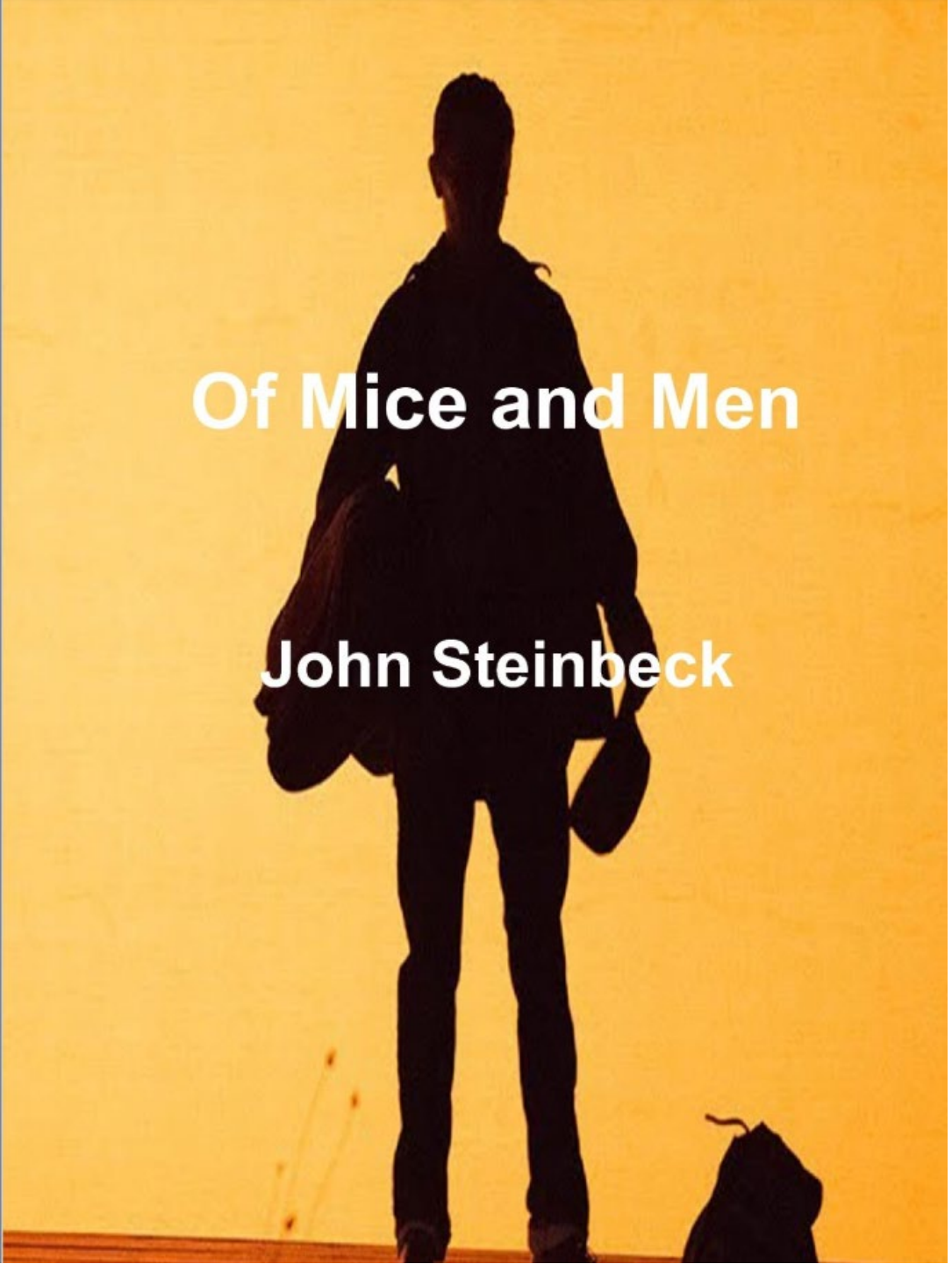
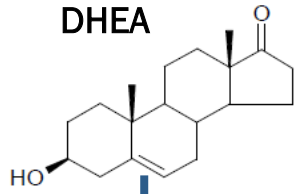
bioVie



In 2000, we asked "Why does DHEA show anti-inflammatory, insulin-sensitizing and anti-aging effects in mice, and not in humans?"



MICE — INFLAMMATION — MEN

√ ACTIVE

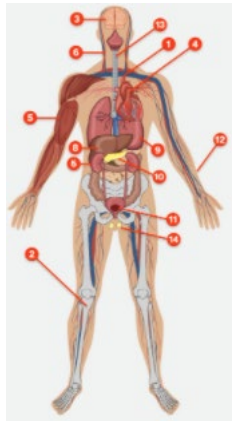
X INACTIVE

Harvard Health Publishing
HARVARD MEDICAL SCHOOL

NFκB
cytokines
chemokines
IRS1/2 p-Serine
insulin resistance

amyloid β
cognitive decline
Alzheimer's
neuron death

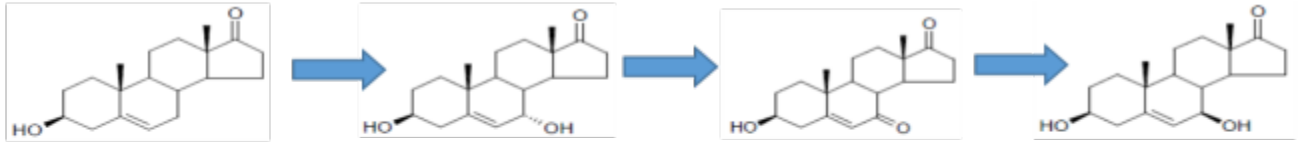
hypertriglyceridemia
adipogenesis
obesity
diabetes
longevity



insulin resistance
hyperglycemia
obesity
dyslipidemia
osteoporosis
cognitive decline
Alzheimer's
decreased quality of life

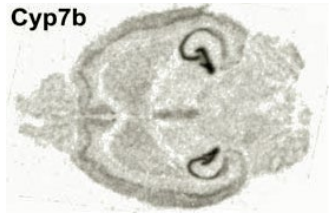
Thousands of publications

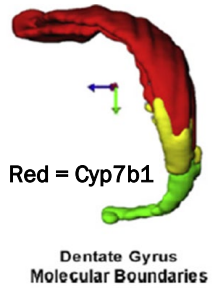
Differential Metabolism of Androst-5-ene-3,17-diol Between Rats, Canines, Monkeys and Humans



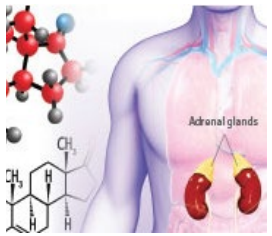
DHEA → 7α-hydroxyl-DHEA → 7-keto-DHEA → 7β-hydroxyl-DHEA

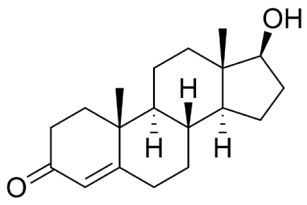
5-Androstene diol (AED) → 5-Androstene, 3β,7α,17β-triol (αAET) → 7-keto-AED → **5-Androstene, 3β,7β,17β-triol (βAET)**[1,2]



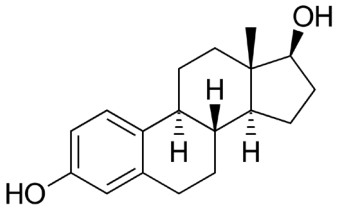


Neurosteroid: Cyp7B defines hippocampal dentate gyrus[3,4]

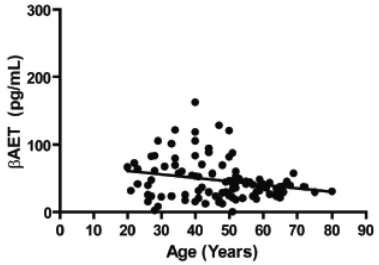




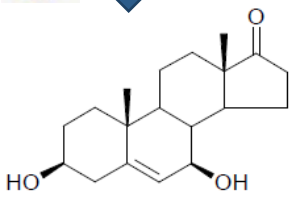
Testosterone



Estradiol



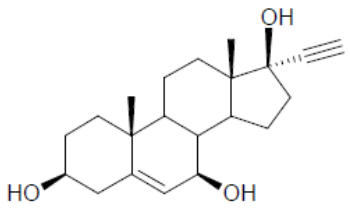
Oxidation



7β-hydroxyl-DHEA

INACTIVE

Medicinal Chemistry



17α-ethynyl-βAET (bezisterim)

Metabolic stability
Oral biovailability
Blood-brain permeable

Background

- Bezisterim (formerly NE3107)
  - Binds ERK[5] and inhibits inflammatory,[6,7] but not homeostatic ERK, NF-κB and TNF signaling[8]
  - Decreased inflammatory mediators and insulin resistance in animal models and subjects with obese impaired glucose tolerance or type 2 diabetes[9]
  - Improved motor activity and decreased neurodegeneration in a Parkinson's disease (PD) Marmoset model[10] and in a phase 2a PD study[11]
  - Improved neurological, neuroimaging, and biomarkers in an open-label 14-week phase 2 study in MCI and mild AD[12,13]
  - Has a well-tolerated safety profile to date[11,12]

Does Bezisterim Modify Metabolic Inflammation and "Longevity" Relevant to Processes Underlying Dementia?

- The primary goal of our study was to investigate the associations between metabolic inflammation, biological aging, and dementia in a human clinical investigation
- The specific aim tested whether bezisterim could impact physiologic processes consistent with neurocognitive decline and diseases of aging
- Methodology included:
  - Epigenetic methylation (clock: Horvath Epigenetic Clock Development Foundation[14])
  - Inflammation (clock: HURDLE/Chronomics analyses[15-18])
  - Principal component analyses
  - Divergent correlational analyses
- **Data from these analyses suggests that bezisterim may induce epigenetic remodeling associations between metabolic inflammation, biological aging, and dementia**

Patient Disposition and Data Source for This Presentation

- The trial started during the COVID-19 pandemic and enrolled a total of 439 subjects through 39 sites
- We reported that upon trial completion, the Company found significant deviation from protocol and Good Clinical Practice violations at 15 sites, causing the Company to exclude all subjects from these sites
- After exclusions for GCP violations, 57 subjects remained in the Per-Protocol population; those assigned to bezisterim were verified to have taken study drug from pharmacokinetics data, and 7 subjects randomized to placebo discontinued before day 150
- Baseline and completion data were available for 50 subjects (bezisterim, n=24 and placebo, n=26); and DNA methylation data were available for 33 of this cohort

Safety Findings: Bezisterim Treatment Was Well Tolerated

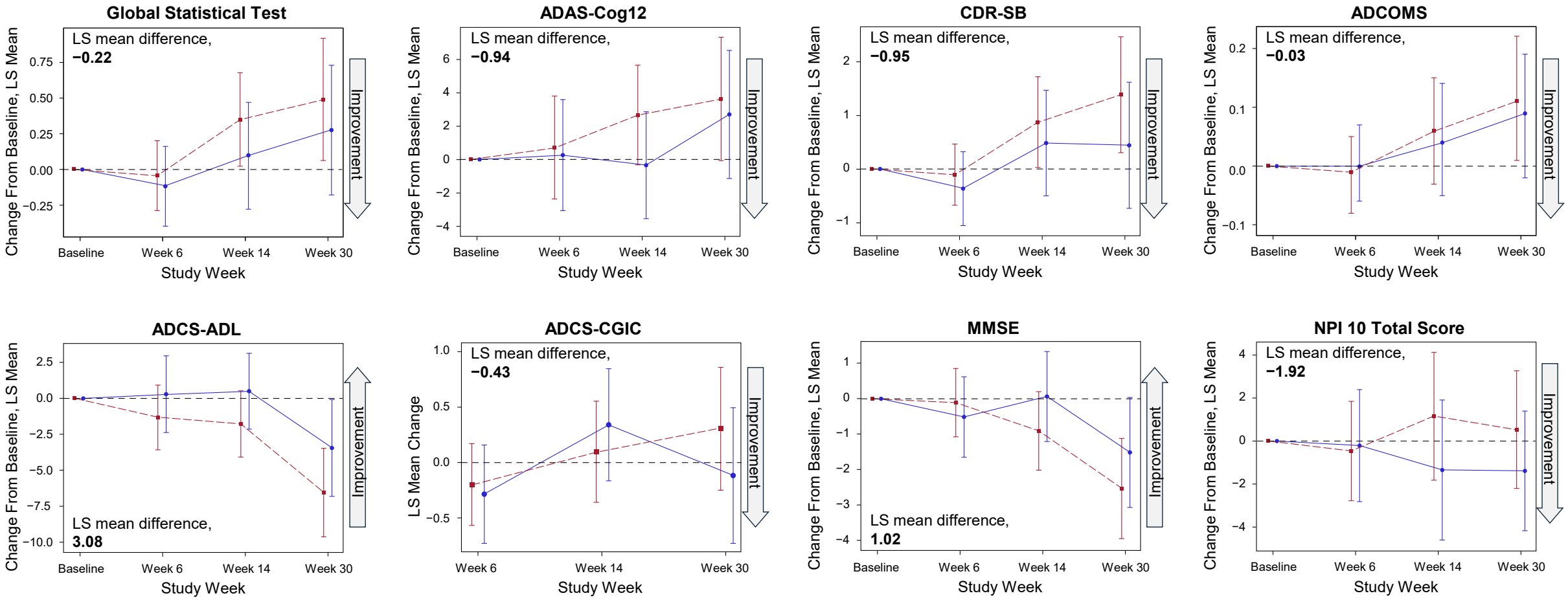
In the Per-Protocol (PP) population (n=57)

- Treatment emergent adverse events (TEAEs) occurred in 62.5% (n=15) of patients in the bezisterim group and 72.7% (n=24) of patients in the placebo group
  - Bezisterim TEAEs ≥5% and > Placebo

    Headache (12.5%; n=3) vs (0%; n=0)
- Treatment-related TEAEs occurred in 12.5% (n=3) of patients in the bezisterim group and 18.2% (n=6) of patients in the placebo group
- Serious AEs occurred in 4 patients (bezisterim, n=1; Placebo, n=3); none were treatment-related
- There was 1 non-treatment–related death in the bezisterim group. The patient was a 70-year-old male who died of a respiratory arrest
- 3 patients in the placebo group and none in the bezisterim group discontinued due to an AE

The PP Population included randomized participants who took at least 1 dose of study intervention and had a baseline and at least 1 post-baseline efficacy assessment. Participants from study sites who were identified as being in persistent violation of GCPs or who had a major protocol deviation (such as improper rater certification) that could impact primary efficacy were excluded. This analysis is to be based on the actual treatment the participant received.

Bezisterim Showed Improvements Over Time in Primary and Secondary Endpoints



Treatment Modified Change From Baseline in Primary and Secondary Endpoints Comparable to Approved Medications

- After the exclusions, the study was no longer powered for endpoints, but week 30 data suggest bezisterim vs placebo is comparable to results reported from clinical trials by approved medications

	Bezisterim vs placebo	Published data at 1.5 years[a]
Co-Primary		
CDR-SB (Lower=improvement)	−0.95 (68%)	−0.45 (27%)[19] −0.39 (22%)[20]
ADAS-Cog12 (Lower=improvement)	−0.94 (26%)	−1.44 (25%)[19] −1.40 (27%)[20]
Secondary		
MMSE (Higher=improvement)	+1.02 (40%)	+0.6 (18%)[20]
ADCS-ADL (Higher=improvement)	+3.08 (47%)	+2.0 (36%)[19]
ADCS-CGIC (Lower=improvement)	−0.43 (139%)	
ADCOMS (Lower=improvement)	−0.03 (27%)	−0.05 (23%)[20]

[a]Other published data at 18 months data for lecanemab[19] and aducanumab.[20]

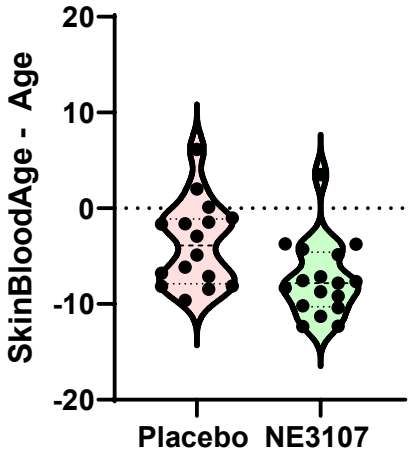
ADAS-Cog12, 12-item cognitive subscale of the Alzheimer's Disease Assessment Scale; ADCOMS, Alzheimer's Disease Composite Score; ADCS-ADL, Alzheimer's Disease Cooperative Study—Activities of Daily Living; ADCS-CGIC, Alzheimer's Disease Cooperative Study—Clinical Global Impression of Change; CDR, Clinical Dementia Rating.

Could Bezisterim Treatment Modify Biological Age in 7 Months?

- Individuals appear to age at different rates
  - Analysis of DNA methylation “clocks” can provide evidence of changes in gene expression related to aging (biological age)
  - The difference between the biological age and the chronological age (dAge) is a measure of age acceleration
- At study completion, available DNA samples from per protocol subjects (n=33) were analyzed by the Epigenetic Clock Development Foundation using the Horvath SkinBlood Clock[14]
- DNA methylation data were also analyzed by Hurdle/Chronomics for PhenoAge, Grim Age, AgeHannum, and their new InflammAge Clock[15-18]

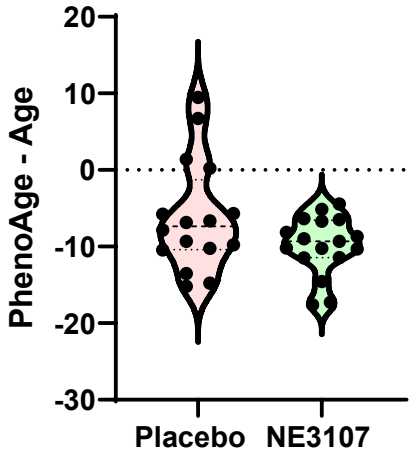
Bezisterim Modified dAge[a] for 5 Aging Clocks

SkinBloodClock dAge



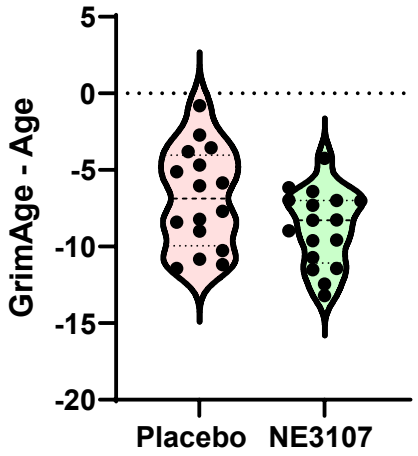
mean -3.70, -7.41
n 16, 17
Δ **-3.68 years**
Welch t-test **p 0.017**

Pheno dAge



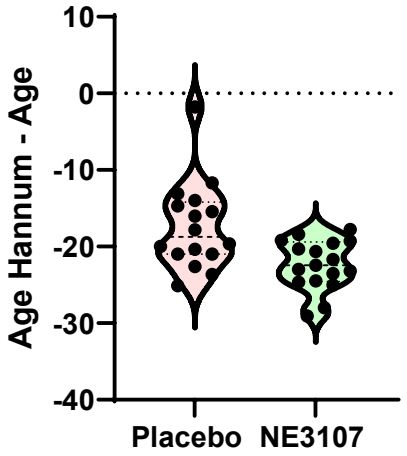
mean -6.14, -9.84
n 16, 17
Δ **-3.71 years**
Welch t-test **p 0.081**

Grim dAge



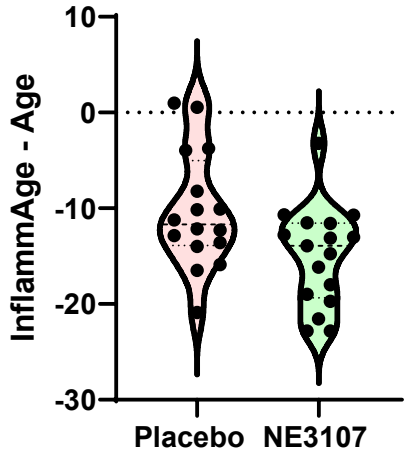
mean -6.85, -8.77
n 16, 17
Δ -1.92 years
Welch t-test **p 0.068**

Hannum dAge



mean -17.4, -22.4
n 16, 17
Δ **-5.00 years**
Welch t-test **p 0.006**

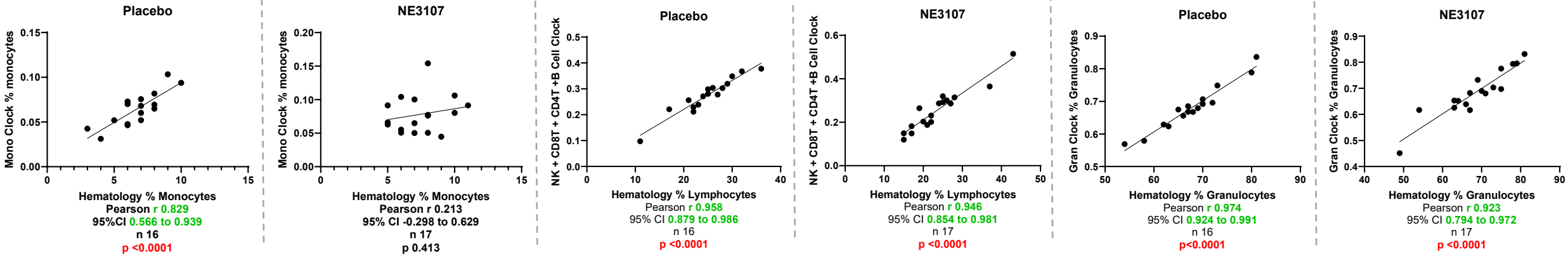
Inflamm dAge



mean -10.25, -15.02
n 16, 17
D **-4.77 years**
Welch t-test **p 0.022**

[a] dAge = Clock age – chronological age

Bezisterim Modification of Monocyte DNA Methylation May Alter SBC Correction for Cell Type



Cell Type	Placebo		Bezisterim			
	Pearson r	P	Pearson r	P	Z'	P[a]
Monocytes	0.829	<0.0001	0.213	0.413	2.51	0.012
Lymphocytes	0.958	<0.0001	0.946	<0.0001	0.334	0.738
Granulocytes	0.974	<0.0001	0.923	<0.0001	1.44	0.149

Correlations of cell type clocks and hematology results:

- Placebo hematology % monocytes is correlated with Mono Clock
- Bezisterim hematology % monocytes is not correlated with Mono Clock
- **Bezisterim's impact on dAge may be explained by modification of monocyte DNA methylome, changing from a pro-inflammatory to an anti-inflammatory state (M1→M2 transition hypothesis)**

[a] Significance between correlations=Z test statistic of Fisher's to Z transformation

Precedent For Epigenetic Memory In Long Covid Monocytes[21]

- Severe COVID-19 programs durable epigenetic changes and hyper-activation in monocytes
- Circulating HSPC capture post-COVID-19 changes in hematopoiesis and stem cell programs
- Post-COVID-19 HSPC convey epigenetic and transcriptional memory to mature progeny cells
- IL-6 contributes to epigenetic reprogramming of mouse and human HSPC and myeloid cells

Correlations and Principal Components

- Correlations
  - For DNA methylation data and neurological assessments from per-protocol subjects with available DNA methylation data that passed QC (n = 33), the data was normalized[a] in separate correlation matrices for bezisterim and placebo
  - For clinical measure correlations, data from per-protocol subjects that completed >150 days treatment (n = 50) were normalized in separate matrices for bezisterim and placebo

- Principal Component Analysis
  - Principal component analysis (PCA) is a dimensionality reduction and machine learning method used to simplify a large data set into a smaller set while still maintaining significant patterns and trends[22]
  - PCA was performed to reduce data dimensionality for the dAge correlations

[a] Missing data values were Bayesian imputed, outliers were removed, and results were converted to Z' scores.

Bezisterim Decreased Age Acceleration Was Correlated With Improvements In Neurologic Assessments

SkinBlood Clock	Neurologic Assessment	Placebo n = 16		Bezisterim n = 17	
		Pearson r	p	Pearson r	p
dAge	GST	0.209	0.438	0.473	0.055
	CDR-SB	0.110	0.686	0.413	0.099
	ADAS-Cog12	0.167	0.537	0.455	0.067
	MMSE	−0.011	0.967	−0.580	0.015
	ADCOMS	0.134	0.621	0.469	0.058
	ADCS-CGIC	0.017	0.952	0.467	0.059

- Placebo completion
  - **No correlations** with neurologic assessments
- Bezisterim completion
  - Decreased age acceleration **correlated with improvements**
    - GST, MMSE, CDR-SB, ADCOMS, ADAS-Cog12, ADCS-CGIC

Bezisterim Modified Interdependence of Neurological Assessments

Placebo was correlated with worsening biomarkers/measures

Dementia biomarkers

↑GFAP with ↑GST, ↓MMSE, ↑CDR-SB, ↑ADCOMS, ↑Cog12

↑pTau217 with ↑GST, ↑ADCOMS, ↑Cog12, ↑CGIC

Metabolic measures

↑Blood Pressure with ↑GST, ↑CDR-SB, ↑ADCOMS

↑Glucose with ↑CDR-SB

↑Beta cell insulin production with ↓ADL

Inflammatory measures

↑CRP with ↑CDR-SB, ↓ADL

↓C1q with ↑CGIC

Bezisterim yielded inverse correlations with improvement vs placebo

- ↓Fructosamine with ↑ADL (placebo: ↓Fructosamine with ↓ADL) p=0.003[a]
- ↑RANTES with ↓CDR-SB (placebo: ↑RANTES with ↑CDR-SB) p=0.003[a]

Bezisterim correlated with additional improvements

- ↓NfL with ↓GST, ↑MMSE, ↓CDR-SB, ↓Cog12, ↓ADCOMS, ↑ADL
- ↓Cholesterol with ↓GST, ↑MMSE, ↓CDR-SB, ↓Cog12
- ↑RANTES with ↓GST, ↑MMSE, ↓ADCOMS, ↓Cog12

For neurological assessments Red = Decline, Green = Improvement.

[a] Z test statistic of Fisher Z transformation.

Placebo dAge Principal Component Analysis:

Divergent PC1 vs PC2 suggestive of systems dysregulation in placebo-treated patients

PCA1 included metabolic, inflammation, & dementia biomarkers

- Metabolic

(Glc, HOMA2, Trig, Chol, WHR, Ins, Fruc)

- Inflammation

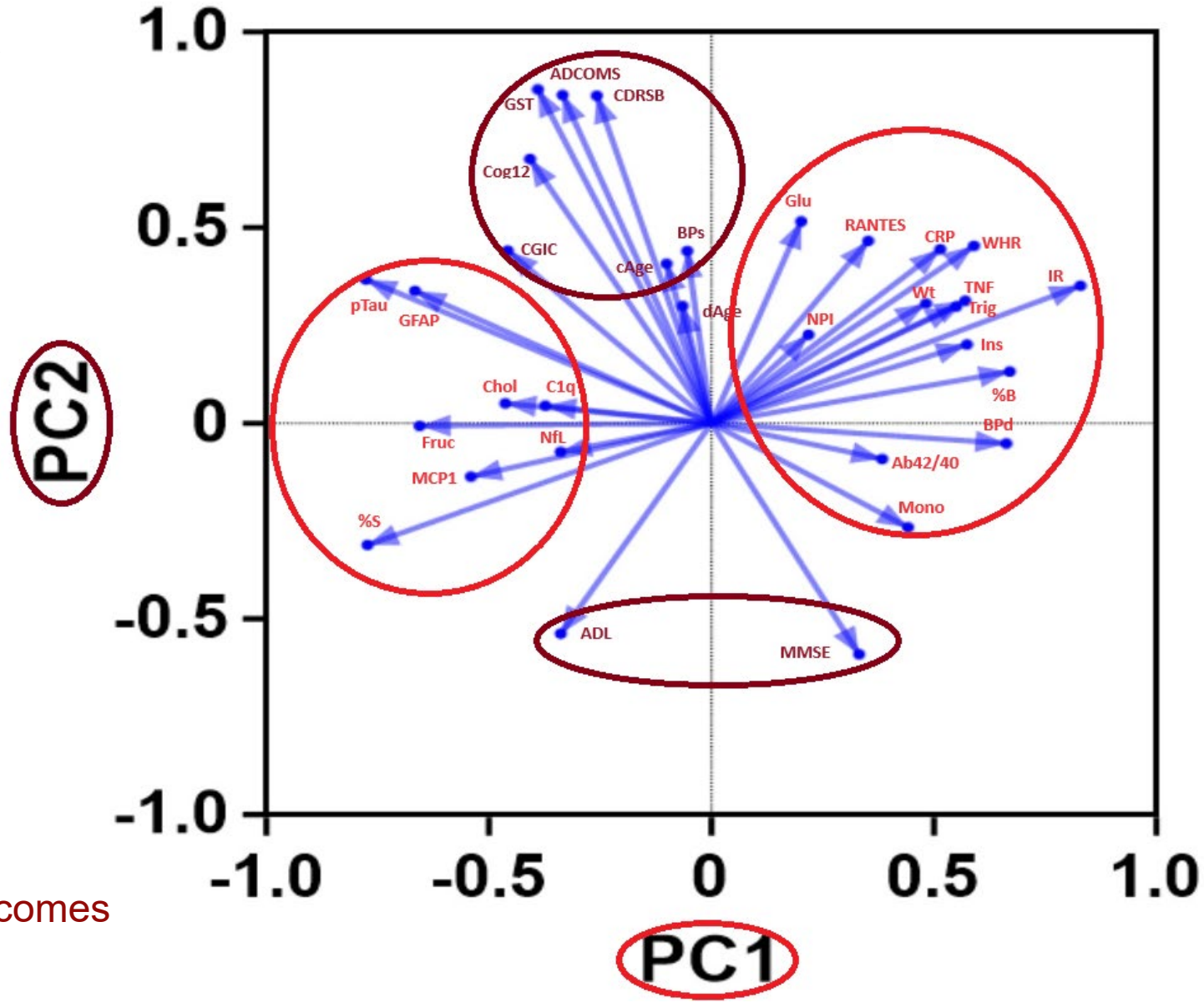
(TNF, CRP, RANTES, MCP1, C1q)

- Dementia Biomarkers

(Ab42/40, pTau, GFAP, NfL)

PCA2 included

- Neurological assessments
- Age
- DNA methylation
- dAge was only weakly associated with outcomes



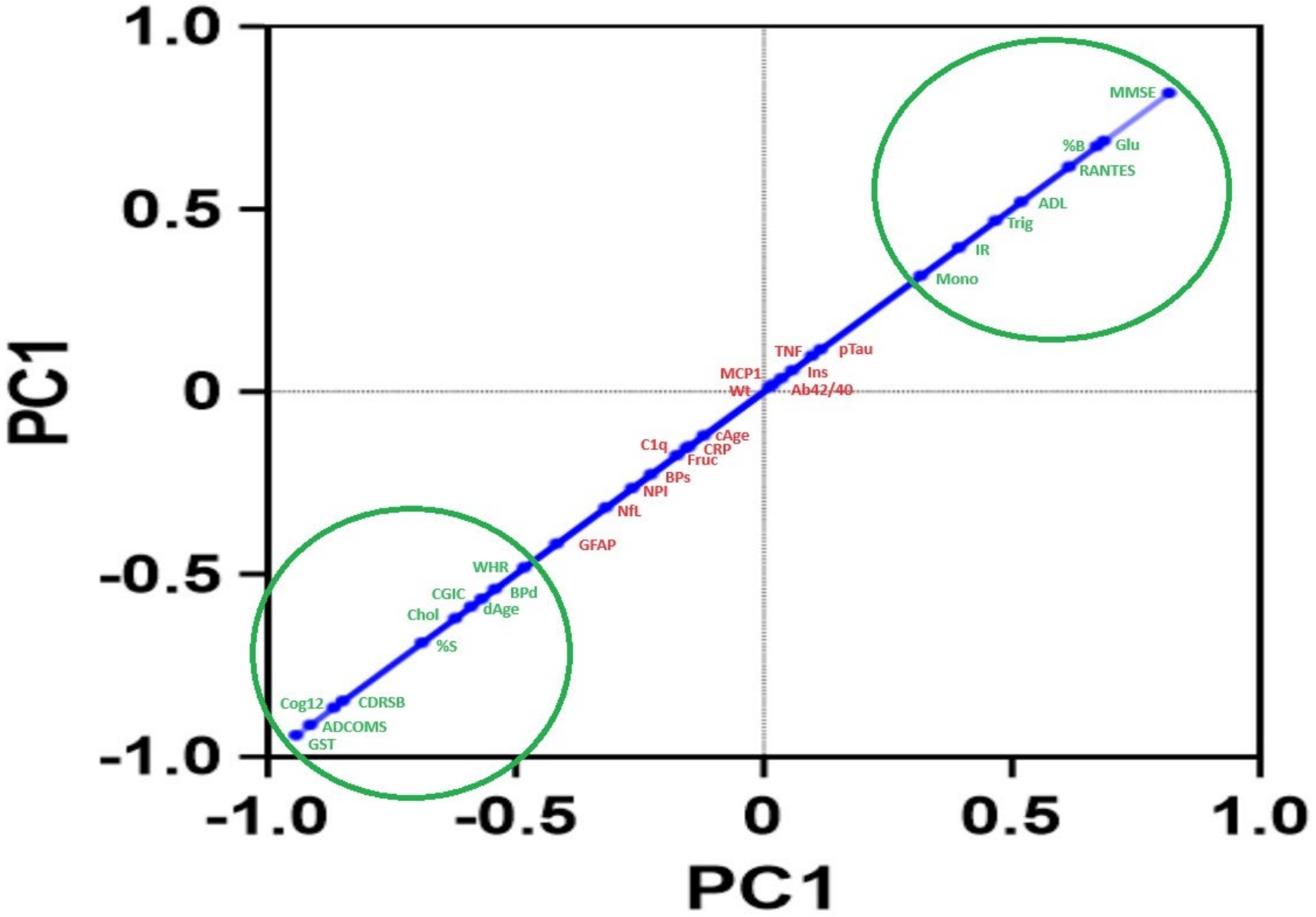
Bezisterim Modified dAge PCA:

Single PC1 suggestive of re-regulation in Bezisterim-treated patients

- Only one PCA Identified

Neurological assessments
Metabolic
(Glc, Insulin Sensitivity, Trig, Chol, BP)
Microglia-related
(% Mono, RANTES)
DNA methylation
(dAge)

Excludes
Inflammatory Biomarkers
(CRP, C1q, TNF, MCP1)
Dementia Biomarkers
(pTau, Ab42/40, NfL, GFAP)

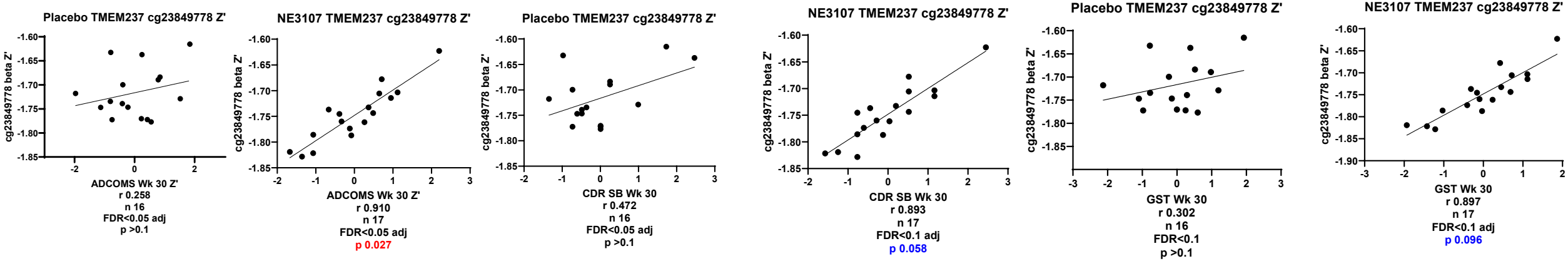


Treatment-Mediated PCA dAge Summary

- For placebo-treated subjects at study completion
  - PC1 identified metabolic, inflammation, and dementia biomarkers
  - PC2 identified chronological age and neurological assessments; age acceleration was only weakly associated with outcomes

- For bezisterim-treated subjects
  - Only PC1 was identified, combining metabolism, innate immunity, and age acceleration, and excluding inflammatory and dementia biomarkers
  - All 31 measures were significant in principal component regression (PCR) for dAge with bezisterim, with no significance for placebo

Bezisterim Modified Correlations Between Clinical Measures and DNA Methylation of AD-Associated Genes



EXAMPLE: Decreased **Transmembrane protein 237** is associated with neurodevelopment delays. Decreased TMEM237 Promoter methylation (increased expression) correlates with improved ADCOMS

- ~21 significant correlations with individual gene CpGs
  - 15 were significant for bezisterim and not for placebo
  - 6 were significant for placebo and not for bezisterim
  - 15 correlations were in promoters, enhancers of CpG islands
- These correlations extend the Principal Component Analyses
  - Aging clocks; clinical correlations; epigenetic modification of AD-related genes

These modifications were significant at FDR <0.05, and Fisher transformation (p<0.05)

Divergent Correlations Were Observed With RANTES

- Placebo Week 30
  - Increased RANTES
    - Significant correlates with CDR-SB (decline), TNF, Triglycerides
    - Trending correlations with baseline WHR, increased CRP & C1q
- Bezisterim Week 30
  - Increased RANTES
    - Significant correlates with improved MMSE, CDR-SB, ADCOMS, ADAS-Cog12, & GST
    - Trending correlations with improved ADCS-CGIC, Weight and decreased TNF

Proinflammatory and Anti-inflammatory Myeloid Cell States[23]

- Monocytes, macrophages, astrocytes and microglia can exist in a continuum between the extremes (A1 and M1: proinflammatory cytokines and chemokines, ROS, tissue destruction, to A2 and M2: anti-inflammatory cytokines, phagocytic, tissue repair)
- MCP1 (chemokine CCL2) recruits monocytes and microglia; depending on the milieu, they can be M1 or M2 biased
- Aβ, pTau, ROS and proinflammatory cytokines and chemokines activate astrogliosis leading to A1 astrocytes and M1 microglia

RANTES (CCL5), Receptor = CCR5

- Proinflammatory[24]
  - CCL5 is a “Janus” chemokine contributing to both pro- and anti-inflammatory programs
  - Low grade inflammation can stimulate inflammatory cytokine and chemokine production
  - This drives A1 astrocytes and M1 microglia (NF-κB, cytokines, oxidative stress)
  - These insults are associated with AD progression

- Anti-inflammatory
  - CCL5 important in neurotransmission, neuron development and learning and memory
  - CCL5 is decreased in AD plasma
  - Deficiency induces astrocyte activation, Aβ deposit, impaired memory function
  - CCL5 is neuroprotective against ROS in neurons
  - CCR5 deletion associated with earlier dementia onset
  - CCL5 recruits microglia M2 immune response
  - CCR5 antagonist decreases CCR2-induced NF-kB & Aβ; CCL5 ko mouse microglia have increased NF-κB, MCP1 and Aβ production

Inhibition of NF-κB can result in A2 and M2 transitions, including stimulation of anti-inflammatory cytokines, tissue repair and Aβ phagocytosis

Summary

- In this small per-protocol sample, compared to placebo, bezisterim appeared to:
  - Improve neurological assessments
  - Decrease biological age
  - Realign biological aging with neurological assessments
- Correlations, PCA and PCR (Principal Component Regression) are consistent with the hypothesis that bezisterim, by decreasing TNF- and MCP1-stimulated NF-κB and neuroinflammation, might promote a transition of microglia from inflammatory and destructive to anti-inflammatory, phagocytic and restorative cells
- In the absence of NF-κB and TNF signaling, RANTES signaling may transition astroglia and microglia to anti-inflammatory, phagocytic (degrading Aβ), restorative phenotypes
- Bezisterim appeared to change the monocyte phenotype and DNA methylome
- These data suggest bezisterim may improve probable AD via pathways related to inflammation
- An additional trial under strict GCP oversight will be required to confirm these findings
- It is possible that DNA methylation aging clocks may prove to be valuable biomarkers for neurodegeneration

Collaborators

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CLOCK FOUNDATION

Juozas Gordevicius, Ph.D.
Bobby Brooke

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Lisa Schmunk Ph.D.
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Quanterix
Discovery Fueled by Ultra-Sensitivity

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p-value communications

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Abbreviations

- Aβ42/40, ratio of amyloid β 42 to amyloid β 40
- AD, Alzheimer's disease
- ADAS-Cog12, 12-item cognitive subscale of the Alzheimer's Disease Assessment Scale
- ADCOMS, Alzheimer's Disease Composite Score
- ADCS-ADL, Alzheimer's Disease Cooperative Study—Activities of Daily Living
- ADCS-CGIC, Alzheimer's Disease Cooperative Study—Clinical Global Impression of Change
- BP, blood pressure
- cAge, chronological age
- CDR-SB, Clinical Dementia Rating Sum of Boxes
- Chol, cholesterol
- COVID-19, coronavirus 2019
- CRP, C-reactive protein
- CSF, cerebrospinal fluid
- dAge, age acceleration (Horvath SkinBlood Clock age – chronological age)
- ERK, extracellular signal-regulated kinase
- GFAP, glial fibrillary acidic protein
- GST, Glutathione S-transferase
- HOMA2, Homeostatic Model Assessment 2
- IGT, impaired glucose tolerance
- IR, HOMA2 insulin resistance
- MCP1, monocyte chemoattractant protein-1
- MMSE, mini-mental state exam
- Mono, monocytes
- NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells
- NfL, neurofilament light chain
- MDS-UPDRS, Movement Disorder Society-Unified Parkinson's Disease Rating Scale
- NPI, Neurological Pupil Index
- %B, HOMA2 % beta cell function
- %S, HOMA2 % insulin sensitivity
- PCA, Principal Component Analysis
- PD, Parkinson's disease
- pTau217, phosphorylated tau
- RANTES, Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted
- TNF, tumor necrosis factor alpha
- Trig, triglycerides
- TSH, thyroid-stimulating hormone